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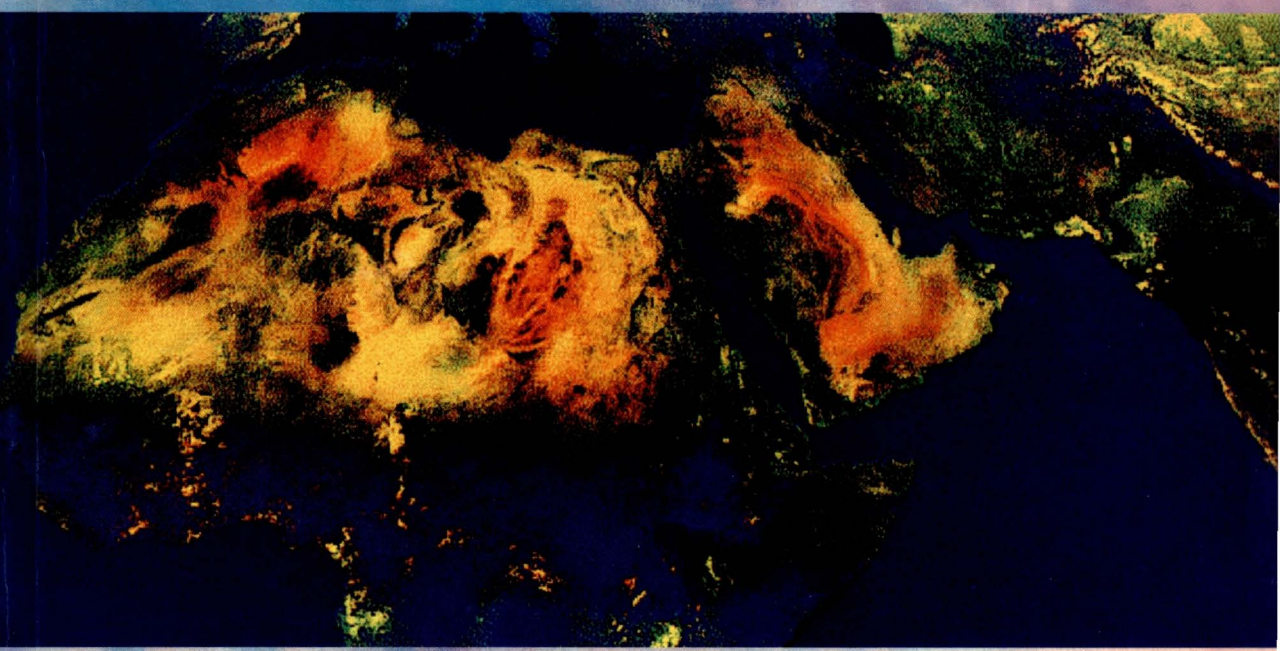

المكتب الإقليمي لشرق المتوسط
Regional Office for the Eastern Mediterranean
Bureau régional de la Méditerranée orientale

منظمة الصحة العالمية
World Health Organization
Organisation mondiale de la Santé

المجلة الصحية لشرق المتوسط

هي المجلة الصحية الرسمية التي تصدر عن المكتب الإقليمي لشرق المتوسط بمنظمة الصحة العالمية. وهي منبر لتقديم السياسات والمبادرات الجديدة في الخدمات الصحية والترويج لها، ولتبادل الآراء والمفاهيم والمعطيات الوبائية ونتائج الأبحاث وغير ذلك من المعلومات، وخاصة ما يتعلق منها بإقليم شرق المتوسط. وهي موجهة إلى كل أعضاء المهن الصحية، والكليات الطبية وسائر المعاهد التعليمية، وكذا المنظمات غير الحكومية المعنية، والمراكز المتعاونة مع منظمة الصحة العالمية والأفراد المهتمين بالصحة في الإقليم وخارجه.

EASTERN MEDITERRANEAN HEALTH JOURNAL

IS the official health journal published by the Eastern Mediterranean Regional Office of the World Health Organization. It is a forum for the presentation and promotion of new policies and initiatives in health services; and for the exchange of ideas, concepts, epidemiological data, research findings and other information, with special reference to the Eastern Mediterranean Region. It addresses all members of the health profession, medical and other health educational institutes, interested NGOs, WHO Collaborating Centres and individuals within and outside the Region.

LA REVUE DE SANTE DE LA MEDITERRANEE ORIENTALE

EST une revue de santé officielle publiée par le Bureau régional de l'Organisation mondiale de la Santé pour la Méditerranée orientale. Elle offre une tribune pour la présentation et la promotion de nouvelles politiques et initiatives dans le domaine des services de santé ainsi qu'à l'échange d'idées, de concepts, de données épidémiologiques, de résultats de recherches et d'autres informations, se rapportant plus particulièrement à la Région de la Méditerranée orientale. Elle s'adresse à tous les professionnels de la santé, aux membres des instituts médicaux et autres instituts de formation médico-saniaire, aux ONG, Centres collaborateurs de l'OMS et personnes concernés au sein et hors de la Région.

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ALL ARTICLES ARE PEER REVIEWED

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COVER: Remote sensing image of the area of the world that includes the Eastern Mediterranean Region. Cover image produced by the Canada Centre for Remote Sensing, Ottawa, Canada. (NOAA AVHRR Composite) Reproduced with permission

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Contents

Eastern Mediterranean Health Journal

Letter from the Editor

The devastating earthquake in Bam, Islamic Republic of Iran on 26 December 2003 was a profound tragedy inflicting great loss and suffering on the people. The world responded with help and Dr Hussein A. Gezairy, the Regional Director for the WHO Eastern Mediterranean Regional Office affirmed the commitment of WHO to provide necessary technical and material support to the Islamic Republic of Iran. This disaster served to illustrate what little control we often have, but at the same time emphasized the need to be prepared.

Unfortunately, it is often the actions of people themselves that affect the health and well-being of others. In this issue we present two papers on the impact of sanctions on the health of children in Iraq. While sanctions are no longer an issue, sadly the beleaguered people of Iraq are now experiencing other health dangers as a result of the ongoing fighting within the country. It is to be hoped they will come through these troubled times and come to enjoy peace and stability. WHO continues to work to assist the country in its provision of health care for its people. Today we also face the man-made threat of bioterrorism and included here are two papers on anthrax, which has already been used as an agent for bioterrorism and remains a potential threat.

Not only do people suffer from risks from outside, but they can endanger their own well-being by their actions and life styles. On this aspect, this issue contains papers on risky health behaviour among university students in Egypt, opioid use in the Islamic Republic of Iran, and respiratory function in chronic smokers in Turkey.

It is reported that 7 million children around the world are born annually with severe genetic disorders or birth defects and 90% of infants born with such disorders are found in developing countries. Thus included here are a number of papers concerned with genetic conditions such as Down syndrome, fragile X syndrome and Rothmund Thomson syndrome and an Arabic paper on handicapped and mute children.

These are just some of the papers published in this first issue of Volume 10. Other areas covered are communicable diseases (such as malaria, tuberculosis, hepatitis, typhoid fever and leprosy), health systems and population health data.

As ever, therefore, EMHJ presents an eclectic mix of papers from many countries both within and outside the Region, and we hope our readers will find much to inform and stimulate interest.

رسالة من المحرِّر

كان الزلزال المدمِّر الذي ضرب مدينة بام في جمهورية إيران الإسلامية في السادس والعشرين من كانون الأول/ديسمبر 2003 مأساة مفجعة، أدَّت إلى خسائر جسيمة ومعاناة كبيرة بين الناس. وقد استجاب العالم لهذا الحدث بتقديم العَوْن، وأكَّد الدكتور حسين الجزائري، المدير الإقليمي لمنظمة الصحة العالمية لشرق المتوسط، على التزام منظمة الصحة العالمية بتقديم الدعم التقني والمادي الضروريَّيْن لجمهورية إيران الإسلامية. وقد ألْقَتْ هذه الكارثة الضوء على كمِّ السيطرة المحدود الذي يتوافر أمامنا لمواجهة هذه الكوارث، ولكنها في نفس الوقت أكَّدت الحاجة إلى الاستعداد.

والمؤسف أنَّ ما يقوم به الناس أنفسهم هو الذي يؤثِّر غالباً على صحة الآخرين ومعافاتهم. ويطالع القارئ في هذا العدد مقالتَيْن عن أثر العقوبات على صحة أطفال العراق. صحيح أن العقوبات لم تَعُدْ قضية ذات شأن بعد الاحتلال، إلا أن من المحزن أن السكان الذين أنهكهم الحصار المديد في العراق يعانون الآن من الأخطار الصحية الأخرى نتيجةً للحرب الدائرة في بلدهم. ونسأل الله أن يعينهم على تجاوُز هذه المحنة وعلى التمتُّع بالسلام والاستقرار. وتواصل منظمة الصحة العالمية بَذْلَ ما في وُسعها لمساعدة هذا البلد العزيز في تقديم الرعاية الصحية لأهله. أما التهديد بالإرهاب البيولوجي الذي صنعه الإنسان، فتتناوله مقالتان عن الجمرة الخبيثة، التي تمثِّل تهديداً ممكناً.

على أن معاناة الناس لا تقتصر على ما يحيط بهم من مخاطر خارجية، بل إنهم يعرِّضون صحتهم وعافيتهم الشخصية للخطر نتيجة لأفعالهم وأنماط حياتهم. وفي ما يخص هذا الجانب، يتضمَّن هذا العدد من المجلة مقالات حول السلوكيّات المحفوفة بالمخاطر على الصحة بين طلبة الجامعات في مصر، وحول تعاطي الأفيون في جمهورية إيران الإسلامية، وحول الوظائف التنفسية بين مدمني التدخين في تركيا.

لقد أبلغ عن أن سبعة ملايين طفل في العالم يولدون سنوياً وهم يحملون اضطرابات وراثية أو عيوباً خلقية وخيمة، وأن 90٪ من الصبية الذين يولدون بمثل هذه الاضطرابات يعيشون في البلدان النامية. ولذلك يحتوي هذا العدد من المجلة على عدد من المقالات المهتمة بالحالات الوراثية مثل متلازمة داون، ومتلازمة الإكس الهشَّة، ومتلازمة روثمند — طومسون، ومقالة باللغة العربية عن الأطفال المعوقين البُكم.

ومن المواضيع الأخرى التي يغطِّيها هذا العدد الأول من المجلد العاشر من المجلة: الأمراض السارية (كالملاريا، والسل، والالتهاب الكبدي، والحمى التيفية، والجذام)، والنُظُم الصحية، والمعطيات الصحية للسكان.

وهكذا فإن المجلة الصحية لشرق المتوسط تقدِّم كالعادة مزيجاً متنوِّعاً من المقالات العلمية من العديد من البلدان سواء من داخل الإقليم أو من خارجه، ونأمل أن يجد قراؤها كمّاً لا بأس به من المعلومات القيِّمة التي تثير اهتمامهم.

Hepatitis B infection among Iraqi children: the impact of sanctions

H.Y.M. Ali[1]

العدوى بالتهاب الكبد البائي بين الأطفال العراقيين: مغبّة العقوبات

هشام علي

الخلاصة: درست مَغبّة العقوبات على توافر لقاح التهاب الكبد البائي ومعدل حدوث التهاب الكبد الفيروسي البائي بين الأطفال العراقيين، وذلك في الفترة بين حزيران/يونيو 2000 وحزيران/يونيو 2001، وشملت الدراسة أسر المرضى الذين يزورون مختبر الصحة العمومية في الموصل للمتابعة حول التهاب الكبدي البائي وذلك بإجراء اختبارات المقايسة المناعية للممتز المرتبط بالأنزيم (الإليزا) لتحري المستضد السطحي لالتهاب الكبد والمستضد الغلافي لالتهاب الكبد البائي والمضاد للالتهاب الكبدي البائي. وقد شخصت الدراسة 74 من الأطفال المولودين بين عامي 1994-1998 على أنهم حملة للمستضد السطحي للالتهاب الكبدي البائي. ومن بين هؤلاء أسرع 62 من آبائهم وأمهاتهم لاستشارة مراكز التطعيم، فحصل 21 منهم فقط على جرعة واحدة من اللقاح فيما لم يتمكن 41 منهم من الحصول على أية جرعة من اللقاح. وكان المستضد الغلافي لالتهاب الكبد البائي إيجابياً لدى تسعة أطفال (14.5٪) وكان المضاد للالتهاب الكبدي البائي إيجابياً لدى خمسين منهم (80.7٪). وكانت المقاومة التي أبداها 12 من الوالدين سبباً لعدم تلقيحهم التلقيح. وقد تم توثيق نقص اللقاحات في السنوات التي ولدت فيها الحالات المدروسة وذلك حتى بعد تطبيق القرار 986 الذي أصدره مجلس الأمن.

ABSTRACT Effect of sanctions on hepatitis B vaccine availability and occurrence of viral hepatitis B among Iraqi children was studied. Between June 2000 and June 2001, families of patients attending the Public Health Laboratory, Mosul, for hepatitis B follow-up were screened. Enzyme-linked immunosorbent assay was used to test for HBsAg, HBeAg and anti-HBe. We diagnosed 74 children born 1994–1998 as HBsAg carriers. For 62 of 74 cases, parents had consulted vaccine centres promptly: 41 were not vaccinated and 21 had only one vaccine dose. HBeAg marker was positive for 9 (14.5%) and anti-HBe for 50 (80.7%). Parental reluctance was the reason for non-vaccination for 12. Vaccine shortages during the birth years of cases were documented, even after implementation of United Nations Security Council Resolution 986.

L'infection par le virus de l'hépatite B chez les enfants iraquiens : impact des sanctions

RESUME L'effet des sanctions sur la disponibilité du vaccin contre l'hépatite B et la survenue de l'hépatite virale B chez les enfants iraquiens a été étudié. Entre juin 2000 et juin 2001, les familles des patients consultant au Laboratoire de santé publique de Mosoul pour le suivi d'une hépatite B ont fait l'objet d'un dépistage. La technique ELISA a permis la recherche de l'AgHBs, de l'AgHBe et de l'anti-HBe. Nous avons diagnostiqué 74 enfants nés entre 1994 et 1998 comme porteurs de l'AgHBs. Pour 62 des 74 cas, les parents avaient consulté dans des centres de vaccination rapidement : 41 n'étaient pas vaccinés et 21 n'avaient reçu qu'une dose de vaccin. Le marqueur de l'AgHBe était positif pour 9 patients (14,5 %) et l'anti-HBe pour 50 (80,7 %). La réticence des parents était la raison de la non-vaccination pour 12 enfants. Les pénuries de vaccins durant les années de naissance des cas étaient documentées, même après l'application de la Résolution 986 du Conseil de Sécurité des Nations Unies.

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Introduction

Infection with hepatitis B virus (HBV) is of global importance and is one of the major diseases of humankind. The scale of public health problems posed by HBV is large and over 250 million carriers exist worldwide. In developing countries, infants and children form carrier pools and infection occurs through mother–child transmission or close contact among children [*1*]. The probability of becoming a chronic carrier is substantially greater following infection during infancy and early childhood with increases in incidence of HBV-induced serious liver sequelae [*2*]. Hepatitis B vaccine has an outstanding record of safety and effectiveness. The vaccine has been licensed since 1982 and the World Health Organization strongly urges all countries to use it in national immunization programmes. The cost of the vaccine, wars and sanctions have been obstacles to its introduction and to shortages in many countries.

Before 1990, Iraq was a middle-income country with integrated public health services. The Gulf War and the subsequent sanctions imposed upon Iraq in August 1990 created a humanitarian crisis from the massive degradation of the country's infrastructure and the decline in public health services [*3*]. Infectious disease control programmes were disabled and shortages of medicines and vaccines became common because of the sanctions. Millions of people, especially children, women and the elderly, are still suffering from its consequences. The lifestyle of the Iraqi people has changed and a significant number of families have moved to rural areas to work in agriculture, where public health services are insufficient and shortages of medicines and vaccines are evident. The oil for food programme was implemented by United Nations Security Council Resolution 986 in December 1996 to correct the humanitarian crisis created by the sanctions imposed on Iraq since 1990.

Mosul is the second biggest city in Iraq with a population of approximately 2 million. The main vaccine store of the Health Directory of Mosul is responsible for vaccine delivery to the primary health care centres that vaccinate infants and children. The amount of vaccine received by the city depends on the amount present in the stores of the Ministry of Health. The demand of hepatitis B vaccine in Mosul is estimated to be 250 000 doses per year according to local health records. A humanitarian crisis has been created by the sanctions imposed on Iraq since 1990 and infection control measures have been greatly damaged [*4,5*]. Health authorities are still working, however, through the screening of blood donors and other blood products to prevent parenteral routes of spreading HBV. This has led to the diagnosis of many acute or chronic carrier cases. The screening of contacts and relatives of infected cases has enabled us to identify more carrier cases.

The aim of this study was to investigate the effect of sanctions in Mosul on the availability of hepatitis B vaccine for children and its impact on the occurrence of viral hepatitis B cases among children there.

Methods

From June 2000 to June 2001, 74 hepatitis B surface antigen (HBsAg) positive carrier children were identified and evaluated. All were born during sanctions, between 1994–1998, and their age range was 2–7 years.

It is policy at the blood banks to register the addresses of all blood donors and to

notify positive cases of HBV infection for further evaluation. Health authorities also screen contacts and family members of these cases. Among 419 contacts and family members of 76 cases of HBsAg carrier blood donors and other high-risk groups who attended the Public Health Laboratory, Virology Centre, in Mosul, North Iraq, the main viral hepatitis referral centre in that area, 254 children were screened for HBV infection. From them, 74 HBsAg positive carrier children were identified and evaluated. The vaccine cards of HBV-infected children were inspected and their parents were interviewed about their consultations at vaccine centres at the recommended time of vaccination. We recorded their reasons why the children had not been properly vaccinated. Data regarding the amount of HB vaccine in the main vaccine store and the number of HB vaccine recipients during the birth years of our cases were also collected.

The enzyme-linked immunosorbent assay technique was performed to test for HBsAg, HBeAg and anti-HBe markers. We used Biotest kits (Biotest AG, Dreieich, Germany) for HBsAg and Hepanostika kits (Organon Teknika, Boxtel, the Netherlands) for hepatitis Be antigen (HBeAg) and anti-hepatitis Be (anti-HBe). The assays were performed according to the manufacturer's instructions.

Results

In our study, 74 of 254 screened children (29.1%) were HBsAg positive. There was documentation that for 62 of our 74 cases (83.5%) parents had visited the vaccine centre at the recommended time of vaccination: 41 (66.1%) were not vaccinated and 21 (33.9%) had only 1 vaccine dose. Parental reluctance was responsible for non-vaccination in 12 of 74 cases (16.2%). HBeAg marker was positive for 9 of 62 (14.5%) and anti-HBe for 50 of 62 cases (80.7%). Serologic status, birth year and cause of HB vaccine noncompliance for the 62 cases were compared to the quantity of vaccine received in Mosul during each birth year of infected children (Table 1). More children who were HBsAg positive were born in years with severe vaccine shortages in Mosul (Figure 1). Furthermore, the number of HB vaccine doses that reached the city spiked in 1997, the year of the first shipment of food and medicines into Iraq following the implementation of UN Security Council Resolution 986; there were

Table 1 Serologic status, vaccination uptake and birth years of 62 HBsAg carrier children and number of hepatitis B vaccine doses received during 1994–1998[a]

Variable	HBsAg carrier children	
	No.	%
Serologic status		
HBeAg	9	14.5
Anti-HBe	50	80.7
Vaccination status		
Not vaccinated	41	55.4
Low uptake	21	28.4
Birth year		
1994	10	16.1
1995	17	27.4
1996	12	19.5
1997	8	12.9
1998	15	24.2
HB vaccine doses received in:		
1994	40 000	
1995	6 000	
1996	80 000	
1997	130 000	
1998	20 000	

[a] *The city of Mosul demand of hepatitis B vaccine per year was 250 000 child doses.*

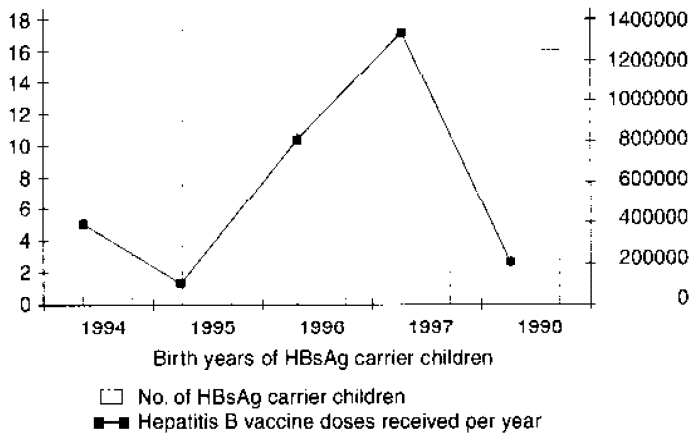

Figure 1 **Number of vaccine doses received during the birth years of 62 HBsAg carrier children (1994–1998). Annual demand of hepatitis B vaccine was 250 000 child doses. United Nations Resolution 986 was implemented in December 1996.**

fewer HBsAg positive children born in that year than in any other year between 1994 and 1998 (Figure 1). However, the number of vaccine doses received fell in 1998, the second year of sanctions, resulting in more HBsAg carrier children being born in that year.

Discussion

Iraq is an area of intermediate endemicity of HBV infection [*6*]. Prevalence rates of HBV infection have been studied throughout the country and among different occupations and the primary routes of transmission that have been proposed are intrafamilial, non-sexual and non-parenteral [*7–9*]. Among adults, the annual sporadic cases of acute and carrier hepatitis B have been mostly from unpredictable sources [*9*]. The HBsAg carrier rate among the normal population was estimated to be 4.3% and the prevalence of anti-HBs was 30%. The frequency of HBeAg and anti-HBe among carriers was estimated to be 20% and 65% respectively [*10*]; the majority represent a state of childhood infection with persistence into adulthood rather than new exposure to the virus [*8–9*]. High carrier rates were detected among hospital workers and foodhandlers with the detection of delta hepatitis viral infection among some carriers [*11,12*]. HBV status among Iraqi children is not well studied but is thought to be similar to other Middle Eastern countries in that HBsAg carrier rates are high between 1 and 2 years of age and HBeAg positivity peaks around the age of 5 years [*7,13*].

Iraq was a pioneer among Middle Eastern countries in introducing the hepatitis B vaccine. The Iraqi Viral Hepatitis Committee hoped that this vaccine together with other strategies would control hepatitis B infection. The importation of sufficient amounts of vaccines in the early 1980s allowed for the vaccination of high-risk groups. In the late 1980s, hepatitis B vaccination was introduced as a 'routine vaccination' as part of the Expanded Programme

on Immunization. Children who escaped vaccination or who did not complete the vaccination schedule had a very low chance of vaccination later in life, because adult vaccination was obligatory only in certain high-risk groups.

After sanctions, efforts were made to maintain childhood vaccination programmes, including hepatitis B vaccination, to prevent infectious disease occurrence among children using the available vaccine doses in the main stores. The breakdown of vaccination programmes was inevitable with the prolonged years of sanctions. Therefore, many children are either not vaccinated against hepatitis B or have escaped one or two doses. Because of this situation, a new susceptible pool of children has been added to the existing reservoir of adult carriers of hepatitis B.

We found that HBV child carrier cases accumulated during sanctions. Our finding is supported by data documenting the drastic shortages of vaccine doses reaching Mosul in the birth years of infected children, especially before and after the launching of UN Security Council Resolution 986. We have included only cases identified through the policy of screening family members and contacts of infected blood donors, the problem might be found to be more serious if a large-scale study were to audit hepatitis B vaccine uptake among children in the years of vaccine shortages. Non-completion of other vaccine types was also recorded. Parental reluctance may play only a small part in these escapes, and perhaps they are also the result of sanctions because of the need to work in rural areas, far from vaccination centres, and not because parents were unwilling to vaccinate their children. Moreover, the presence of HBeAg carrier children carries high risk for the development of serious liver sequelae and the increased tendency for transmission to other contacts. Some of these cases will become carrier mothers themselves and perpetuate the cycle of perinatal transmission, subsequently expanding acute and carrier states and increasing the burden of liver disease in the country.

In conclusion, the present humanitarian crisis in Iraq continues to smoulder, and takes a heavy toll in human lives and health conditions. The oil for food programme designed by UN Security Council Resolution 986 and implemented in December 1996 may have improved the availability of some drugs and certain medical equipment but has not relieved the humanitarian crisis in Iraq. These economic sanctions for this prolonged period appear to have devastating consequences.

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Correction

Relationship between depression and non-adherence to anticoagulant therapy after valve replacement. A.S. El-Gatit and M. Haw. *Eastern Mediterranean Health Journal*, 2002, Vol. 9 Nos 1/2, pages 12–19.

The name of the first author should read: A.M. El-Gatit in English and عبد القطيط الغتيت in Arabic.

Supplementary feeding of malnourished children in northern Iraq

S.Y. Agha[1]

الإطعام التكميلي للأطفال المصابين بسوء التغذية في شمال العراق

سعد يونس أغا

الخلاصة: تم تقييم فعالية برنامج الإطعام التكميلي للأطفال المصابين بسوء التغذية والذين تتراوح أعمارهم بين 6 شهور و3 سنوات في محافظة دهوك شمال العراق. وقد كانت معايير إدراج الأطفال في الدراسة أن يكون وزن الطفل أقل من الوزن المعياري بالنسبة للوزن بمقدار انحرافين معياريين أو أكثر. وقد بدأ إدراج الأطفال في البرنامج منذ كانون الثاني/يناير 2001 وتمت متابعتهم على مدى 7 شهور وكان الأطفال يتلقون البسكويت الغني بالبروتين والطاقة في الشهر الأول، ثم يعطى كل طفل حصة شهرية لتحضير مزيج فول الصويا طيلة الأيام التالية. وكانت أسر هؤلاء الأطفال تتلقى حصص الطعام في الأشهر الأربعة الأولى. وقد لوحظ تحسُّن لدى جميع الأطفال ولاسيما في الشهر الأول. وقد ظهرت المشكلات في الحصص وفي وحدات رصد نمو الأطفال مما أدى إلى قدر كبير من تسرب الأطفال من الدراسة. وقد يكون استخدام بطاقة نمو معيارية أحد طرق التغلب على هذه المشكلات، وينبغي توزيع البسكويت الغني بالبروتين على نطاق واسع بدلاً من مزيج فول الصويا.

ABSTRACT The effectiveness of the supplementary feeding programme for malnourished children aged 6 months to 3 years in Dohuk province, northern Iraq was evaluated. The enrolment criterion was child weight ≥ 2 standard deviations below standard weight-for-age. Children enrolled in the programme in January 2001were followed over 7 months. Children received high-protein high-energy biscuits in the first month and a monthly child ration for preparing soyabean mix throughout. Their families received food rations in the first 4 months. Improvement was noticed for all children, particularly in the first month. Problems with the rations and within the growth monitoring units resulted in significant drop-out. Use of standard growth charts may be a way to overcome this problem. High-protein biscuits should be distributed throughout instead of the mix.

L'alimentation supplémentaire des enfants malnutris dans le nord de l'Iraq
RESUME L'efficacité des programmes d'alimentation supplémentaire pour les enfants malnutris âgés de 6 mois à trois ans dans la province de Dohouk (nord de l'Iraq) a été évaluée. Le critère d'admission était un poids de l'enfant en dessous du rapport poids-âge standard de 2 écarts-types ou plus. Les enfants inscrits au programme en janvier 2001 ont fait l'objet d'un suivi pendant sept mois. Les enfants ont reçu des biscuits à haute teneur protéinique et énergétique pendant le premier mois et une ration mensuelle pour la préparation d'un mélange de graines de soja pendant toute la période. Les familles ont reçu des rations alimentaires pendant les quatre premiers mois. Une amélioration a été notée chez tous les enfants, notamment au cours du premier mois. Des problèmes avec les rations et au sein des services de surveillance de la croissance ont entraîné d'importantes défections. L'utilisation de courbes de croissance standard peut être un moyen de surmonter ce problème. Des biscuits à haute teneur en protéines devraient être distribués pendant toute la période au lieu du mélange.

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Introduction

Improvement in the nutritional status of young children reduces morbidity and mortality [*1,2*]. Recognizing this fact, in 1994 the World Food Programme, in coordination with UNICEF and the directorates of health in Northern Iraq, initiated a supplementary feeding programme (SFP) for children under 5. Since then the programme has been evaluated through a nutrition surveillance system set up in all the primary health care centres involved in the SFP as well as through UNICEF-sponsored Regional nutrition surveys conducted every 6–12 months [*3*]. This study is aimed at the evaluation of the SFP in Dohuk province.

Methods

The study was conducted at the primary health care level under the supervision of the Directorate of Health and UNICEF. After the simple random selection of 9 (15%) of 62 primary health care centres which have growth monitoring units, the evaluation study started in Dohuk province on 1 January 2001. Two of the 9 units were located in urban areas, the rest in rural areas. The growth monitoring staff of these centres, plus 1 supervisory mobile nutrition team, were retrained in a 3-day UNICEF-sponsored course. Retraining of growth monitoring staff is a routine process, done almost every year.

The SFP routinely consists of assessing the nutritional status of children aged 6 months to 3 years according to weight-for-age criteria. Children are weighed wearing light underclothing and without shoes. Readings are taken to the nearest 100 g. Standard scales, model MP25, were supplied by UNICEF. If weight is ≥ 2 standard deviations (SD) below the standard weight-for-age, then the child receives a weekly ration of high-protein high-energy biscuit (0.8 kg/week) for the next month. The biscuit contains wheat flour, soyabeans, glucose, vegetable oil, multivitamins and minerals. At the same time, the child is registered to receive, over the next 7 months, a monthly child ration of 5 kg soyabean flour plus vegetable oil (0.6 kg) and sugar (0.6 kg) for making a high-protein high-energy mix. The family should, theoretically, also receive 4.5 kg vegetable oil, 3 kg sugar, 3 kg pulses and 13 kg rice monthly. This family ration is for the first 4 months only. Food rations are received from a ration agent and not from the growth monitoring unit.

At least once monthly, before distributing any rations, the staff of the growth monitoring unit should see all the children who are enrolled and record follow-up information, in particular the child's weight. Staff are also supposed to give health and nutrition education to the mothers. The supervisory team and the author monitored and supervised staff throughout the period of the study.

Height was measured for every malnourished child enrolled in this study (if a child's weight is ≥ 3 SD below standard weight-for-age, height is measured routinely). Children < 2 years were measured lying down, and those ≥ 2 years were measured standing, all without shoes. Shore infant/child length/height measuring boards supplied by UNICEF were used. Readings were taken to the nearest 0.1 cm. If weight is ≥ 3 SD below standard weight-for-height, then the child is referred to a hospital nutritional rehabilitation centre and admitted and given therapeutic milk plus medical care.

During the current evaluation study, the growth monitoring staff were requested to routinely record monthly height measurements for every malnourished child en-

rolled in the SFP according to weight-for-age criteria.

Weight-for-age, weight-for-height and height-for-age standards used were those of the World Health Organization [*4,5*].

Another aspect of the current evaluation was a 1-month field trial to study the increase in the weight of a sample of 27 malnourished children who were receiving high-protein high-energy biscuits. They were compared with 27 normal control children in the same age range (6 months to 3 years). The control children were visiting the primary health care centres for routine immunization or growth monitoring. Participants were selected by taking the first 3 malnourished and the first 3 healthy children who presented to the primary health care centres involved in the study after 20 June 2001. Informed consent was obtained orally from parents, and there were no refusals. After 1 month, 2 malnourished children and 3 controls were lost to follow-up. Unpaired *t*-test for 2 independent samples with unequal variances (*SPSS*, version 10) was used to determine the increase in weight of children receiving high-protein high-energy biscuits compared to controls.

Results

Malnourished children enrolled in the SFP in January 2001 were followed up till July 2001. There was significant drop-out, particularly in the last 2 months (Table 1).

Throughout the study, we saw few children with weight ≥ 2 SD below standard weight-for-height or height ≥ 2 SD below standard for age. The wasting or stunting in those children, therefore, showed no significant changes. Since the SFP basically uses the weight-for-age index for the purposes of enrolling children, results of follow-up were constructed using weight-for-age criteria (Table 2 and Figure 1). Eighteen children had been introduced erroneously into the programme, i.e.

Table 1 **Reduction in number of children attending the supplementary feeding programme at primary health care centres in Dohuk province, Iraq, 2001**

Primary health care centre	January	April		May		July	
	No. children enrolled	No. children covered	%	No. children covered	%	No. children covered	%
Bardarash	16	14	87.5	11	68.8	3	18.8
Seeri	21	20	95.2	19	90.5	10	47.6
Hojava	9	9	100	9	100	8	88.9
Mangesh	16	15	93.8	14	87.5	4	25.0
Razgary	13	13	100	10	76.9	2	15.4
Dairabon	13	13	100	9	69.2	1	7.7
Moqibla	7	7	100	5	71.4	6	85.7
Qasrok	43	43	93.0	35	81.4	23	53.5
Heizel	39	36	92.3	26	66.7	0	0.0
Total	177	167	94.4	138	78.0	57	32.2

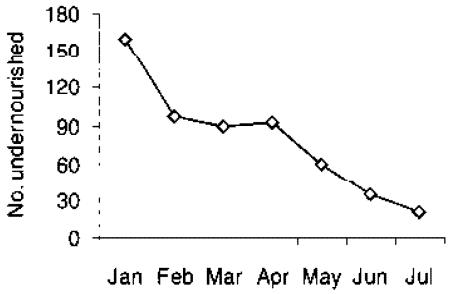

Figure 1 **Number of underweight children more than 2 standard deviations below standard weight-for-age, January–July 2001 (numbers represent only children on the programme ≥ 2 standard deviations below normal weight-for-age at dates indicated, excluding drop-outs and the 18 initially normal children)**

they initially had normal weight-for-age measurements, and therefore were excluded from the results in Table 2 and Figure 1.

The mean increase in weight for 25 children who received the biscuits for 1 month was 440 g, compared to 315 g increase in the controls, a mean difference of 125 g (95% CI: –18.6 to 269.5). The *t*-value was 1.772, therefore the difference was not statistically significant ($P > 0.05$). However, scrutiny of the data reveals that for 1 child enrolled in Sceri, weight decreased 200 g over the first month while he was receiving the high-protein high-energy biscuit. The follow-up data showed that he was having persistent diarrhoea. Exclusion of this child from analysis makes the difference statistically significant (mean difference in weight increase of 152.1 g, 95% CI: 16.4 to 287.8, *t*-value 2.241, $P < 0.05$).

Discussion

The household nutritional status survey of 1994 revealed a high prevalence of malnutrition in Northern Iraq, with 25.8% of children being underweight (≥ 2 SD below standard weight-for-age) [*6*]. Since 1994, there has been a general improvement in the nutritional situation [*3*]. The nutritional sta-

Table 2 **Progress of 159 children enrolled in January 2001 on the supplementary feeding programme according to weight-for-age criteria**

Month	Weight below normal for age (standard deviations)						Total no.
	<2.0		2.0–2.9		≥ 3.0		
	No.	%	No.	%	No.	%	
January	0	0.0	150	94.3	9	5.7	159
February	51	34.4	92	62.2	5	3.4	148
March	37	29.1	80	63.0	10	7.9	127
April	63	40.6	79	51.0	13	8.4	155
May	62	51.2	49	40.5	10	8.3	121
June	45	57.0	28	35.4	6	7.6	79
July	31	59.6	17	32.7	4	7.7	52

Eighteen children were excluded as they were initially normal weight but had been erroneously enrolled in the programme.

tus survey of November 1999 indicated prevalence of underweight of 9.5% [7]. The same trend of general improvement is shown by the nutrition surveillance data compiled by the directorates of health for growth monitoring units throughout Northern Iraq [8]. Distribution of rations by UNICEF and the directorates of health nutrition programme with the World Food Programme has contributed to improvements in the nutritional status of children [3]. However, the current evaluation reveals, more specifically, the difficulties experienced in improving the nutritional status of malnourished children through the existing SFP.

The most prominent feature of the present study is the significant drop-out, starting in May 2001, and becoming quite significant in June and July. From May onwards, the family ration was no longer given and families of enrolled children were only receiving the small child ration, the value of which is much lower than the expense of the trip from many villages to primary health care centres. Despite the existence of good general food rations, families had become dependent on this ration to justify their visit to the primary health care centre. Providing the special ration for a malnourished child and ignoring the family may decrease food availability within the family, and make the mother less capable of breast-feeding her child.

An important factor in causing drop-out from the SFP may have been the poor palatability and difficult preparation method of the soyabean mix, the main item of the child ration. These were repeatedly mentioned by parents during the course of the study. Some families used to feed the mix to poultry rather than their children.

Another factor which contributed to drop-out from the SFP, was the instruction given by World Food Programme and Directorate of Health mobile teams to the growth monitoring staff to weigh children and record monthly data only if the ration arrived. Due to the irregular arrival of the ration, such instructions resulted in an interruption of the monitoring process. Deficiencies and changes in the staff, coupled with overcrowding in some primary health care centres, e.g. Heizel and Seeri, also contributed to the drop-out. In Heizel, for instance, a trained female staff member took a long "motherhood leave" and was replaced by an untrained health worker. Growth monitoring of enrolled children and all other children was carried out not more than once or twice weekly. The result was that, despite supervision, such problems with the staff led to the erroneous enrolment of 18 children in the SFP. This is the reason for different totals in Tables 1 and 2.

Other factors that could have increased the drop-out rate include the hot weather of June and July, which may have discouraged families from travelling. These months also coincide with the harvest season, when rural families are too busy to bring their children to the growth monitoring units (Figure 1).

The current child ration (apart from the high-protein high-energy biscuit given in the first month) lacks adequate complementary food items like animal protein and micronutrients, in particular iron and vitamin A [9]. Iron deficiency anaemia and vitamin A deficiency are considered significant health problems in the region [10,11].

There was a relatively steep decline in the number of underweight children more than 2 SD below standard weight-for-age during the first month of the SFP (Table 2, Figure 1). This may be attributed to the effect of the high-protein high-energy biscuit. Apart from the first month, follow-up of malnourished children showed a gradual

and slow, rather than dramatic, improvement in nutritional status. This is obvious for the period from February to May (Table 2 and Figure 1). Afterwards, the attendance rate to the growth monitoring units was too low for a conclusion to be drawn. It may be concluded from the current study that the soyabean mix has less effect on weight gain than the biscuits, as the rate of decline in malnutrition was slower after the first month.

Further evidence for the effectiveness of the high-protein high-energy biscuit comes from the short follow-up study. After excluding 1 child who had persistent diarrhoea, children receiving the biscuits over 1 month displayed significantly higher weight gain than controls.

Lack of remarkable improvement, except for the first month of the SFP, could also reflect the emphasis by the growth monitoring staff on just giving the food ration. Other activities, in particular health education, seemed to be considered of less importance, taking into consideration the difficulties they encountered.

It is known that nutritional problems are multi-factorial with roots in many sectors of development such as health, education, demography, agriculture and rural development [*2,12*]. Lack of a widely based intersectoral approach toward malnutrition may explain the slow trend of improvement.

Recommendations

Weighing of children enrolled in the programme and recording of data must be done monthly, even if the ration arrives late. This will compensate for the incomplete registration of follow-up data and reduce the drop-out rate. Families should be educated on how to carry out the process. Since neither the growth monitoring staff nor the families used the small growth chart present on the immunization card, its importance should be emphasized for monitoring the growth of every child under 5.

Children enrolled in the SFP should preferably be given high-protein high-energy biscuits throughout the period of enrolment instead of the soyabean mix. It would then be possible to reduce the period of enrolment to 4 or 5 months because of the more rapid catch-up in growth.

Staff at primary health care centres, particularly growth monitoring staff, can be very useful in providing health and nutrition education to all caregivers provided they are well trained and retrained, supervised, supported and motivated.

A broad intersectoral and integrated approach involving all sectors of development is needed to tackle nutritional problems. Community participation programmes, using for example the Women's Union and traditional birth attendants, are needed to educate families on the use of growth charts and supplementary food items to tackle malnutrition.

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Training workshop to develop nutrition surveillance capabilities of Member States to improve the monitoring and evaluation capacities in nutrition programmes

The World Health Organization organized the above-mentioned training workshop at the High Institute of Public Health, Alexandria University, Alexandria, Egypt from 7 to 15 March 2004 in order to develop nutrition surveillance capabilities of Member States and improve the monitoring and evaluation capacities in nutrition programmes. The objective of the workshop was to prepare Member States to establish national nutrition surveillance systems with focus on micronutrient deficiencies. Participants from Bahrain, Islamic Republic of Iran, Jordan, Kuwait, Morocco, Oman attended this workshop as well as representatives from the Centers for Disease Control and Prevention, Atlanta and the United Nations Children's Fund.

Pakistan's experience of a bioterrorism-related anthrax scare

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خبرة باكستان في الخوف من الجمرة الخبيثة المرتبطة بالإرهاب البيولوجي

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الخلاصة: تلقى المعهد الوطني للصحة في إسلام آباد، باكستان خلال الفترة بين تشرين الثاني/نوفمبر 2001 وآذار/مارس 2002، 230 عينة من 194 مصدراً مختلفاً لتحليلها بحثاً عن أبواغ الجمرة الخبيثة. وقد أخذت هذه العينات من رسائل وطرود اشتبه بأنها تحتوي على الجمرة الخبيثة، ومن الأشخاص الذين تعرضوا لتلك الرسائل والطرود البريدية. ولدى زراعة العينات على الأغار بدم الخراف، أنتجت 141 عينة نموات توحي بأنواع من العصويات. وانطلاقاً من خصائص تلك النموات مثل غياب الانحلال الدموي البيتائي وغياب الحركية أو الشك بوجودها إلى جانب الخصائص الشكلية للمستفردات الملونة بملوّن غرام، فقد اعتبر 62 من المستفردات مشتبهاً وتم تلقيح القُبَيْعات به. وقد بقيت الحيوانات الملقحة بالمستفردات المشبوهة بصحة جيدة مدَّةً أطول من المدّة المطلوبة للمراقبة والتي تقدر بخمسة أيام، مما أدى إلى القول بسلبية جميع النماذج وعدم احتوائها على العصويات الجمريه. وقد نوقش نظام التعامل مع المواد التي يشتبه بتلوثها بجراثيم الجمرة وتحليلها.

ABSTRACT From November 2001 to March 2002, the National Institute of Health, Islamabad, Pakistan, received 230 samples from 194 different sources for analysis for anthrax spores. These samples were taken from letters/packages suspected of containing anthrax and from individuals exposed to them. When cultured on sheep blood agar, 141 samples yielded growth suggestive of *Bacillus* species. On the basis of growth characteristics, absence of beta-haemolysis, absent or doubtful motility and morphological characters of the isolates on Gram stain, 62 isolates were considered suspicious and were inoculated into guinea-pigs. Inoculated animals remained healthy well beyond the required observation period of 5 days. All the samples were therefore reported as negative for *B. anthracis*. Systems for handling and analysing suspected anthrax-contaminated materials are discussed.

Expérience d'une alerte au charbon liée au bioterrorisme au Pakistan

RESUME De novembre 2001 à mars 2002, l'Institut national de la Santé d'Islamabad (Pakistan) a reçu 230 échantillons de 194 sources différentes à analyser pour détecter la présence de spores du charbon. Ces échantillons provenaient de lettres/paquets suspectés de contenir le bacille du charbon, et d'individus exposés. Lorsqu'ils ont été mis en culture sur gélose au sang de mouton, 141 échantillons ont produit une croissance faisant penser à des espèces du bacille. Sur la base des caractéristiques de la croissance, de l'absence de bêta-hémolyse, de l'absence de motilité ou de son aspect douteux et des caractères morphologiques des isolats à la coloration de Gram, 62 isolats ont été considérés comme suspects et ont été inoculés à des cobayes. Les animaux inoculés sont restés sains bien au-delà de la période d'observation requise de 5 jours. Tous les échantillons ont donc été déclarés négatifs pour *B. anthracis*. Des systèmes pour la manipulation et l'analyse des matériels suspectés d'être contaminés par le bacille du charbon sont examinés.

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Introduction

Described as a disease of antiquity, anthrax is primarily a zoonosis predominantly affecting sheep, goats, cattle and other herbivores from which man is secondarily infected [*1*]. Caused by a Gram-positive, spore-forming bacillus called *Bacillus anthracis*, the human disease occurs in 3 main clinical forms: cutaneous anthrax, inhalation (or pneumonic) anthrax and gastrointestinal anthrax. The cutaneous form is the most common and carries the least mortality while inhalation anthrax is much less common but is almost 100% fatal if untreated [*2*].

Because of the long life of its spores and the fact that the spores can be prepared in bulk in powder form and can be used as a dispersible aerosol, anthrax has long been considered an ideal weapon for biological warfare [*3*]. Although no major deployment of anthrax as a biological weapon has been recorded, the Japanese used it in the Manchurian War in the early 1940s [*2*] and there is evidence that during the Second World War, the Allies came close to using anthrax 'spore bombs' against the Germans [*4*]. Recently, there have been widely publicized reports of letters containing anthrax spores being mailed to individuals and organizations in an apparent spate of terrorist attacks that are analogous to explosive 'letter bombs' that are used to target individuals [*1*].

Since the September 11 2001 terrorist attacks in the United States of America (USA), there have been a number of incidents of anthrax-related bioterrorism in the USA and other countries of the world. In Pakistan, the first package suspected of containing anthrax was found in Islamabad in late October 2001, and was followed by similar incidents throughout the country during subsequent months. Soon after the first incident, the National Institute of Health in Islamabad was designated as the National Reference Centre for the diagnosis and management of anthrax. This paper documents the experience of the National Institute of Health in handling and analysing samples of suspected anthrax from letters/packages and from the individuals exposed to them.

Methods

Sample collection and transport

Samples collected were of 2 types: non clinical/environmental samples and clinical samples.

Three types of non-clinical and environmental samples were collected: the suspected powder; the envelope, letter or package in which the powder was received; and wipe swabs taken from inanimate objects or surfaces thought to have been contaminated by the suspected powder. Universal precautions were followed according to the National Institutes of Health and World Health Organization (WHO) guidelines for the collection and transport of contaminated samples [*5–7*].

Clinical samples collected from exposed individuals consisted of nasal swabs from anterior nares and skin swabs from areas of skin in contact with the suspect powder or the letter, envelope or package. Wherever possible, swabs were collected in duplicate, 1 for culture and 1 for direct staining. Blood samples, rarely indicated, were taken directly into blood culture bottles using standard protocols for blood culture.

Detailed instructions were issued to all relevant people about the proper transport of samples, preferably through a personal courier [*5*]. The senders were instructed to ship the sample containers sealed in an outer container made of non-porous material.

This was enclosed in yet another container made of strong thick plastic or metal.

Specimen handling in the laboratory

Samples were processed according to the USA Centers for Disease Control (CDC) guidelines [7]. All procedures, from the opening of sample containers to the issuing of the final report, were carried out inside a class II biological safety cabinet (BSL–II), in a separate room specified for this purpose. Only designated members of laboratory staff handled the samples using protective clothing, gloves, masks, etc. All items, including instruments and disposables used in the test, were discarded into a container of 10% sodium hypochlorite. Subsequently, reusable items were autoclaved and disposables were incinerated. After finishing work, all surfaces were wiped with hypochlorite solution.

Non-clinical/environmental samples

For analysis of powders, a small amount was suspended in 1.0 mL sterile distilled water in a sterile universal container, then 0.1 mL of suspension was inoculated onto sheep blood agar plate and incubated at 37 °C for 18–24 hours.

For analysis of envelopes, the envelope was opened and any powder inside was cultured as above. The letter was pulled out and a piece 2.5 × 2.5 cm was cut out, preferably from an area free from writing. The piece was transferred to 1.0 mL of sterile distilled water or saline and shaken for about 2 minutes. Then 0.1 mL of this fluid was inoculated on a sheep blood agar plate and incubated as above.

For analysis of soil or dusts, about 2.0 g of material was suspended in sample processing solution (phosphate buffered saline with 0.3% polysorbate-20 or normal saline), shaken vigorously and left for 2–3 minutes. The supernatant was divided into 2 aliquots, 1 of which was heated at 65–70 °C for 10 minutes and allowed to cool, then 0.1 mL from heated and unheated aliquots was streaked on sheep blood agar and incubated as above.

Environmental swabs were placed in 3 mL of sample processing solution or normal saline and shaken. The fluid was divided into 2 aliquots, 1 of which was heated at 65–70 °C for 10 minutes, then 0.1 mL from both aliquots was streaked on sheep blood agar and incubated.

Clinical samples

Swabs from anterior nares and skin were streaked onto sheep blood agar plates and incubated for 18–24 hours. From the second swab (or from the same swab used for streaking), smears were made on slides and stained with Gram stain for direct microscopic examination.

Blood cultures were incubated for up to 7 days. From those showing growth, subcultures were made on sheep blood agar.

Analysis of cultures

After incubation for 18–24 hours, inoculated plates showing growths were examined for colony characters, haemolysis, morphology, spore formation, motility and catalase test. If these tests indicated growth of *Bacillus* species, the isolates were then subjected to tests for species characterization. A 3-step strategy was adopted for identification of suspected isolates.

Level A

Cultures yielding large (3–5 mm) colonies especially those with ground-glass or medusa head appearance, which were non-beta haemolytic and on Gram stain showing large spore-forming bacilli in chains, were non-motile and catalase positive, were considered as suspicious and subjected to further (level B) tests.

Level B

Representative colonies from the above plates were subcultured on MacConkey agar, bicarbonate agar for capsule formation (trypticase soy agar containing 0.8% $NaHCO_3$, incubated in a candle jar) and a gelatin stab culture. Mueller–Hinton agar was inoculated in duplicate, 1 for penicillin susceptibility (10 U) and 1 for the pearl-string test.

For the pearl-string test, a heavy single streak was made on Mueller–Hinton agar. A 10 U penicillin disc was applied over the streak, which was overlaid with a coverslip. After incubation for 3–6 hours, growth from beneath the coverslip was examined microscopically for the presence of strings of spherical cellular forms of the organism. Presence of such 'strings of pearls' is considered characteristic of *B. anthracis* [*8*].

B. anthracis does not grow on Mac Conkey agar, forms mucoid colonies on bicarbonate agar [*9*], gives an inverted fir-tree appearance in gelatin stab culture after a 2–3 day incubation, is susceptible to 10 U penicillin and may yield a positive pearl-string test. An india ink preparation was made from any mucoid colonies on bicarbonate agar and examined microscopically for capsule formation. The capsule appears as a well-defined clear zone around the bacilli. Also a smear from mucoid colonies was stained with polychrome methylene blue (McFadyean reaction) and examined microscopically for capsule formation [*7,10*]. A presumptive identification of *B. anthracis* could be made on the results of these tests.

Level C

For final confirmation, all presumptive positive cultures were inoculated into guinea-pigs. Guinea-pig is highly susceptible to *B. anthracis*. Inoculation of 0.5 mL of a 24-hour broth subcutaneonsly will usually kill the animal within 48 hours [*11*]. Bacilli can be seen in a direct smear from heart blood of the dead animal and can be isolated from blood and other organs [*11*]. Colonies were emulsified in 1.0 mL of sterile normal saline to give a slightly turbid suspension. Then 0.5 mL of this suspension (or 0.5 mL of an overnight broth culture) was injected subcutaneously over the thigh area of each of 2 guinea-pigs. A third animal was injected with 0.5 mL of sterile saline as control. Animals were observed for a minimum of 5 days. To speed up the results, all primary cultures giving suspicious growth (level A) were also directly inoculated into guinea-pigs along with level B tests.

Smears were made from swabs, powder suspensions and from suspicious growth on blood agar and stained with malachite green (5% aqueous) for 45 minutes and counterstained with safranin for the demonstration of spores. Malachite green stains the spores green while the bacilli are stained red by safranin [*7*].

Results

Over a 5-month period from November 2001 to March 2002, a total of 230 samples from 194 sources were sent to the National Institute of Health for analysis. Samples were sent from all parts of the country. Table 1 lists the organizations and individuals who were recipients of parcels/letters suspected of containing anthrax.

Details of the types of clinical and non-clinical samples processed are given in Table 2. When cultured on sheep blood agar, 141 samples yielded growth suggestive of *Bacillus* species (Table 3). On the basis of growth characteristics, absence of beta-haemolysis, absent or doubtful motility and morphological characters of the isolates on

Table 1 **Recipients of parcels/letters suspected of containing anthrax**

Type of organization	No. of samples
Foreign missions	18
Foreign media organizations, banks and multinational corporations	17
Institutions/universities/hospitals	61
Government officials	48
Individuals/miscellaneous	50
Total	194

Gram stain, 62 isolates were considered suspicious for *B. anthracis* and were therefore inoculated into guinea-pigs. Inoculated animals remained healthy well beyond the required observation period of 5 days. All the samples were therefore reported as negative for *B. anthracis*.

None of the isolates yielded mucoid colonies on bicarbonate agar, inverted fir-tree appearance in gelatin stab or a positive pearl-string test. However, for unexplained reasons a limited number of isolates were found to be susceptible to penicillin.

Table 2 **Types of samples collected for analysis of suspected anthrax**

Source	No. of samples
Site of clinical samples	
Anterior nares	42
Skin	26
Blood	2
Culture for identification	1
Total	71
Source of non-clinical and environmental samples	
Powders[a]	135
Swabs from inanimate objects/ surfaces	10
Papers, envelopes, packages, plastics	12
Miscellaneous	2
Total	159
Total	230

[a] *Includes a few samples of wood shavings, flour or soil.*

Discussion

One of the most reliable criteria for the preliminary screening of *Bacillus* isolates for *B. anthracis* is non-motility of the isolate. Any *Bacillus* isolate exhibiting motility may be safely assumed to be a species other than *B. anthracis*. The reverse, however, is not always true as some strains of *B. mycoides* (*B. cereus* var *mycoides*) are also non-motile [*12*].

Susceptibility to gamma phage has been widely accepted as a reliable test of identification for *B. anthracis* [*7*]. However, a number of *B. mycoides* strains are also reported to be susceptible to gamma phage [*13,14*]. Experience at the Pakistan National Institute of Health during the present study has shown that, in the absence of supplies of gamma phage (which CDC has been unable to provide) or a polymerase chain reaction (PCR) facility, animal inoculation remains the mainstay of a definitive identification of *B. anthracis*. It is not uncommon to isolate saprophytic members of the *Bacillus* group from the environment. In fact, they are the commonest contaminants in a clinical laboratory. But no other member of this group, except *B. anthracis*, will kill a guinea-pig within 48 hours when injected with a pure culture [*11*]. Our experience also underlines the difficulties inherent in the definitive diagnosis of anthrax by a routine microbiology laboratory. Without facilities for animal inoculation (or PCR or

Table 3 **Results of cultures and animal inoculation with suspected samples of anthrax**

Type of sample	Type of isolate					
	Suggestive of *Bacillus* spp. (*n* = 230)		Suggestive of *B. anthracis*[a] (*n* = 230)		Positive on guinea-pig inoculation (*n* = 62)	
	No.	%	No.	%	No.	%
Clinical samples	14	6	5	2	0	–
Powders	120	52	56	24	0	–
Environmental samples	7	3	1	< 1	0	–
Total	141	61	62	27	0	–

[a] *Isolates with typical morphology on Gram stain, non-motile non-beta haemolytic, catalase-positive.*
n = *total number of cultures tested.*

gamma phage), a presumptive diagnosis of *B. anthracis* based on inconclusive findings could have led to unforeseen complications.

It maybe no coincidence that since 11 September 2001, the USA has experienced a number of anthrax-related attacks with 5 deaths that were directly attributable to these attacks. All died of inhalation anthrax; all but one received or had contact with letters containing anthrax spores [*15*]. It has been speculated that Pakistan could also be a target for bioterrorism. Fortunately, in spite of a number of suspected incidents, this has proved to be mere speculation. It is well known that production of weapons-grade anthrax is a highly sophisticated technology. Our experience underlines the fact that the capability to produce anthrax parcel/letter bombs is not easily available to a potential terrorist; the only success of the perpetrators of these incidents has been to create an atmosphere of panic among the general public.

Although the incidents described in this report proved to be hoaxes in every case, they nonetheless served to demonstrate the public health implications of an actual or potential bioterrorism attack as well as the strengths and weaknesses of the systems in place to deal with it. The threats called for a prompt and coordinated response on the part of several government agencies, notably in health, law enforcement and information. In spite of the fact that Pakistan has never faced a bioterrorism attack before, its systems performed surprisingly well. Facilities for laboratory testing and prompt reporting were fully in place right after the first incident. In some cases, the law enforcement agencies not only investigated the incident but also collected and delivered the suspect letter or package to the testing laboratory from distant regions. Communication and coordination with the original recipients of the object and the general public on the one hand and the public health, the law enforcement and mass media on the other, demonstrated the professionalism and confidence with which the episodes were handled by the authorities concerned, particularly in the Ministry of Health and the National Institute of Health. Availability of a first-rate laboratory,

backed by animal testing facilities, made this task considerably easier.

Conclusions

It is concluded on the basis of strong scientific evidence that all the incidents of suspected anthrax parcel/letter bombs in Pakistan were hoaxes. Nevertheless, standard operating procedures for disaster management in a bioterrorism setting should be formulated and their periodic review should be ensured. Isolates which are provisionally labelled as *B. anthracis* on the basis of presumptive tests should not be reported unless confirmed by a reference laboratory. It should be noted that animal inoculation, although no longer generally practised in clinical microbiology laboratories, still has a place in public health laboratory practice in developing countries with scarce resources.

Acknowledgements

The authors take this opportunity to gratefully acknowledge the generous assistance and active participation of WHO Pakistan, during all phases of this study, both in the field as well as in the laboratory, particularly in terms of provision of equipment and supplies. A mobile laboratory provided by WHO under the Health Laboratory Support project played a pivotal role in handling and transportation of suspected samples. Dr Khalif Bile Mohamud, WR Pakistan, and Dr Faizullah Kakar, WHO Epidemiologist, deserve our special thanks for their valuable advice and guidance in this regard. The contribution of the following staff at the National Institute of Health is gratefully acknowledged: Mr Sohail Zaidi, I/C Immunology and Virology Department, Drs Altaf Bosan and Zafar Toor, Epidemic Investigation Cell, Dr Mohammad Hussain, I/C Animal House, and Mr Mohammad Iqbal, I/C Transport. We are profoundly indebted to the following members of the laboratory staff for their invaluable technical help during this study: Mr Tariq Mahmood, Mr Mohammad Jamshed and Mr Abdul Basit.

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Public health response to biological and chemical weapons: WHO guidance

This second edition of WHO's 1970 publication *Health aspects of biological and chemical weapons* includes information designed to guide preparedness for and response to the deliberate use of biological and chemical agents that affect health. While noting that the probability of an attack with such weapons may be low, the guide underscores the magnitude of potential impacts on civilian populations and the corresponding need for public health authorities, in close cooperation with other parts of government, to develop contingency plans. For such plans to be effective, collaborative arrangements involving all partners have to be established and tested well before an incident or emergency occurs. Recommendations and advice draw on the expertise of many specialists around the world.

The publication can be obtained from: Marketing and Dissemination, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 2476; fax: +41 22 791 4857; email: *bookorders@who.int*). It is also available on line at: http://www.who.int/csr/delibepidemics/biochemguide/en/index.html

Anthrax: pathological aspects in autopsy cases in Shiraz, Islamic Republic of Iran, 1960–2001

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الجمرة الخبيثة: المظاهر الباثولوجية في حالات من فتح الجثة (الصفة التشريحية) في شيراز، إيران، 1960-2001

سيد صياء الدين نابعي، علي أمين، أشكان مولا، سيد علي أكبر نبوي زاده، علي رزمكن

الخلاصة: إن الجمرة الخبيثة تشبه السل من حيث عودة انتشارها من جديد في البلدان الصناعية، مما يظهر بعض الجوانب الغامضة في هذا المرض ويفرض تحديات على الطب. وقد سجلت جامعة شيراز للعلوم الطبية إجراء 7130 من حالات فتح الجثة (الصفة التشريحية) خلال السنوات الأربعين المنصرمة، كان من بينها 33 حالة بسبب الجمرة الخبيثة. وقد راجعنا جميع الشرائح الباثولوجية لهذه الحالات وصنفنا العضو المصاب بحثاً عن موجودات مجهرية لم يمكن التعرف عليها من قبل. وقد كان أكثر أسباب الموت شيوعاً الإنتان الناجم عن إصابة الأعضاء والتسمم الخلوي المباشر بالعصويات الخمرية وما تنتجه من ذيفان خارجي. ومن الموجودات المستجدة تشكل أغشية هيالينية في حالات إصابة الجهاز التنفسي، وهذه الأغشية تشبه ما يحصل في متلازمة العسرة التنفسية الحادة لدى البالغين إلى جانب بيّنات على إصابة بدئية في جهاز الهضم مما يوضح قدرة الجرثوم على عبور الحاجز المعدي.

ABSTRACT Anthrax, like tuberculosis, shows a new epidemic spread in industrialized countries, revealing some ambiguous aspects to the disease and providing new challenges to medicine. Shiraz University of Medical Sciences has records of 7130 autopsies performed in the past 40 years, 33 of which are anthrax cases. We reviewed all the pathology slides of these cases and classified the organs involved in a search for unrecognized microscopic findings. The most common cause of death was sepsis, caused by organ involvement and direct cytotoxicity of *Bacillus anthracis*, in addition to its exotoxin production. Novel findings included hyaline membrane formation in respiratory system cases that is similar to acute (adult) respiratory distress syndrome and evidence of primary gastrointestinal involvement, showing the ability of the organism to pass the gastric barrier.

Maladie du charbon : aspects pathologiques dans les cas d'autopsie à Chiraz (République islamique d'Iran), 1960-2001

RESUME A l'instar de la tuberculose, la maladie du charbon connaît une nouvelle poussée épidémique dans les pays industrialisés, révélant certains aspects ambigus de la maladie et posant de nouveaux défis à la médecine. L'Université des Sciences médicales de Chiraz a des dossiers de 7130 autopsies réalisées au cours des 40 dernières années, dont 33 concernent des cas de maladie du charbon. Nous avons examiné toutes les lames pathologiques de ces cas et avons classifié les organes atteints à la recherche de résultats de l'examen microscopique méconnus. La septicémie était la cause la plus courante de décès, due à l'envahissement des organes et à la cytotoxicité directe de *Bacillus anthracis*, en plus de sa production d'exotoxines. Les nouvelles découvertes comprenaient la formation de membrane hyaline dans les cas impliquant le système respiratoire qui est similaire au syndrome de détresse respiratoire aiguë (adulte) et l'évidence d'une atteinte gastro-intestinale primaire, révélant la capacité du micro-organisme à passer la barrière gastrique.

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Introduction

Anthrax, an acute infection caused by *Bacillus anthracis*, is acquired through contact with anthrax-infected domestic animals, or anthrax-contaminated animal products [*1,2*], and has the potential to be used in biological weapons [*3,4*]. While there is no clear evidence of direct human-to-human spread, the possibility has been discussed [*5,6*].

The disease is now extremely uncommon among humans but causes serious morbidity and mortality when encountered. The majority of descriptions of the pathology of anthrax date from the end of the nineteenth and beginning of the twentieth centuries. The preventive measures that were introduced at that time have almost eradicated the disease in some parts of the world and little attention has been paid to its epidemiology recently. Despite improved diagnostic and treatment techniques, anthrax, like other infections believed to have been eradicated, has emerged in a new epidemic that has gained worldwide attention [*7,8*]. This serves to remind us of our limited knowledge about the disease and the need for better early recognition and diagnosis that could result in more successful treatment of respiratory and gastric anthrax. It is worth noting that in current practice complete autopsy of confirmed (and even suspicious) cases of anthrax is no longer recommended, in order to decrease the risk of contagion [*9*].

As in other parts of the world, the number of anthrax cases in the Islamic Republic of Iran markedly decreased in the late twentieth century, but individual cases are still occasionally reported [*10*], especially among farmers and those who have direct contact with domestic animals or their products. The incidence of anthrax during 1996 was reported to be 0.54 per million population [*11*].

Shiraz University of Medical Sciences, founded in 1949, is a regional referral centre for all diseases and currently has an archive of about 7130 autopsy records from the last 40 years. Many of the anthrax cases have been presented earlier in the literature [*12–14*]. Our aim was to review the pathological findings in these autopsy records for unrecognized microscopic findings that could raise new concepts and to review the literature for theories about the pathogenesis of anthrax. We believe this would help improve diagnosis and treatment of the disease.

Methods

A review of records from the pathology ward of Shiraz University of Medical Sciences found 33 autopsies of anthrax cases performed between the years 1960–2001. All cases had been diagnosed by the clinico-pathological methods available at the time, such as Gram staining of fluids or tissues, different cultures and spore staining of the isolated organisms. All of the diagnoses had been confirmed by a complete autopsy, either through bacteriological investigations or the relevant organic histopathology findings.

The haematoxylin-eosin stained slides and special stains including tissue Gram stain were reviewed again to confirm previous findings and to look for new concepts. Cases were classified principally into cutaneous, pulmonary and gastrointestinal cases, according to light microscopy histopathology findings. Related findings in other organs were also considered. Then the data was analysed and reported, followed by a brief review of the literature.

Results

There was no significant gender bias among the 33 autopsy records: 16 (48.5%) were from males and 17 (51.5%) females. All patients were aged between 1 year and 65 years, with a mean age of 28.4 years old. Most (63.6%) were between 20–40 years of age. Thirty out of 33 patients had been living in rural areas, and were thus likely to have been in close contact with domestic animals.

Under light microscopy, 28 cases out of 33 (84.9%) had respiratory manifestations of anthrax (Figure 1). Most cases (26) had pulmonary congestion and haemorrhage with dilated interalveolar vessels and patchy parenchymal haemorrhage. Figures 2 and 3 show examples of marked exudation of serosanguinous fluid in the alveolar spaces, accompanied by intra-alveolar haemorrhage and mild fibrin deposition. Typical hyaline membrane formation was noted in 5 cases indicative of acute (adult) respiratory distress syndrome (ARDS). Leukocyte infiltration consisted mostly of neutrophils; just 1 case had prominent lymphoplasmacytic infiltration. The inflammation was intra-alveolar in 10 cases and interstitial in 6 cases. Five patients showed parenchymal necrosis and 5 cases hyperplasia of the alveolar wall and intra-alveolar haemosiderin-laden macrophages.

Only 3 of those who had pulmonary infection showed evidence of pleural involvement, in the form of effusion and leukocyte infiltration. Well-formed acute lobar or patchy pneumonia was scarce. Eleven cases (one-third of all cases) had evidence of upper airway involvement and inflammation: 1 case in this group had cutaneous anthrax of the neck without any evidence of septicaemia or internal organ involvement, and died of asphyxia due to severe upper airways obstruction from oedema.

Cutaneous anthrax was seen in 23 out of 33 cases (69.7%) but was the cause of death for only 1 patient (mentioned above). Several cases of cutaneous anthrax in our series had been treated for insect bites before developing the characteristic skin lesion of anthrax (a coal-black, scar-forming lesion). Ulceration and necrosis of the skin

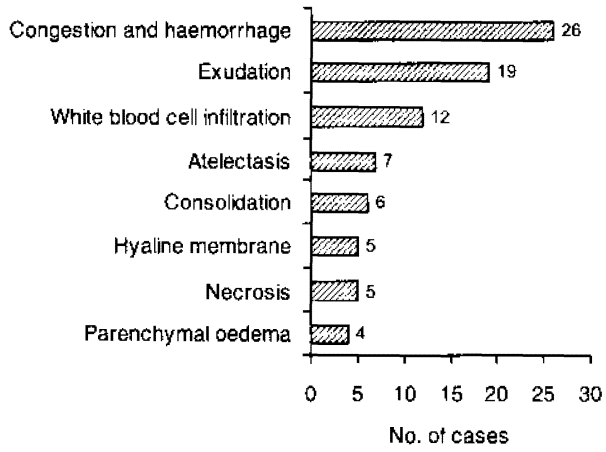

Figure 1 **Histopathology findings in 28 autopsy cases with pulmonary anthrax**

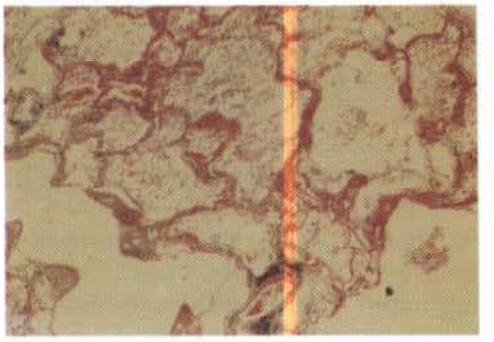

Figure 2 Low power view of acute respiratory distress syndrome in lung tissue. Note necrosis, congestion and inflammation with hyaline membrane formation in the alveolar tissue

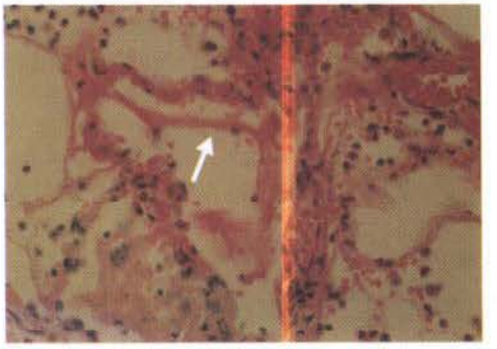

Figure 3 High power view of alveolar tissue, revealing the hyaline membranes (arrow)

was the most common finding (17 cases), involving all layers of the skin (Figure 4). Other typical histological findings were: oedema (15 cases), vascular congestion and haemorrhage (12) and white blood cell infiltration (8).

Twenty-two out of 33 cases (66.7%) had gastrointestinal manifestations of anthrax, commonly the small bowel (19 cases), the stomach (7 cases) and the large bowel (4 cases). The histopathology findings are summarized in Figure 5. Only 1 case had ascites, while 6 of them had evidence of peritoneal involvement, mostly in the form of oedema and leukocyte infiltration. Gastrointestinal involvement was the single cause of septicaemia and death in one-quarter of patients with gastrointestinal anthrax (15% of all cases), all of whom showed peritoneal involvement and a degree of haemorrhagic oedema (causing abdominal protrusion in a few cases) and leukocyte infiltration.

Lymphadenopathy was detected in 13 of the 33 cases (39.4%). The lymph nodes affected were adjacent to the organs involved: the mesentric nodes in gastrointestinal cases and the cervical, axillary and parahilar nodes in pulmonary cases. Generalized lymphadenopathy was seen in a few cases. No specific histological changes were present in the slides. The most severe cases showed haemorrhagic lymphadenitis and necrosis, with extension to the adjacent mediastinum or mesentery. Congestion and neutrophil infiltration were more common. Bacilli were only rarely identified in the involved lymph nodes.

Splenic congestion was a major finding, evident in 17 cases (51.5%). Five patients showed various degrees of congestion and infiltration of acute and chronic inflammatory cells in areas of red pulps indicative of acute septic splenitis, and 3 patients (including the one with typhoid fever) showed splenic infarction. Significant splenomegaly was noted in 5 cases on autopsy.

Hepatic findings were noted in 17 cases (51.5%), mostly in the form of sinusoidal dilatation and congestion, mainly parenchymal and rarely subcapsular, producing hepatocellular necrosis in some areas, probably due to a pressure effect. Fourteen

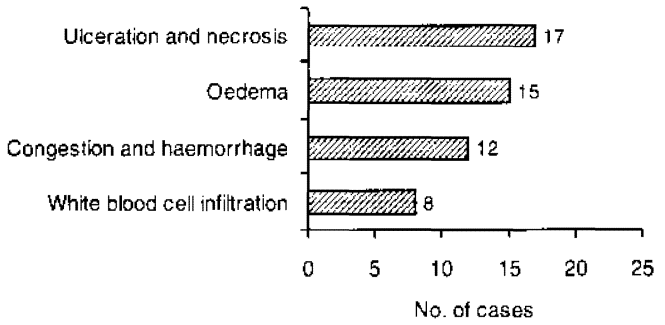

Figure 4 **Histopathology findings in 23 autopsy cases with cutaneous anthrax**

of these cases showed various degrees or parenchymal neutrophilic infiltration indicating hepatitis. Abscess formation was noted in 1 case.

Three out of 33 cases (9.1%) had histopathological findings of meningeal involvement showing mainly leptomeningeal congestion and neutrophile infiltration. One patient had a haematoma in the subarachnoid space.

Kidney findings consisted of congestion and intraparenchymal haemorrhage in 6 patients (18.2%) with signs of tubular necrosis in 3 of them.

The adrenal glands showed fat depletion and cortical tubular formation in 5 cases (15.2%) indicating septicaemia, and 3 cases showed congestion and haemorrhage of the adrenal parenchyma.

Bacillus anthracis was detected in 13 cases (39.4%) using haematoxylin-eosin or other special stains on the tissues. It is noteworthy that 1 of the cases was simultaneously seropositive for typhoid fever

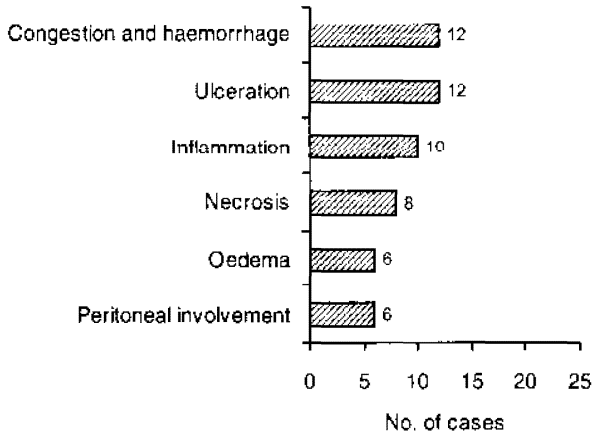

Figure 5 **Histopathology findings in 22 autopsy cases with gastrointestinal anthrax**

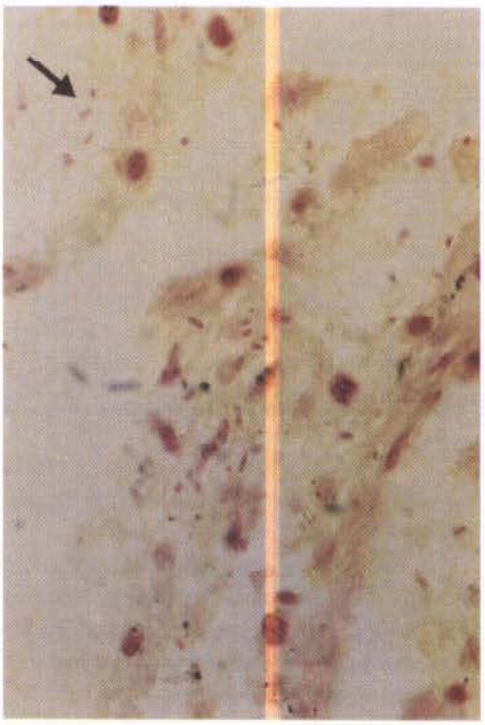

Figure 6 **Anthrax bacilli inside a skin lesion (arrow). Note the spore-forming bacilli in a necrotic background**

(both O and H antigen titres were about 1:320) with evidence of splenic haemorrhagic infarction and liver congestion. This patient died of anthrax septicaemia not typhoid fever.

Discussion

The causative agent of anthrax, *Bacillus anthracis*, is a large, non-motile, facultative and spore-forming Gram-positive rod (Figure 6). A glutamyl-polypeptide capsule is present that inhibits phagocytosis, and has a major role in the agent's pathogenic capabilities [*15,16*]. Anthrax toxin is well known as the major toxic component of the bacillus, but non-virulent toxin-producing strains have also been isolated [*15*]. These mutant strains fail to produce the polyglutamic acid capsule.

According to the current classification of bacterial exotoxins, anthrax toxin has an AB pattern [*17–20*]. Two potent exotoxins, called 'oedema factor' and 'lethal factor' constitute the A domain which gains entry into the host cells by the B domain ('protective antigen'). Antibodies against protective antigen seem to confer immunity [*16*]. After entry, oedema factor produces tissue oedema through increasing adenylate cyclase activity [*18,19*]. It may also suppress neutrophil function [*20,21*], and hence leukocytes are rarely encountered in anthrax lesions regardless of the organs involved and the severity. Lethal factor has recently been shown to possess an endoprotease activity, interrupting a vital cell-signalling pathway that brings about direct cell death and cytokine production (including tumour necrosis factor-α and interleukin-1β). It also inhibits mitogen-activated protein kinase kinase (MAPK) and prevents cell proliferation [*22–24*].

Both the organism and its spore can be infective. The most common route of entry for anthrax is the skin (95% of cases). Other routes of entry are inhalation of airborne spores causing pulmonary anthrax (wool-sorter's disease) or ingestion of the organism or spores in contaminated food leading to gastrointestinal anthrax. The former is the most lethal type, killing 100% of the patients if untreated; the latter is the least common type but is also very dangerous.

Skin involvement in anthrax is characteristically painless but pruritic [*25*], which may be due to destruction of nerve endings in the affected areas of skin. Cutaneous manifestations are more prevalent in exposed areas of skin, most commonly on the head and neck [*5*]. Stings may play a rule in

contagion, as some of the skin lesions begin as an insect bite [*6,26*]. Several cases of cutaneous anthrax in our series had been treated for insect bites before developing anthrax skin lesions. The most common histological findings of cutaneous anthrax consist of intense prolonged oedema, vascular congestion, haemorrhage and necrosis. Necrosis of the skin was the most common finding among our cases, involving all layers of the skin. The necrosis is mainly due to direct cytotoxicity of the organism [*27*] rather than ischaemia due to vascular compression caused by severe oedema. Cutaneous anthrax was recorded as the cause of death for only 1 patient, as death among cutaneous anthrax patients results mainly from the systemic dissemination of the disease [*1,2,9,10*].

In pulmonary anthrax, an acute alveolar or lobular pneumonia may develop, forming an extensive serofibrinous exudation throughout the parenchyma [*28,29*]; the striking characteristic of this pneumonia is the relative paucity of inflammatory cells. Haemorrhagic necrosis of the alveolar septum with a large number of bacteria may be present. Mucosal oedema may involve any level, from the oropharynx down to the alveoli. Acute mediastinitis and mediastinal widening due to lymphadenitis and haemorrhagic oedema in the mediastinum may be present. Occasionally, intravascular thrombosis may be a cause of death in respiratory anthrax [*30*].

In our series of patients, pulmonary congestion was the most common finding. Many of those with pulmonary involvement showed dilated interalveolar vessels and patchy parenchymal haemorrhage. Exudation of serosanguinous fluid in the alveolar spaces accompanied by intra-alveolar haemorrhage and fibrin deposition was characteristic of the early stages of ARDS, leading to hyaline membrane formation followed by respiratory failure. Typical hyaline membrane formation was noted in 5 cases: the other patients died before the hyaline membrane could form (3–7 days). Short survival also explains the low frequency of parenchymal necrosis in these patients, since necrosis was found only in those who survived longer. Very few cases showed hyperplasia of the alveolar wall and intra-alveolar haemosiderin-laden macrophages. Well-formed acute lobar or patchy pneumonia was scarce in our cases.

Oedema of the upper respiratory airways may be caused by primary mucosal infection with bacilli [*7*], but there was evidence of secondary spread of oedema from the skin surface to the deeper soft tissues in 1 of our patients. As previously mentioned, this patient had cutaneous anthrax lesions on the neck and died from asphyxia caused by severe oedema of the soft tissue and upper airways. No sign of systemic involvement was evident in this patient.

The alimentary canal may be involved in anthrax primarily from the mouth down to the large bowel. It seems that the symptoms are caused by bacilli in the tonsils and the gut-associated lymphatic tissue [*8*], but light microscopy shows full thickness involvement of the gut. Although massive oedema and ulceration with vascular congestion and haemorrhage is mentioned in the literature, other gastrointestinal findings are not much discussed. However, ascites and involvement of mesentery have also been reported.

In our series, the most and least frequently involved gastrointestinal sites were the small and large bowel respectively; the most common was the duodenum, followed by the jejunum and the ileum. The rate of stomach involvement (7/21 cases) was higher than previously reported [*8,31,32*], revealing that the bacillus can survive the usual gastric defence mechanisms such as gastric acid secretion. This

finding, and the large number of cases where stomach manifestations of anthrax were associated with septicaemia and death, suggest primary gastrointestinal involvement in anthrax, which contradicts previous reports in the literature [*7,8*].

Hepatic involvement was detected in half of our cases, showing some non-specific leukocyte infiltration in the liver parenchyma. Only 1 patient had significant abscess formation accompanied by the presence of many bacilli in the liver parenchyma. Hepatocellular necrosis, if present, was focal and scant.

Histopathological findings in the spleen were mainly due to systemic infection in those patients with anthrax septicaemia; therefore, no definite conclusions can be drawn about splenic involvement. Various degrees of congestion and infiltration of acute and chronic inflammatory cells in areas of red pulps were indicative of acute septic splenitis. Five cases showed splenomegaly on autopsy but there was little correlation between spleen size and the pathology findings.

Lymph node involvement adjacent to the primary organ involved was present in nearly 40% of cases, with a few cases of generalized lymphadenopathy. In its most severe form, haemorrhagic lymphadenitis and necrosis were present, with extension to the adjacent mediastinum or mesentery; hence the signs of mediastinal widening in chest X-rays [*33*]. Congestion and neutrophil infiltration were more commonly found. Bacilli were rarely detectable in the involved lymph nodes (or other organs) and this might be due to antibiotic therapy before death. Besides, bacilli engulfed by inflammatory cells are more difficult to see. Some investigators recommend new immunostaining techniques to reveal the bacilli, particularly the intracellular ones [*34,35*].

Meningeal involvement was found in a few cases in the form of non-specific congestion (mainly) and leukocyte infiltration. One case had subarachnoid haemorrhage. A case of anthrax accompanied by subarachnoid haemorrhage has been mentioned in the literature [*36*].

The adrenal glands showed non-specific signs of septicaemia, congestion and haemorrhage and were rarely infected directly by the organism. Involvement of the kidneys was notably non-specific among our cases, showing congestion and haemorrhage of the parenchyma.

Nearly all cases died sometime after antibiotic treatment was started, so the rate of organism cultivation was low, since *Bacillus anthracis* was sensitive to most antibiotics current at the time [*15,16*]. It has been reported that culturing before 21 hours of antibiotic therapy yields the organism in nearly all patients [*8*].

Conclusion

Although there have been great advances in our knowledge about the pathogenesis of anthrax in recent years, more research is still needed. Our review of autopsy findings was consistent with previous experimental reports. However, we observed manifestations of anthrax in some organs not previous discussed, such as the spleen. The major findings were an ARDS-pattern of pulmonary involvement and a primary involvement of gastrointestinal tissue including the stomach.

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Coût des soins de santé de base dans une circonscription sanitaire en Tunisie

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تكاليف الخدمات الصحية الأساسية في إحدى مناطق تونس

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الخلاصة: تعد المعلومات حول تكاليف الخدمات الصحية ضرورية للتخطيط والإدارة الجيدة والاستخدام الأمثل للموارد. وقد قمنا بحساب التكاليف الإجمالية لتشغيل مركز للرعاية الصحية الأولية لمدة عام (1995) في إحدى المناطق في إنفيذه، في تونس، وقدرنا الكميات المستخدمة طيلة العام من كل منتج صحي؛ ثم قدرنا التكاليف الإجمالية لكل منتج صحي على أساس مستويات الانتفاع به وفقاً لنمط الخدمة المقدمة. ثم استنتجنا وسطي تكلفة كل وحدة. وقد بلغ الإنفاق السنوي 099 219 1 ديناراً تونسياً، وكان الإنفاق العام لكل فرد 17.494 ديناراً تونسياً (كان كل دولار أمريكي يعادل 0.950 ديناراً تونسياً عام 1995). وقد أنفق 65.31٪ من التكاليف الإجمالية على العاملين فيما أنفق 17.03٪ منها على الأدوية. وإذا نظرنا إلى التكاليف من زاوية أخرى فسنجد أن 84.96٪ من التكاليف قد أنفقت على الخدمات العلاجية وأن 14.04٪ منها قد أنفقت على الخدمات الوقائية. وقد كانت تكاليف الاستشارات للرعاية العلاجية 6.847 ديناراً، وللرعاية في الفترة المحيطة بالولادة 2.764 ديناراً، وللتمنيع 3.680 ديناراً، وللزيارات في المدارس 6.680 ديناراً. وتساعد الدراسة في التعرف على الطرق التي يمكن لتحليل التكاليف أن تستقصي بها كفاءة استخدام الموارد وكفايتها في المنطقة المدروسة.

RESUME L'objectif de notre étude est de calculer le coût de fonctionnement des différentes structures de santé en première ligne et les coûts moyens des services fournis dans ces structures durant un année (1995). La région de l'étude est la circonscription sanitaire d'Enfidha (Tunisie). Le coût global de fonctionnement de la circonscription sanitaire s'élève à 1 219 099 dinars tunisiens (TND) et les dépenses publiques annuelles par habitant supportées par la circonscription sanitaire sont de TND 17,494 (un dollar US = TND 0,950 en 1995). Les frais de personnel représentent 65,37 % du coût global et ceux des médicaments 17,03 %. Par ailleurs, 14,04 % sont dépensés pour le préventif et 84,96 % pour le curatif. Les coûts unitaires sont de TND 6,847 pour la consultation curative, de TND 3,680 pour l'acte de vaccination, de TND 2,764 pour la consultation périnatale et de TND 6,680 pour la consultation de médecine scolaire. Disposer d'informations à propos des coûts permet un meilleur usage des ressources, une meilleure équité et une prestation de qualité.

Costs of basic health services in a health district in Tunisia

ABSTRACT Information on the cost of health services is essential for good planning and management and the efficient use of resources. We calculated the total costs incurred in running primary health services for one year (1995) in the health district of Enfidha (Tunisia). The yearly operating expenditure for the health district was 1 219 099 Tunisian dinars and the cost per inhabitant was 17.494 dinars (US$ 1 = Tunisian dinar 0.950 in 1995); 65.37% of total costs went on staff and 17.03% on drugs. Looked at another way, 84,96% went on curative services and 14.04% on preventive services. The cost of a consultation for curative care was 6.847dinars, for perinatal care was 2.764 dinars, for immunization was 3.680 and for school visit was 6.680 dinars. The study helps to identify ways in which cost analysis can be used to explore efficiency and resource adequacy in the district.

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Introduction

Le progrès des techniques médicales d'une part, l'évolution démographique et la transition épidémiologique d'autre part ont induit une importante croissance de la demande et de l'offre de soins, particulièrement dans les pays en développement. Il en a résulté une augmentation continue des dépenses de soins, à l'origine d'une crise du financement public de la santé dans la plupart de ces pays [*1*]. Devant les limites des moyens, il est ainsi devenu impératif d'optimiser l'utilisation des ressources mobilisées, d'opérer des choix à différents niveaux d'intervention dans le système de santé et de décider des degrés de priorité entre différentes alternatives (la prévention, les soins curatifs, et autres) [*2*]. Pour y parvenir, encore faut-il disposer d'un minimum d'informations à propos des coûts induits. L'étude des coûts, l'un des outils essentiels de la gestion des ressources, nous permet en effet de chiffrer les dépenses, de déterminer les grandes lignes de planification et de choisir entre différentes alternatives stratégiques [*3*]. Ceci est d'autant plus vrai au niveau le plus périphérique du système, celui de la circonscription sanitaire. Celle qui est censée être la porte d'entrée et en même temps l'unité fonctionnelle du système de santé se caractérise en effet par une certaine faiblesse dans la gestion, entre autre traduite par une mauvaise utilisation des ressources allouées aux prestations de soins [*2*].

L'objectif de ce travail est ainsi de calculer le coût de fonctionnement des différentes structures de santé composant la circonscription sanitaire (l'hôpital de circonscription, les centres de santé) de même que le coût moyen des services fournis au niveau de ces structures. A travers cette étude, nous espérons contribuer à fournir des informations sur les coûts en première ligne, ce qui permettra aux gestionnaires du niveau central et du niveau local d'analyser le fonctionnement des structures et de prendre les décisions appropriées.

Méthodes

La région de l'étude est la circonscription sanitaire (CS) d'Enfidha, une CS rurale située au centre-est de la Tunisie qui comporte 69 688 habitants. Le secteur sanitaire privé y est très peu développé et le secteur public est représenté par deux niveaux de structures opérationnelles. Le premier niveau est constitué par 26 centres de santé de base (CSB) dont 46 % sont de petits centres, desservant chacun en moyenne une population d'environ 2000 habitants et fonctionnant avec un personnel paramédical, une sage-femme (1/6 du temps). Une consultation médicale y est assurée à raison de deux fois par semaine, les autres jours étant consacrés aux consultations de la sage-femme ou aux soins (dont la vaccination). Le deuxième niveau ou niveau de recours comporte l'hôpital de circonscription avec des consultations spécialisées, un service de médecine interne (20 lits), une maternité (15 lits), un service d'urgence, une unité de radiologie et un laboratoire d'analyses biologiques.

L'Etat contribue au financement de la quasi-totalité des dépenses (89 %) (salaires du personnel et une subvention de TND 288 000 par an). Le reste du budget de fonctionnement de la circonscription est fourni par les recettes propres des structures sanitaires (contribution directe des patients aux frais de consultations externes, d'hospitalisation et des examens complémentaires, remboursement par les caisses d'assurance).

Le personnel technique est composé de 10 médecins, d'un pharmacien, de deux chirurgiens dentaires, de neuf sages-

femmes et de 122 infirmiers et aides-soignants.

Notre étude a considéré l'ensemble des dépenses induites durant une année pour la totalité des structures de santé publique de la circonscription, soit 26 centres de santé de base, les consultations spécialisées et les trois services hospitaliers de l'hôpital de la circonscription. La démarche suivie lors du calcul du coût est inspirée de la méthodologie utilisée par l'UNICEF et collaborateurs dans l'initiative de Bamako [*1*]. Elle consiste à :

a) identifier les ressources utilisées pour produire les services dont les coûts sont à calculer ; cette catégorie de ressources (renouvelables ou de fonctionnement) englobe le personnel (administratif et technique), les fournitures (médicaments, vaccins, seringues, petit équipement), l'utilisation et l'entretien des véhicules, l'utilisation et l'entretien des bâtiments, la formation continue et la supervision ;

b) estimer la quantité consommée pour chaque ressource ou intrant durant l'année 1995 ;

c) attribuer des valeurs monétaires à chaque unité d'intrant et en calculer le coût total ;

d) répartir proportionnellement le coût de chaque intrant entre les activités où il intervient ;

e) utiliser les mesures de la production de chaque activité pour le calcul des coûts unitaires.

Résultats

Dépenses totales

Le coût global de fonctionnement de la circonscription est de TND 1 219 099 (un dollar US = TND 0,950 en 1995), ce qui équivaut à une dépense par habitant et par an au niveau des structures étatiques de la CS de TND 17,494 (Tableau 1).

Répartition du coût selon le niveau de soins

Les dépenses ont été réparties entre :

- les CSB (70,40 %) avec un coût moyen par CSB de TND 32 793 ;
- l'hôpital (26,83 %) dont 46,94 % pour le service des urgences, 32,16 % pour la maternité et 20,90 % pour le service de médecine interne ;
- et les consultations spécialisées (2,77 %).

On dépense donc par habitant TND 12,314 au niveau des CSB, TND 0,484 au niveau des consultations spécialisées et TND 4,694 au niveau de l'hôpital (Tableau 1).

Répartition du coût selon le type d'activité

L'analyse du coût par type d'activité montre que les soins curatifs correspondent à 84,96 % du total du coût au niveau de la circonscription, soit une dépense de TND 14,862 par habitant et par an. Les soins préventifs forment le reste (15,04 %), soit une dépense de TND 2,632 par habitant et par an (Tableau 2). La répartition du coût des soins préventifs montre

Tableau 1 **Dépenses publiques par habitant et par type de structure (en dinars)**

Type de structure	Coût global (TND)	Coût par habitant (TND)	%
CSB (28 CSB)	858 246	12,314	70,40
Hôpital	327 084	0,484	26,83
Consultation spécialisée	33 769	4,694	2,77
Total	1 219 099	17,494	100

Tableau 2 **Répartition du coût selon le type d'activité (en dinars)**

Activités	Coût global (TND)	Coût par habitant (TND)	%
Curatives	1 035 723	14,862	84,96
Préventives	183 376	2,632	15,04
Total	1 219 099	17,494	100

que 29,46 % du coût est relatif à la vaccination, 23,89 % à la médecine scolaire, 26,64 % à la consultation de périnatalité, 13,70 % à l'hygiène du milieu, 3,42 % à d'autres programmes nationaux (la tuberculose, la rage, la lutte contre les envenimations, le paludisme) et 2,90 % à l'éducation pour la santé.

Les composantes du coût

Les frais du personnel (des services généraux de soutien, technique, du laboratoire et du service de radiologie) représentent 65,37 % du coût global et les médicaments 17,03 % (Tableau 3). On dépense ainsi par habitant et par an TND 11,961 pour le personnel et TND 2,979 pour les médicaments. Le coût fixe représente quant à lui 69,04 % du coût de fonctionnement. Selon le type de structure, il varie de 67,09 % au niveau de l'hôpital à 88,05 % au niveau de la consultation de 2e degré et il est de 69,33 % au niveau des CSB.

Le coût unitaire par activité

Les coûts unitaires moyens des différentes prestations fournies au niveau des CSB sont de TND 6,847 pour la consultation curative, de TND 3,680 pour l'acte de vaccination, de TND 2,764 pour la consultation périnatale (avec TND 2,610 pour la consultation prénatale, TND 2,604 pour la consultation postnatale, TND 2,997 pour la consultation de planning familial et TND 2,656 pour la consultation de gynécologie), de TND 6,399 pour la consultation en médecine scolaire et de TND 7,492 pour la consultation de stomatologie (Figure 1).

Au niveau de l'hôpital, le coût d'une journée d'hospitalisation est de TND 15,426 en médecine interne et de TND 67,744 à la maternité. Si on considère les accouchements, le coût moyen d'un accouchement à la maternité s'élève à TND 174,184 (environ le SMIG en Tunisie). Le coût moyen d'un malade hospitalisé au service de médecine est de TND 133,521. Le coût d'un lit budgétaire s'élève à TND 2734,520 au service de médecine et à TND 7013,800 à la maternité. Pour le service des urgences, le coût d'une consultation est de TND 8,930. Les coûts unitaires des consultations spécialisées varient quant à eux de TND 6,339 à 10,092.

Discussion

Sachant que les études relatives aux coûts des services des soins de santé de base en Tunisie sont rares, nous avons eu pour objectif d'analyser comment le budget de la

Tableau 3 **Composantes du coût**

Rubrique	Coût en dinars	%
Personnel	796 964	65,37
Médicaments	207 555	17,03
Laboratoire	40 834	3,35
Matériel médical	23 186	1,90
Programmes nationaux	32 323	2,65
Radiologie	22 740	1,87
Autres	95 487	7,83

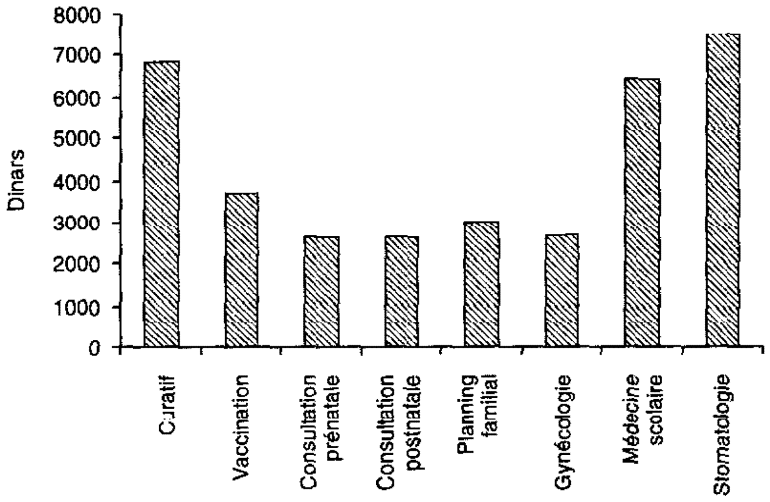

Figure 1 **Coût unitaire par activité (en dinars)**

CS était consommé. Pour ce faire, nous avons utilisé une méthodologie qui a fait ses preuves puisqu'elle a été essayée dans plusieurs pays, en particulier à travers l'initiative de Bamako (Bénin, Gambie, Niger, Ouganda, Zaïre, Burundi, etc.) [*4*]. Par contre, notre étude présente des limites du fait qu'elle n'a concerné que le secteur public et qu'elle a considéré uniquement le coût de fonctionnement supporté par la circonscription. De même, dans le but d'obtenir les estimations les plus proches de la réalité, nous avons choisi nos clés de répartition en nous référant, d'une part, aux données de la littérature et d'autre part, aux résultats des entretiens avec l'équipe de la circonscription à propos du déroulement des activités et de la gestion des ressources au sein de la circonscription. De ce fait, le choix de la répartition de certaines ressources entre les structures ou entre les activités pourrait être entaché d'une certaine subjectivité. En l'absence d'autres alternatives, ce choix peut être considéré comme justifié.

Par rapport à l'année de notre étude (1995), les dépenses totales de santé en Tunisie ont été évaluées à 1072,9 millions de dinars, soit 5,9 % du PIB [*5*] et représentent une dépense annuelle moyenne de TND 120 par habitant [*6*]. Depuis 1990, on a en effet dépassé l'objectif fixé par l'OMS pour les dépenses de santé (5 % du PIB). Par ailleurs, au niveau du ministère de la santé, la part du budget de fonctionnement (sans compter les salaires et les indemnités du personnel) des établissements publics destinée aux établissements de première ligne (hôpitaux de circonscription et groupements de CSB) était de 22,7 % [*7*].

En comparant les résultats de notre étude à ceux observés dans la circonscription de Medjez El Bab au nord-ouest de la Tunisie, nous constatons que les dépenses par habitant et par an sont plus élevées à Medjez (TND 26,304 contre TND 17,494) [*8*]. Cette différence est due essentiellement à des dépenses plus importantes au niveau de l'hôpital (les dépenses par habi-

tant occasionnées par l'hôpital sont de TND 14,309 à Medjez et de TND 5,178 à Enfidha). Ceci pourrait s'expliquer par la capacité et les activités relativement restreintes à l'hôpital d'Enfidha. Cette situation serait à l'origine d'un taux d'orientation-recours élevé et d'une prise en charge plus coûteuse par le troisième niveau, d'où l'intérêt de doter l'hôpital de circonscription des ressources nécessaires pour qu'il puisse, avec une bonne gestion, jouer son rôle d'orientation-recours et assurer la continuité des soins curatifs aux malades. En effet, lorsque les centres de santé et les hôpitaux de premier recours fonctionnent de manière satisfaisante, ils peuvent offrir la possibilité de répondre à plus de 90 % des demandes des soins de santé et de réduire ainsi jusqu'à 30 % la charge de morbidité [*9*]. La part des dépenses de l'hôpital est à peu près de 50 % en Zambie (district de Monze) et de 73,53 % au Cameroun (district d'Obala) [*10,11*]. Il est difficile néanmoins de porter un jugement sur cette répartition qui dépend de plusieurs facteurs.

En ce qui concerne les composantes du coût, nos résultats concordent avec ceux retrouvés dans la circonscription de Medjez El Bab où le personnel représente 64,6 % et les médicaments 17,3 % du coût total [*8*]. Selon la littérature, on note en outre une variation de la part du coût du personnel qui est de 38 % en Zambie [*10*], de 83 % au Cameroun [*11*], de 54 % en Namibie [*12*] et de 43,3 % au Burundi [*13*]. Quant au coût fixe, il représente 70 % au Mali [*14*]. Il faut cependant noter qu'au Bénin et en Guinée, la part du personnel qui était de 80 % en 1986, soit avant la mise en oeuvre de l'initiative de Bamako, est passée ensuite à 49 % [*15*].

En fait, dans la majorité des pays en développement, la part des salaires dans le budget n'a cessé de croître à la suite du développement des systèmes de santé et de l'augmentation des effectifs entre les années 70 et 80, entraînant ainsi une diminution des crédits de fonctionnement courant. En Afrique, les coûts de personnel sont ainsi le principal poste budgétaire du ministère de la santé, et plus de 60 % de la totalité des ressources publiques consacrées à la santé sont, dans l'ensemble, allouées aux salaires [*16*].

Les médicaments ont formé 17,03 % du coût de fonctionnement de la circonscription, soit une dépense de TND 2,979 par habitant. Il s'agit là uniquement du coût des médicaments délivrés par les structures publiques. Si, en Afrique, les dépenses consacrées aux produits pharmaceutiques représentent généralement de 20 à 30 % du total des coûts de fonctionnement des établissements sanitaires publics et privés et ne sont dépassées que par les dépenses de personnel [*17*], il apparaît néanmoins important d'observer qu'en Tunisie, par rapport aux autres coûts de la santé, la part des médicaments dans le budget de l'Etat décroît régulièrement. Elle est ainsi passée de 40,68 % en 1987 à 28,85 % en 1994 malgré l'augmentation de l'enveloppe des médicaments. Dès lors, un effort de réflexion et de clarification a été engagé à plusieurs niveaux et ce, dans le cadre de la définition d'une nouvelle politique pharmaceutique [*18*].

L'analyse de la répartition des coûts par type d'activité apporte au gestionnaire des informations plus précises sur les déséquilibres constatés. Ainsi, au niveau de la circonscription d'Enfidha, les soins préventifs ne représentent que moins d'un cinquième du coût global, faisant que la dépense annuelle moyenne par habitant est sept fois plus élevée pour les soins curatifs comparés aux soins préventifs. Sachant l'importance de la prévention, sur le plan national la politique de l'Etat va dans le sens

de l'augmentation des crédits alloués à la médecine préventive, lesquels sont passés de 2,990 millions de dinars en 1992 à 7,990 millions de dinars en 1996, soit une augmentation annuelle de 33,4 % [*19*]. Dans cette perspective, l'Etat compte ainsi prendre en charge les actions sanitaires prioritaires entre autres, en renforçant les actions préventives en première ligne. En effet, ces actions constituent une composante essentielle pour améliorer l'état de santé de la population, et ceci par le développement des activités de dépistage et d'éducation pour la santé.

Conclusion

Notre étude nous a permis d'estimer les coûts des structures et le coût moyen des services fournis en première ligne dans une circonscription sanitaire en Tunisie, une des informations essentielles pour la gestion des ressources, d'autant plus que la stratégie de la circonscription sanitaire, pierre angulaire du système de santé tunisien, vise à rendre les circonscriptions sanitaires fonctionnelles, répondant aux critères de qualité de service, de bonne gestion et d'efficience que les populations sont en droit d'attendre. Les études des coûts permettent en effet, en assurant un meilleur usage des ressources, de garantir une meilleure équité et une prestation de qualité pour la population. Que cela soit au niveau national ou local, il est impératif, pour rationaliser les choix budgétaires et oeuvrer pour une meilleure allocation des ressources humaines et financières, de disposer de données de base et d'indicateurs pertinents relatifs à la sécurité, l'efficacité et les coûts des techniques utilisées et des services fournis à la population.

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Life expectancy and cause of death in the Kuwaiti population 1987–2000

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مأمول الحياة وأسباب الوفيات لدى السكان الكويتيين 1987-2000

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الخلاصة: تم تحليل معطيات التعداد والمعطيات الصحية لمعرفة التغيرات في مأمول الحياة عند الولادة في الفترة 1987-2000 لدى السكان الكويتيين وربط ذلك مع معدلات الوفيات السنوية المرتبطة بسبب معين. وقد ارتفع مأمول الحياة من 73.3 عاماً إلى 75.5 عاماً مع وجود فجوة بين الرجال والنساء اتسعت من 2.2 إلى 4.5 سنوات. وتكون معدلات الوفيات لجميع الأسباب باستثناء فرط ضغط الدم أعلى لدى الرجال منها لدى النساء. والأسباب الرئيسية للوفاة بين الرجال هي مرض القلب الإقفاري وحوادث الطرق والسرطان، أما بين النساء فهي السرطان ومرض القلب الإقفاري وفرط ضغط الدم. إن المشكلات المرافقة لتشيخ السكان ستحتاج لمزيد من الاهتمام في تخطيط السياسات الصحية في الكويت.

ABSTRACT Census and health data were analysed to determine changes in life expectancy at birth during 1987–2000 in the Kuwaiti population and to correlate these with cause-specific annual mortality rates. Life expectancy at birth rose from 73.3 to 75.5 years with a gap between females and males, which increased from 2.2 to 4.5 years. For all causes of death except hypertension and ill-defined conditions, males had higher mortality than females. The leading causes of death in males were ischaemic heart diseases, traffic accidents and cancer, while in females they were cancer, ischaemic heart diseases and hypertension. The problems of an ageing population will need to be considered in planning the health policies of Kuwait.

Espérance de vie et causes de décès dans la population koweïtienne, 1987-2000

RESUME On a analysé les données de recensement et données sanitaires pour déterminer les changements survenus dans l'espérance de vie à la naissance entre 1987 et 2000 dans la population koweïtienne et les corréler avec les taux annuels spécifiques de mortalité par cause. L'espérance de vie à la naissance est passée de 70,0 à 75,5 ans, l'écart entre les femmes et les hommes étant passé de 2,2 à 4,5 ans. La mortalité était plus élevée chez les hommes que chez les femmes pour toutes les causes de décès sauf pour l'hypertension. Les cardiopathies ischémiques, les accidents de la circulation et le cancer étaient les principales causes de décès chez les hommes, tandis que chez les femmes, c'étaient le cancer, les cardiopathies ischémiques et l'hypertension. Les problèmes d'une population vieillissante devront être pris en compte dans la planification des politiques de santé au Koweït.

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Introduction

The epidemiological transition is the most important historical change affecting the level and pattern of human mortality. The transition refers to the decline of acute infectious disease and the rise of chronic degenerative disease over time [*1*]. Consequently, life expectancy has been increasing around the world. It is one of the key indicators of population health and economic development. Early and rapid gains in life expectancy in the early 20th century were due to improvement in living standards and organized efforts to control the spread of infectious disease [*2*]. The rise of life expectancy during the second half of the 20th century was slower because it depended on the reduction of death rates at older ages [*3*]. The most significant component of the mortality decline at older ages is the reduction of the rates of death from cardiovascular diseases, including heart disease and stroke, and from cancer [*4*].

The causes of change in life expectancy are many and varied. Epidemiological and public health factors such as lifestyles and behaviours, along with the pattern of diseases, play major roles. The historical stability of biological and other factors affecting mortality decline suggests that the most reliable method of predicting the future is merely to extrapolate past trends. Such methods suggest that life expectancy at birth in industrialized countries will be about 85–87 years at the middle of the 21st century [*5,6*].

In the Eastern Mediterranean Region, published studies about trends of life expectancy at birth are scarce [*7–12*]. In a study carried in North Africa, it was found that life expectancy at birth was 50–52 years in 1970 and rose to 64–70 years in 1993, a change that was attributed to the decrease of infant and female mortality [*7*]. In the Arabian peninsula, due to the increased socioeconomic levels and gradual improvement in health status, life expectancy at birth has increased markedly [*10,11*]. The health of Kuwaitis has improved considerably in the past 20 years, owing to economic and social transformations that have ameliorated the problems of feeding, sanitation, hygiene, housing and social conditions in general, as well as health services [*12*]. It was postulated, in a study in 1984, that the potential gain in life expectancy of the Kuwaiti population was due to elimination of infectious diseases. Since this date, no study to determine trends in life expectancy has been conducted.

In this study, we aimed to define trends in life expectancy at birth in the Kuwaiti population during 1987 2000 and correlate the trends with changing patterns of causes of death during the same period.

Methods

Study design

This study was conducted in the Department of Health and Vital Statistics, Ministry of Health, Kuwait. Data on all deaths in Kuwait between 1987 and 2000 inclusively were collected retrospectively from copies of original Kuwaiti death notification forms and the computer database in the department. Data were analysed by sex and cause of death to examine trends in life expectancy at birth and cause-specific patterns of mortality among the Kuwaiti population over this period.

Data coding

Death notification forms in Kuwait are sent by health care facilities to 4 registration offices distributed regionally to cover all governorates in the country. Copies of the original death notification forms are then sent to the Department of Health and Vital Statistics for processing. First, the forms

are revised and corrected through feedback channels with the registration offices and health care facilities, then the cause of death is coded, followed by data entry, tabulation and reporting. Up to 1994, causes of death were coded according to the *International classification of diseases, 9th revision (ICD-9)* [*13*], after which the coding changed to *ICD-10* [*14*]. For the purpose of this study, to avoid incompatibility of the data, we recoded the underlying causes of death from 1995 to 2000 according to *ICD-9*. There were no valid data for the year 1990 because it was the year of the Iraqi invasion of Kuwait.

Causes of death were aggregated into 10 broad classes: ischaemic heart diseases; hypertension; diseases of the pulmonary circulation and other forms of heart diseases; lower respiratory diseases; cancer; congenital anomalies; perinatal conditions; endocrine and metabolic diseases; traffic accidents; and ill-defined conditions (symptoms, signs and abnormal clinical and laboratory findings not elsewhere classified).

Statistical analysis

Mortality rates were computed for all causes of death combined and for the 10 leading cause groups, ranked according to the mean number of deaths during the study period. For the selected causes of death, we calculated the mortality rates over the 13 years, for each sex and for both sexes combined, per 100 000 Kuwaiti population. Mid-year population estimates were obtained from the Ministry of Planning, Central Statistical Office.

Life tables were used to determine the life expectancy at birth, i.e. how long the people live on average in a population [*15*]. It was calculated for each year using standard life table techniques. Annual abridged life tables are constructed using age-specific death rates derived from vital registration and census population data. Population data were aggregated into 5-year age intervals except for the first and last intervals. The first interval contained infants less than 1 year and the last those aged 85 years and over. As a result, the life table closed with the category 85 years and over. Calculation of the abridged life table was derived from the probability of death (q_x), which depends on the number of deaths (D_x) and the mid-year population (P_x) for each age interval (x) observed during the calendar year of interest [*16*].

The number of deaths in each age category was adjusted proportionally to account for those whose age was not stated. An assumption was made that deaths with unreported age were distributed among the various age groups in the same proportions as those for which age was reported.

The numbers of deaths in this study represent complete counts of this event. As such, they were not subject to sampling error, although they were subject to non-sampling error in the registration process. However, in the comparison of rates over time and among different groups, the results were subject to random variation and were compared according to certain statistical assumptions. The difference between 2 rates was regarded as statistically significant at the 5% level if it exceeded

$$2\times\sqrt{(\{R^2_1 / N_1\} + \{R^2_2 / N_2\})}$$

where R is the rate corresponding to N number of events. Detailed information on random variation may be found in the technical appendix of *Vital statistics on the United States* [*17*].

Correlation coefficients between life expectancy at birth and cause specific mortality rates during the study period were determined using Pearson's correlation coefficient (r). Manipulation and analysis of data were performed using *Excel* and *SPSS*, version 9 computer packages.

Results

During the period 1987–2000, the total number of deaths per year in the Kuwaiti population rose from 1050 to 1448 among males and from 725 to 972 among females. However, life expectancy at birth increased from 73.34 years to 75.49 years for the total Kuwaiti population (Table 1). The increase in life expectancy was higher among Kuwaiti females (3.33 years) than males (1.05 years). The difference in life expectancy between males and females during each year was in favour of females and from 1987 to 2000 the difference in life expectancy between females and males increased from 2.19 years to 4.47 years (Table 1).

During the study period, life expectancy in both sexes reached its lowest value in 1992. After 1992, however, a steady upward trend in life expectancy at birth was recorded which was more marked in females (Figure 1). Life expectancy for males was 72.22 in 1987, decreased to 70.47 years in 1992 then increased to reach 73.27 in 2000. The corresponding figures in females were 74.41, 72.47 and 77.74 years (Table 1).

Table 1 **Life expectancy at birth among males and females in the Kuwaiti population 1987–2000**

Year[a]	Life expectancy at birth (years)			
	Male	Female	Female–male difference	Overall
1987	72.22	74.41	2.19	73.34
1988	72.14	73.80	1.66	73.00
1989	72.32	73.10	0.78	73.27
1991	71.38	75.14	3.76	73.10
1992	70.47	72.47	2.00	71.68
1993	73.94	75.25	1.31	74.67
1994	74.10	75.91	1.81	74.99
1995	72.69	77.42	4.73	74.91
1996	73.70	77.75	4.05	75.63
1997	73.25	77.14	3.89	75.21
1998	72.51	77.09	4.58	74.79
1999	73.32	77.79	4.47	75.40
2000	73.27	77.74	4.47	75.49

[a]*No data available for the year 1990.*

Table 2 shows the mean annual rate of mortality for the study period, ranked by the cause of death in the 10 groups of conditions. The mean annual mortality rates varied considerably for males and females. For all causes of death, males showed higher mortality rates than females except for hypertension and ill-defined conditions. The excess in mortality rates among males over females was statistically significant for ischaemic heart diseases (58.17 per 100 000 males versus 31.20 per 100 000 females; $P < 0.05$), traffic accidents (39.01 per 100 000 males versus 8.18 per 100 000 females; $P < 0.05$), pulmonary circulation and other heart diseases (22.60 per 100 000 males versus 15.89 per 100 000 females; $P < 0.05$). Although females showed a higher mortality rate from hypertension and ill-defined conditions than males, these differences were not statistically significant.

During the study period, the leading causes of mortality among males were ischaemic heart diseases (58.17 per 100 000 males), traffic accidents (39.01 per 100 000 males) and cancer (37.75 per 100 000 males). Meanwhile, the leading causes of mortality among females were cancer (31.23 per 100 000 females), ischaemic heart diseases (31.20 per 100 000 females) and hypertension (30.52 per 100 000 females).

Table 3 shows the correlations between life expectancy at birth and cause specific mortality rates from the leading causes of death. Overall, there were significant negative correlations between life expectancy at

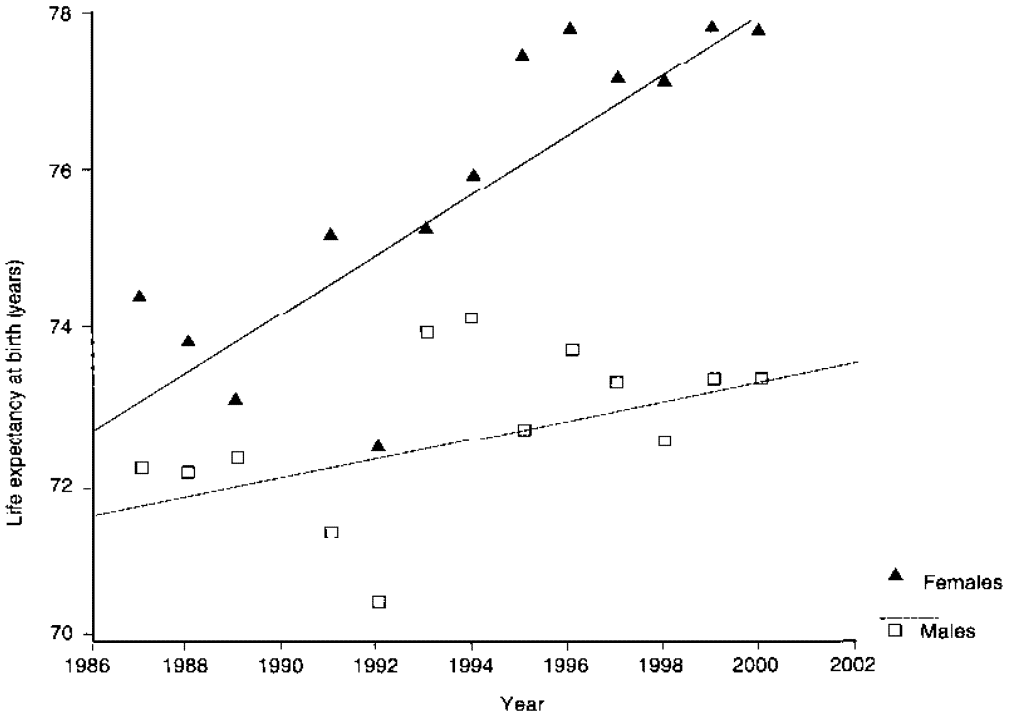

Figure 1 **Trends of life expectancy at birth among males and females in the Kuwaiti population 1987–2000**

birth and the mortality rates from ischaemic heart diseases, lower respiratory diseases and ill-defined conditions. Among males, only traffic accidents and lower respiratory diseases were negatively correlated with life expectancy. On the other hand, life expectancy among females was indirectly correlated with mortality rates from lower respiratory diseases, perinatal conditions and ill-defined conditions. However, mortality from pulmonary circulation and other heart diseases, which was ranked as the 7th leading cause of death in females, was positively correlated with female life expectancy. Figures 2 and 3 show the trends of these leading causes of death from 1987 to 2000.

Mortality from these causes changed during the study years. In males, the mortality rate from traffic accidents was 40.93/100 000 in 1987 and 41.13/100 000 in 2000, while the mortality rate from lower respiratory diseases decreased from 29.84/100 000 in 1987 to 16.55/100 000 in 2000. In females, the mortality rate from pulmonary circulation and other heart diseases was 9.01/100 000 in 1987, increasing to 22.57/100 000 in 2000. Mortality from lower respiratory diseases and from perinatal conditions during the study years fell from 20.76/100 000 and 29.77/100 000 to 14.50/100 000 and 9.27/100 000 respectively (Table 4).

Table 2 **Mean annual rates of mortality ranked by cause of death among males and females in the Kuwaiti population 1987–2000**

Cause of death	Mean annual mortality rate (per 100 000)			
	Overall	Male	Female	Female–male difference
Ischaemic heart diseases	44.70	58.17	31.20	–26.97*
Cancer	34.49	37.75	31.23	–6.52
Hypertension	29.08	27.66	30.52	2.86
Traffic accidents	23.61	39.01	8.18	–30.82*
Pulmonary circulation and other heart diseases	19.24	22.60	15.89	–6.72*
Lower respiratory diseases	18.88	20.87	16.88	–3.99
Congenital anomalies	18.55	20.44	16.66	–3.78
Perinatal conditions	18.87	21.11	16.63	–4.48
Endocrine diseases	16.27	17.28	15.28	–1.99
Ill-defined conditions[a]	16.39	16.27	16.54	0.27
Other causes	72.41	89.14	55.63	–33.82*
All causes	312.50	370.30	254.64	–115.66*

[a] *Includes symptoms, signs and abnormal clinical and laboratory findings not elsewhere classified.*
* $P < 0.05$.

Discussion

One aim of this study was to examine the changes in life expectancy over time for both sexes of the Kuwaiti population. Our results indicate that life expectancy at birth has increased from 73.34 years in 1987 to 75.49 years in 2000. This gain of life expectancy (2.14 years) was mainly due to a decrease in crude death rates over the years of the study from 3.44/1000 to 2.91/1000 [*18*].

In spite of the 36.3% increase in the actual number of deaths from 1775 in the 1987 to 2420 in 2000, there was a progressive decrease in mortality rates per 100 000 Kuwaitis of both sexes during this period. This decrease can be mainly attributed to the considerable improvement of health status in the past 20 years. Economic and social transformations have led to the progressive amelioration of health problems and improvements in health services. The reduction in rates of transmitted diseases and in infant, perinatal and maternal mortality rates have also contributed [*12,18*]

According to the World Health Organization report of life expectancy for 191 countries in 2000, Japan had the highest life expectancy for both males (77.5 years) and females (84.7 years). Sierra Leone had the lowest male life expectancy (37.0 years) while Malawi had the lowest female life expectancy (37.8 years) [*19*]. Kuwait was ranked as the 19th for male and 45th for female life expectancy. Differences in life expectancy between countries can be attributed to many epidemiological and public health factors [*20,21*], including the relationship between low life expectancy and poor income [*22,23*].

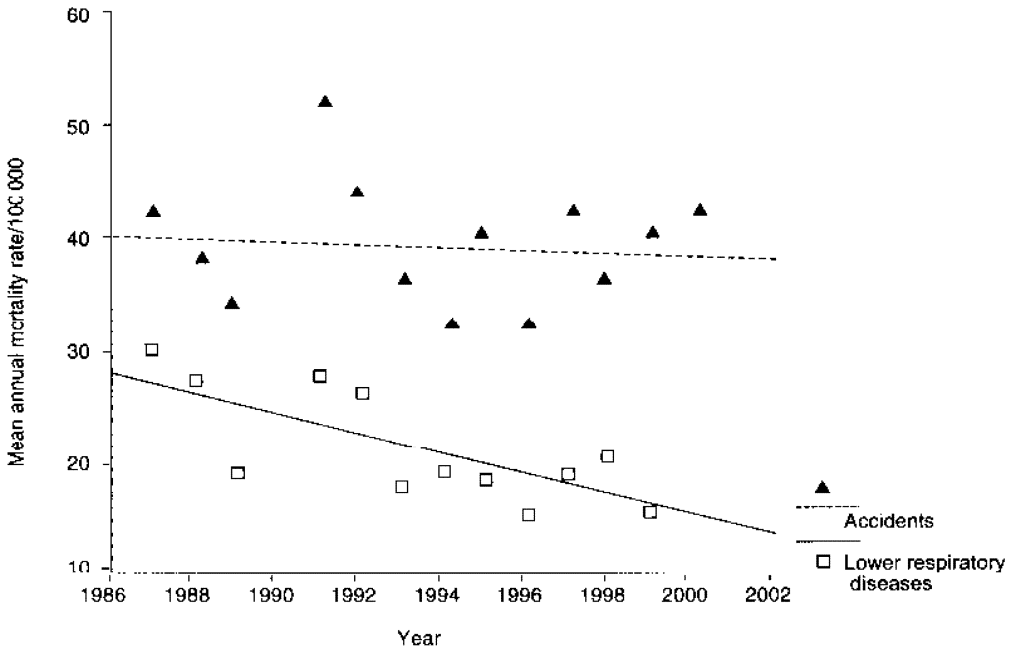

Figure 2 **Trends of cause-specific mortality rates among males in the Kuwaiti population 1987–2000**

Regarding sex differences, our results revealed that changes in life expectancy at birth were in favour of females. While the improvement of life expectancy was 3.33 years in females, it was only 1.05 years in males during the study years. The difference in life expectancy between males and females has doubled over the 13-year period (from 2.19 to 4.47 years). This gender gap in life expectancy can be attributed to increasing male mortality from ischaemic heart disease and lung cancer, presumably due the early and widespread adoption of cigarette smoking by men [*24*]. It has also been postulated that although women live longer than men in all areas the difference is most marked in poorer areas [*25*].

An additional goal of the study was to study the relationship between patterns of cause of death and life expectancy in Kuwait. Cause-specific annual mortality varied from males to females. Our study revealed that for all causes except hypertension and ill-defined conditions, males had higher mortality than females. The leading causes of death in males were ischaemic heart diseases, traffic accidents and cancer. Meanwhile cancer, ischaemic heart diseases and hypertension were more common in females. This pattern of mortality mirrors the reported process of health transition in the developing world. The ageing of the population, reductions in fertility, improved preventive and therapeutic control of infectious diseases, and more affluent lifestyle may all contribute to a decrease in communicable diseases and to an increase in degenerative and 'man-made' diseases and injuries [*1,26,27*].

Table 3 **Correlations between life expectancy at birth and cause-specific mortality rates due to leading causes of death, among males and females in the Kuwaiti population 1987–2000**

Cause of death	Overall		Male		Female	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Ischaemic heart diseases	–0.61	0.03	–0.47	0.11	–0.50	0.08
Cancer	0.40	0.18	0.27	0.38	0.30	0.32
Hypertension	0.27	0.37	0.08	0.80	0.01	0.98
Traffic accidents	–0.49	0.09	–0.62	0.02	–0.13	0.68
Pulmonary circulation and other heart diseases	0.57	0.04	0.19	0.53	0.73	0.005
Lower respiratory diseases	–0.89	< 0.001	–0.75	0.003	–0.72	0.005
Congenital anomalies	0.17	0.59	0.14	0.65	0.14	0.65
Perintal conditions	–0.52	0.07	–0.25	0.41	–0.64	0.02
Endocrine diseases	0.27	0.38	0.06	0.84	0.29	0.34
Ill-defined conditions[a]	–0.78	0.002	–0.51	0.08	–0.71	0.006
Other causes	–0.64	0.02	–0.49	0.09	–0.84	< 0.001
All causes	–0.77	0.002	–0.77	0.002	–0.77	0.002

r = *Pearson's correlation coefficient.*
[a]*Includes symptoms, signs and abnormal clinical and laboratory findings not elsewhere classified.*

Our data showed that male life expectancy was negatively correlated with death rates due to traffic accidents and lower respiratory diseases. The increase in life expectancy in males during the study years was associated with a decrease in mortality due to lower respiratory disease from 29.84/100 000 in 1987 to 16.55/100 000 in 2000. However, the mortality rate from traffic accidents did not change during the study years. This means that by lowering the death rate from accidents, life expectancy for men could be improved.

Female life expectancy was negatively correlated with mortality rates for perinatal conditions, lower respiratory diseases and ill-defined conditions and positively correlated with the death rate from pulmonary circulation and other heart diseases. All these causes of death showed decreased rates in females over the study period, except mortality from pulmonary circulation and other heart diseases, which showed an upward trend over time. Increasing death rate from pulmonary circulation and other heart diseases was compensated by falling death rates from lower respiratory diseases and perinatal conditions.

Overall, for both sexes combined, death rates due to ischaemic heart diseases and lower respiratory diseases were negatively correlated with life expectancy. The decline in mortality from these 2 causes was significantly associated with increasing life expectancy. This fits with the fact that 73% of the decline in total death rates over this time period was due to a reduction in cardiovascular disease mortality. The cause

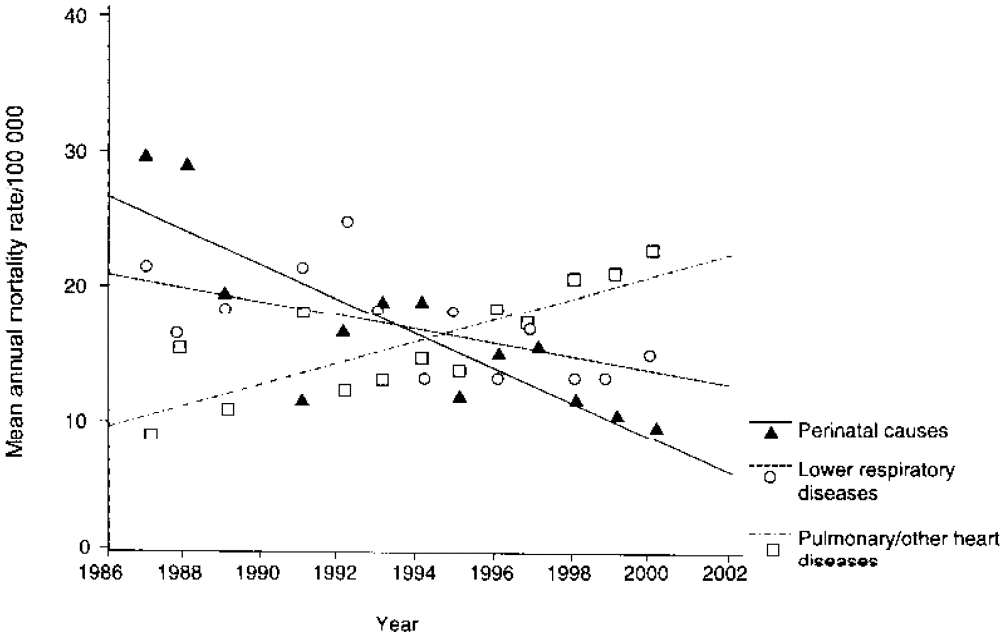

Figure 3 **Trends of cause-specific mortality rates among females in the Kuwaiti population 1987–2000**

of decline in cardiovascular disease mortality is likely to be due to a combination of factors, including improvements in medical care in the form of better diagnosis and treatment of heart disease and stroke, development of effective medications for treatment of hypertension and hypercholesterolaemia and an increase in coronary care units and in emergency medical services for heart disease and stroke [*4*].

Increased life expectancies at birth lead to population growth and an increase in the proportion of the population who are elderly. When there are more old people, *per capita* medical costs for a country will tend to be higher [*2*]. This raises the importance of defining health policies and programmes that will reduce the burden of ageing populations on society and its economy. The development of more long-term and geriatric care facilities should also be considered [*28*].

Conclusion

Life expectancy at birth has increased in the total Kuwaiti population, more among females than males. Falling death rates from ischaemic heart diseases and lower respiratory diseases were correlated with rising life expectancy. With this continuing increase in life expectancy, survival to advanced ages is much more likely, leading to problems of an ageing population that must be taken into consideration in planning the health services in Kuwait.

Table 4 **Mean annual rates of mortality from the leading causes of death among males and females in the Kuwaiti population 1987–2000**

Year[a]	Mean annual mortality rate (per 100 000)				
	Males		Females		
	Traffic accidents	Lower respiratory diseases	Pulmonary circulation and other heart diseases	Lower respiratory diseases	Perinatal conditions
1987	40.93	29.84	9.01	20.76	29.77
1988	37.48	27.19	15.42	16.17	28.96
1989	34.29	18.74	10.85	17.72	19.53
1991	51.46	27.53	18.10	21.45	11.40
1992	43.91	26.22	12.28	24.55	16.47
1993	36.27	17.68	13.09	18.08	18.70
1994	32.14	19.17	14.75	13.24	18.66
1995	39.47	18.28	13.71	17.43	11.71
1996	32.77	15.13	18.43	12.38	14.85
1997	42.80	18.69	16.67	16.93	15.35
1998	35.30	20.66	20.56	12.94	11.42
1999	39.11	15.64	21.10	13.25	10.06
2000	41.13	16.55	22.57	14.50	9.27

[a]*No data available for the year 1990.*

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Capture–recapture methods for estimation of fertility and mortality In a rural district of Turkey

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طرق الالتقاط وعودة الالتقاط لتقدير الخصوبة والوفيات في المناطق الريفية في تركيا

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الخلاصة: استخدمت هذه الدراسة طرق الالتقاط وعودة الالتقاط لمعرفة فيما إذا كانت المعلومات حول الولادات والوفيات واستخدام وسائل تنظيم الأسرة والمستمدة من مصدرين للمعطيات تقدم المعلومات ذاتها أو معلومات أكثر اكتمالاً مما هو متوافر من مصدر واحد. وقد استمدت المعلومات من خمسة مصادر مختلفة هي: مختار القرية، المتطوعون لعمل الاجتماعي الصحي، مراكز الرعاية الصحية الأولية، وحدات رعاية الأمهات والأطفال، والوحدات الإدارية المحلية (رؤساء المجموعات الصحية) في عشر قرى منتقاة في منطقة ريفية في تركيا في الفترة بين أيار/مايو وتشرين الأول/أكتوبر 1999. ورغم أن أعداد الوفيات والولادات كانت تقديرية، فلم يكن هناك أي تقدير لعدد اللواتي يستخدمن وسائل منع الحمل. وتوضح الدراسة بعض المشكلات في جمع المعطيات في نظام الترصد في تركيا وتوصي بتقوية نظام الترصد الروتيني.

ABSTRACT The study used capture–recapture methods to determine if information on births, deaths and family planning use obtained from two data sources provides the same or more complete information than that available from a single source. Five different data sources used were: village heads (*mukhtars*), community health volunteers, primary health care centres, maternal and child care units and local administrative units (health group presidencies) in 10 selected villages in a rural area of Turkey from May to October 1999. Although the numbers of deaths and births were estimated, no estimation of the number of women using any family planning method could be made. The study highlights some data collection problems of the surveillance system in Turkey and recommends that the routine surveillance systems be strengthened.

Méthodes de capture-recapture pour l'estimation de la fécondité et de la mortalité dans un district rural en Turquie

RESUME L'étude a utilisé des méthodes de capture-recapture pour déterminer si les informations concernant les naissances, les décès et le recours à la planification familiale obtenues auprès de deux sources de données fournissent les mêmes informations ou des informations plus complètes que celles provenant d'une seule source. Cinq différentes sources de données ont été utilisées : les chefs de village (*mukhtars*), les volontaires de santé communautaires, les centres de soins de santé primaires, les services de soins de santé maternelle et infantile et les administrations sanitaires locales dans 10 villages sélectionnés dans une zone rurale de la Turquie de mai à octobre 1999. Bien que le nombre de décès et de naissances ait été estimé, aucune estimation n'avait pu être faite concernant le nombre de femmes utilisant des méthodes de planification familiale. L'étude met en évidence certains problèmes liés au recueil de données du système de surveillance en Turquie et recommande que le système de surveillance systématique soit renforcé.

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Introduction

Capture–recapture methods (dual enumeration) were first developed by ecologists for estimating total animal populations and are now used in many fields of science. Although use of the methodology in human populations was first documented in the literature in 1949 by Chandra-Sekar and Deming [*1*], it was used in population censuses in 18th century France. Since then, capture–recapture has been used in the estimation of the numbers of births and deaths in India and in the population census held in the United States in 1990 [*2,3*]. The methodology can be used for estimating unknown populations in epidemiological studies; some examples include estimating prevalences of spina bifida, congenital rubella syndrome, cancer and intravenous drug use [*4–6*].

In Turkey, factors such as migration from rural to urban areas and financial and technical inadequacies in health care provision may have a negative influence on the efficiency of the surveillance systems in the health service [*7,8*]. It is a common problem in developing countries that health care staff are unaware of how and why records are completed. These factors affect the reliability of the record systems, especially in rural areas. Therefore, another practical method such as capture–recapture can be used to estimate an unknown population

The first objective of our study was to use capture recapture methods to estimate births, deaths and the frequency of family planning use in a small rural area of Turkey from May to October 1999. A second objective was to determine to what extent collection of information on vital events and family planning acceptance from two sources of data provides the same or more complete information than is available from a single source.

Methods

Background

Health services in Turkey

The organization of health care services in Turkey is relatively complex. Health services are provided by both the public and private sectors. In the public sector, the Ministry of Health (MOH) is the leading health care provider at primary, secondary and tertiary levels. Health care at primary level is mainly provided by the MOH through primary health care centres, which provide outpatient health care and primary prevention activities, and collect routine data on maternal and child care, infectious diseases and vital events [*9*]. A number of primary health care centres report to another office under the MOH, the health group presidency (HGP), which facilitates administrative activities at the district level and collects data from the health centres. Public hospitals provide secondary and tertiary health care activities. Other public institutions and private institutions also have important roles in providing health care services in Turkey.

Recording births and deaths in Turkey

In Turkey, the General Directorate of Population and Citizenship Affairs (GDPCA) of the Ministry of Interior is a centralized structure, responsible for keeping population registers such as births, deaths and other vital events. It branches into the country through the office of the district directorate of population (DDP). The DDPs keep population registers and record vital events at the district level then transfer them to the provincial offices of GDPCA. All the data are gathered in the capital office based in Ankara.

There are some differences between the birth and death registration systems in urban and rural areas in Turkey. In urban areas, as soon as a birth occurs, the birth

certificate of the newborn should be registered by the DDP at the district level. In rural areas, if the delivery occurs in hospital, the hospital should issue the birth certificate. But for a home delivery the certificate might be issued either by the nurse from the primary health care centre or by the *mukhtar* who is the village head. The *mukhtar* is also responsible for issuing death certificates when a death occurs in the village. He should then officially inform the DDP at the district level. In urban areas, municipalities issue the burial permits and report deaths to the State Institute of Statistics (SIS) [*10*].

Maternal and child health care services in Turkey

Maternal and child health care services are usually provided by primary health care centres and by maternal and child health care and family planning units (MCHU), also within the MOH. Nurses, midwives and physicians are service providers in all of these units. Midwives and nurses are the staff responsible for determining and following up women of reproductive age living in their working area, improving the use of family planning methods, identifying and following up pregnant and postpartum women and infants as well as following up newborns for the first 6 years and carrying out immunizations.

Study area and population

Polatlý is a district of Ankara province located about 76 km west of the capital Ankara. The rural part of Polatlý includes 90 villages. In recent years, migration from rural to urban areas of Polatlý has increased. Therefore, some vital statistics (crude birth rate, crude death rate, the number of women using family planning, etc.) have been underestimated in the recorded health service data [*11*].

Hacettepe Public Health Foundation has set up a project entitled "Community based reproductive health services for adolescents and adults in Polatlý district, Ankara" in 26 villages of Polatlý. The aim of the project is to increase the health status of the target population by using community participation methods. Within the project, community health volunteers who have the potential to be leaders in their own community are determined and selected from women and men in the study villages. Female health volunteers are responsible for registration of births, identification of women aged 15–49 years, registration of womens' gynaecological complaints, follow-up of pregnant/postpartum women, identification of newborns, distribution of condoms, oral contraceptives and folic acid iron deficiency pills for anaemia, and education of women about reproductive health. Male community health volunteers are responsible for informing men about reproductive health, especially about sexually transmitted diseases, distributing condoms, recording deaths and supporting the women health volunteers. Community health volunteers use data forms to record their activities in the field, including follow-up of women aged 15–49 years; follow-up of pregnancy, postpartum and newborn care; and birth and death records [*11*].

Data estimations

The current study was conducted in the region of the community participation project. Ten out of 26 villages were selected proportionally according to the population sizes of the villages. As it was planned as a method trial, the most important issue was not the representation power of the total 26 villages but obtaining the greatest coverage of the population.

Number of births

Two independent data sources were used to estimate births: the records of the HGPs and the birth records (infant determination forms) from the community health volunteers. The formula used for estimation of births for the capture–recapture method was:

$$N = \frac{(M+1)(n+1)}{(m+1)} - 1$$

where

N = the unknown total number of births
M = number of births in community health volunteer records
n = number of births in HGP records
m = number of births identified in both captures (matches).

The formulas shown below were used to calculate the variance (Var) and 95% confidence interval (CI) for the estimate of N:

$$\text{Var}(N) = M \times n(M-m)(n-m) / m^3$$

$$N = \pm 1.96 \sqrt{\text{Var}(N)}$$

For estimation of births, 5 matching criteria specific enough to define the case were recorded in each data source. The degree of matching was defined based on 5 different standards (A–E). For standard A, all the criteria should be exactly the same in both data sources. For standards B–E, the criteria were progressively reduced so that each subsequent standard required one less criterion for matching.

Number of deaths

In this study, the deaths occurring in the previous 6 months were evaluated using 2 independent data sources: death certificates registered by the *mukhtar* and death records (death determination forms) from the community health volunteers. If all the criteria in both records matched exactly, then the matching standard was accepted as standard A. For standards B–E, the criteria were progressively reduced, so that each subsequent standard required one less criterion for matching, as was done for estimating births.

Number of women using family planning

Data on the forms of community health volunteers, primary health care centres and MCHUs were used to calculate the number of women using family planning methods. The name and surname of the women were used as matching criteria.

$$N = \frac{n}{1 - \prod_{i=1}^{k}(1 - E_i)}$$

where

N = total number of women using family planning methods.
k = number of data sources used.
E_i = the probability that the event occurs in the 'i'th source.
$(1 - E_i)$ = the probability that the event does not occur in the 'i'th source.
n = $(n1 \cup n2 \cup n3) = n1 + n2 + n3 - (n1 \cap n2) - (n1 \cap n3) - (n2 \cap n3) + (n1 \cap n2 \cap n3)$
$n1$ = number of women using family planning methods in community health volunteer forms.
$n2$ = number of women using family planning methods in primary health care unit forms.
$n3$ = number of women using family planning methods in MCHU forms.

Results

In this study, 5 different data sources were used for estimating the unknown numbers of births, deaths and women using any family planning method (Table 1): *mukhtar*,

Table 1 **Data sources and sample sizes in the first capture for each data source (Polatlý, May–October, 1999)**

Variable	Data source				
	CHVs	PHCs	HGPs	*Mukhtar*	MCHUs
No. of births	10	–	17	–	–
No. of deaths	24	–	–	21	–
No. of women using family planning	382	23	–	–	5

CHV = community health volunteer.
PHC = primary health care centre.
HGP = health group presidency.
Mukhtar = *village head.*
MCHU = maternal and child health care and family planning unit.
– *indicates not recorded.*

community health volunteers, primary health care centres, maternal and child care units and health group presidencies.

Births

According to the records of the community health volunteers, 10 births occurred between May and October in the study villages. During the same time period, there were 17 births according to the records of the HGP.

The estimated number of births was 65 ± 31 if all the matching criteria were the same in both data sources. This number was found to be 32 ± 18 when 4 of 5 matching criteria were the same in both data sources. If only 1 criterion of 5 was the same, the estimated number was 21 ± 5. It was assumed that at least 3 of the criteria would define a birth in each data source. From this perspective, standard 'C' was the least accepted category (Table 2).

Deaths

There were 21 deaths recorded in the community health volunteers' forms. This number was 24 according to the registries of the Public Registration Office based on information from the village *mukhtar* between May and October 1999.

The estimated number of deaths was 109 ± 101.4 if all the matching criteria were the same in both data sources (Table 3). This number was 49 ± 16 if 5 of 6 matching criteria were matched and 27 ± 1 if 1 of 6 matching criteria was matched. The least accepted category for death numbers was the standard 'C'. However, in each category 'C', 'D' and 'E' the estimated number

Table 2 **Estimated number of births by the capture–recapture method (Polatlý, May–October 1999)**

Standard	No. of matches (out of 5)	No. of people	Estimated no. of births ± 95% CI
A	5	2	65 ± 31
B	4	5	32 ± 18
C	3	5	32 ± 18
D	2	5	32 ± 18
E	1	8	21 ± 5

Matching criteria: name, surname, name of husband, date of birth, sex of baby.
CI = confidence interval.

Table 3 **Estimated number of deaths by the capture–recapture method (Polatlý, May–October 1999)**

Standard	No. of matches (out of 6)	No. of people	Estimated no. of deaths ± 95% CI
A	6	4	109 ± 101
B	5	10	49 ± 16
C	4	19	27 + 1
D	3	19	27 ± 1
E	2	19	27 ± 1

Matching criteria: name, surname, sex, place of death, date of death, age of death.
CI = confidence interval.

was 19. In other words, there was no difference between the standards in which either 4 or 2 criteria matched. The criteria that did not differ from each other were: name, surname, sex and name of the village. The *mukhtar* reported more deaths than community health volunteers did.

Women using family planning

According to the community volunteers' records, 382 women were using any method of family planning between May and October 1999 in the study villages. This number was only 23 in primary health care unit records and 5 in the records of the MCHU. The number of women using any family planning method could not be estimated.

Discussion

Only 10 villages were included in this study, thus the study population was too small to generalize the results. The study was designed as a trial for assessing the applicability of the capture–recapture methodology in the field, which has not been commonly used before now in Turkey. The capture–recapture method is very sensitive to the matching procedure. The matching criteria developed for estimation should be specific enough to define the cases. The cut-off point for the matching criteria might change the results of the study. If the matching criteria are less strict, the estimated total number of cases is lower. For this reason, matching criteria were selected very carefully in this study. The criteria for births—women's name, surname, husband's name, date of birth and sex of baby—were descriptive enough to define a birth in Turkey.

In Turkey, there are some legal obligations in recording births and deaths. In this study, there were some problems about determining and recording these 2 vital events. Both traditionally and culturally, people give more importance to deaths than to births. As soon as a person dies, traditional ceremonies are performed in the community. This means that community health volunteers are sensitive to the occurrence of a death and therefore their death records might be more accurate than their birth records. Furthermore, the *mukhtar* is legally required to report deaths within a specified time. This is one of the most important reasons why estimation of deaths are more accurate than the estimates of births and family planning.

Although there are legal regulations about registration of births by primary health care centres, no births were registered by these centres. This is why we used the HGP records for estimation of birth numbers.

One of the basic assumptions of the capture–recapture method is that the study population should be closed [5]. In our study, the study region is very close to the capital and the migration rate is very high in the area. In addition, a number of people live in the district centre in winter and re-

turn to their villages for the agricultural activities in summer. These factors might have weakened the property of being a closed population and affected our estimations.

Another key assumption for applying capture–recapture is that the data sources should be independent of one another [*12*]. In this project, we should take into consideration that community health volunteers might refer people who are pregnant or have health problems to the health centres. In this situation, a positive dependence might have caused a lower estimate of '*N*' than the expected values. Nevertheless, the prevalence of referring people to health centres was 4.3% among community health volunteers [*11*]. Therefore, the estimation of birth numbers might be lower than the expected number.

The number of women using any family planning method could not be estimated. The capture–recapture method has usually been used for estimating rare and diagnosable diseases such as birth defects and spina bifida [*2,13*]. In the case of family planning, however, there is the problem that couples may change their family planning method over time. For example, a woman using an intrauterine device at the beginning of the study might have changed this method and begun to use oral contraceptives at the end of the study period. There was also the problem that data sources for family planning method use are widely dispersed and not coordinated within the area. The private sector plays an important role in providing family planning services and it was impossible to match the records of the public and the private institutions. For this reason, estimating the number of women using any family planning method was not possible.

The capture–recapture method has been used extensively for estimating the size of animal populations where marking of animals after capture lessens the possibility of errors. For epidemiological studies with humans, the estimation is more complicated.

In conclusion, it can be said that the method of verification of the number of vital events as used in this project has been pre-tested. We hope that this study highlights some data collection problems of the surveillance system in Turkey. By using capture–recapture, some health measures, especially births and deaths, could be estimated in a rural area in Turkey, but the matching criteria, distribution of the health institutions and other limitations might affect the estimates of the real numbers of deaths and births.

It should be remembered that the capture–recapture method is an alternative method for the estimation of unknown events. The major approach should be to strengthen the routine surveillance systems in developing countries.

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Adolescents' use of health services in Alexandria, Egypt: association with mental health problems

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استخدام المراهقين للخدمات الصحية في الإسكندرية بمصر، وارتباطه بمشكلات الصحة النفسية

مصطفى عفيفي

الخلاصة. قيّمت الدراسة نماذج استخدام الخدمات الصحية من قِبَل المراهقين وارتباط ذلك بمشكلات الصحة النفسية في الإسكندرية، مصر. شملت الدراسة عينة عشوائية متعددة الطبقات تضمّ 1577 من طلاب المدارس الذين تتراوح أعمارهم بين 14 و19 عاماً، استكملوا ملء استمارات تستكمل ذاتياً حول الوضع الصحي والديموغرافي، واستخدام الخدمات الصحية في السنة الماضية، إلى جانب قائمة جرد خاصة بالاكتئاب لدى الأطفال ومقياس العدوانية الذي يستكمل ذاتياً من قِبَل المراهقين. وقد أبلغ 97.1٪ من الطلاب أنهم استخدموا العيادات الصحية المدرسية مرّةً أو أكثر من مرة في السنة التي سبقت الدراسة؛ وأن 93.7٪ منهم قد استخدم مراكز الرعاية الصحية الأولية وأن 16.8٪ منهم قد استخدم خدمات الصحة النفسية وأن 13.6٪ منهم قد استخدم الخدمات الصحية الأخرى. وقد ترابطت المعاناة من مرض عضوي في السنة السابقة (نسبة الأرجحية = 1.8) والمعاناة من أعراض الاكتئاب (نسبة الأرجحية = 2.93) والمعاناة من أعراض عدوانية (نسبة الأرجحية = 5.53) ترابطاً يُعتَدُّ به مع تكرار استخدام الخدمات الصحية (أكثر من 4 زيارات في السنة).

ABSTRACT The study assessed patterns of health service use by adolescents and the association with mental health problems in Alexandria, Egypt. A systematic stratified random sample of 1577 school students aged 14–19 years completed a self-report questionnaire about demographic and health status, use of health services in the previous year, and the Children's Depression Inventory and the Adolescent Self-Report Aggression Scale. Overall, 97.1% of students reported using school health clinics once or more in the year before the study, 93.7% primary health centres, 16.8% mental health services and 13.6% other health services. A history of organic illness in the previous year (OR = 1.80), having depressive symptoms (OR = 2.93) and having aggressive symptoms (OR = 5.53) were significantly associated with frequent use of health services (≥ 4 visits/year).

L'utilisation des services de santé par les adolescents à Alexandrie (Egypte) : association avec les problèmes de santé mentale

RESUME L'étude a analysé les schémas d'utilisation des services de santé par les adolescents et l'association avec les problèmes de santé mentale à Alexandrie (Egypte). Un échantillon aléatoire systématique stratifié de 1577 lycéens âgés de 14 à 19 ans a rempli un questionnaire d'autoévaluation sur la situation démographique et l'état de santé, l'utilisation des services de santé au cours de l'année précédente, ainsi que l'inventaire de dépression chez l'enfant « Children's Depression Inventory » et l'échelle d'évaluation « Adolescent Self-Report Aggression Scale ». De manière générale, 97,1 % des lycéens signalaient avoir utilisé les centres médicaux scolaires une fois ou plus au cours de l'année précédant l'étude, 93,7 % les centres de santé primaires, 16,8 % les services de santé mentale et 13,6 % d'autres services de santé. Des antécédents de maladies organiques au cours de l'année précédente (OR = 1,80), le fait d'avoir des symptômes dépressifs (OR = 2,93) et des symptômes agressifs (OR = 5,53) étaient associés de manière significative avec l'utilisation fréquente des services de santé (≥ 4 visites/an).

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Introduction

Many teenagers depend on multiple sources of health care, and school personnel are important sources of health-related information [*1*]. The expansion of school-based health services since the early 1970s is a specific response to meet the medical needs of youth [*2*]. Adolescents are often vulnerable to particular health risks and face multiple barriers to accessing health care. School-based clinics represent an alternative model of health care that responds to the unique health issues of adolescents by offering preventive, comprehensive services, including mental health and other sensitive services [*3*].

According to Parker and Roy, the chances of becoming depressed in adolescence increased in the latter part of the 20th century and the onset of depression is observed at a younger age than before [*4*]. They added that adolescent depression is manifested either as melancholic symptoms or as irritability and anger.

The association between adolescents' use of health services and their mental health status is controversial. Pastore et al. found that the average users, frequent users and non-users of a school-based health centre did not differ in the mental health problems measured in their study [*5*], whereas others have found that depressive symptoms were associated with increased service utilization [*6*].

The aim of this study was to assess the patterns of use of different health services by secondary school adolescents aged 14–19 years in Alexandria, Egypt, and to examine the association between utilization and adolescents' most common mental health problems, namely depressive and aggressive symptoms.

Methods

Subjects

In a cross-sectional school-based study in 1996, 1577 students of both sexes were selected by systematic stratified random sampling from 12 secondary schools representing the 6 districts of Alexandria, Egypt.

Research instruments

A self-report questionnaire was designed by the researcher to be completed by secondary school students. It included demographic and personal data such as age, sex, birth order, number of friends, number of family members, degree of family coherence, satisfaction with school, history of dropping a class and history of organic illness in the year prior to the study. Respondents were asked how many times they had visited primary care centres, school health clinics, mental health services or other health services (e.g. private clinics, general or military hospitals) in the previous year. The questionnaire also included a question on cigarette smoking (never-, ever-, ex- or current smoker). The Arabic Social Class Scale [*7*] was applied to participants' responses. This uses the degree of parents' education, occupation and crowding index (number of family members divided by number of closed rooms in the accommodation).

To obtain a history of emotional disturbance in the previous year, students were asked if they had had any emotional or psychological problems that made them consult a doctor in the year before the survey. Two further tools were used to assess adolescent depression, which usually manifests either as melancholic symptoms or as irritability and hostility [*4*]:

- The Arabic version of the 27-item Children's Depression Inventory [*8*] covers an array of depressive symptoms. Each of the 27 items of the inventory assesses 1 symptom by presenting 3 choices arranged from 0 to 2 in the direction of increasing psychopathology and total score ranges from 0 to 54. The inventory's test–retest reliability was 0.9 and the cut-off score was 24, which means that adolescents having this score or above were considered to have depressive symptoms.
- The Arabic version of the 14-item Adolescent Self-Report Aggression Scale [*9*], covers an array of aggressive symptoms. Each of the 14 items of the inventory assesses 1 symptom by presenting 3 choices arranged from 0 to 2 in the direction of increasing psychopathology and total score ranged from 0 to 28. The scale's split-half reliability was 0.76 for boys and 0.65 for girls and the cut-off score was 18 for both sexes, which means that adolescents having this score or above were considered to have aggressive symptoms.

It took around 40 minutes for the students to complete the questionnaire and other scales during a class session.

Data processing and statistical analysis

Data coding, entry and management was made using the *Epi-Info* statistical program [*10*], followed by data analysis using *SPSS*, version 6 for Windows [*11*].

Ethical issues

To preserve confidentiality, no direct or indirect identification of respondents was used. The adolescent respondents gave their verbal consent to participate in the study. Pre-testing of the questionnaire was conducted on 100 students of both sexes before running the study.

Results

From the 12 secondary schools in Alexandria, 1577 adolescent students (49.8% male) competed the questionnaires and self-report scales. Their ages ranged from 14 to 19 years with a mean of 15.8 years (SD 1.3).

Table 1 shows the different utilization patterns of health services by adolescents. School health clinics had the highest rate of utilization; 97.1% of students used this facility once or more in the year before the

Table 1 **Utilization of different health services by 1577 adolescents in the year before the study**

No. of visits/year	Respondents visiting: Primary care centres		School health clinics		Mental health services		Other health services	
	No.	%	No.	%	No.	%	No.	%
0	100	6.3	45	2.9	1312	83.2	1362	86.4
1	920	58.3	842	53.4	265	16.8	215	13.6
2	511	32.4	606	38.4	0	0	0	0
3	28	1.8	50	3.2	0	0	0	0
4	18	1.1	34	2.2	0	0	0	0

study, compared with 93.7% for primary health centres, 16.8% for mental health services and 13.6% for other health services. Only 17.0% of the sample used every type of health service studied at least once in the year before the study. About 57% of the sample reported having used more than 1 source of care in the year.

The median number of visits to any of the categories of health services was 3 and the range was 0–10 visits. Adolescents were grouped into frequent users (4 or more visits/year) and infrequent users (0–3 visits/year). Overall, 467 (29.6%) of the sample were frequent users of health services. The percentage of adolescents frequently using health services distributed according to their age is shown in Figure 1. No significant difference in frequent utilization was noticed between different age groups.

The respondents' scores on the depression scale ranged from 1 to 43, with a mean of 13.2 (SD 6.17). After applying the cut-off score of 24, 69 (4.4%) of the sample were considered to have depressive symptoms. The adolescents' scores on the aggression scale ranged from 0 to 26, with a mean of 11 (SD 4.4). Applying the cut-off score of 18, 78 (4.9%) of the sample were considered to have aggressive symptoms.

Overall, 190 (12.0%) students reported a history of emotional disturbance. There were significant associations between having a history of emotional disturbance and having depressive symptoms and aggressive symptoms (Table 2). Such association was examined before entering the history of emotional disturbance in the logistic regression model as a confounder to depressive or aggressive symptoms.

Adolescents who reported frequent use of primary health centres or school health clinics were more likely to have aggressive and depressive symptoms than infrequent users. The correlation coefficient between number of visits to health facilities and depression and aggression scores respectively were 0.14 and 0.139 ($P < 0.001$). The coefficient correlation between depression and aggression scores was 0.428 ($P < 0.001$) (data not shown in tables).

Table 3 shows the variables that were significantly positively associated with frequent use of health facilities in a multiple logistic regression model. These were: a

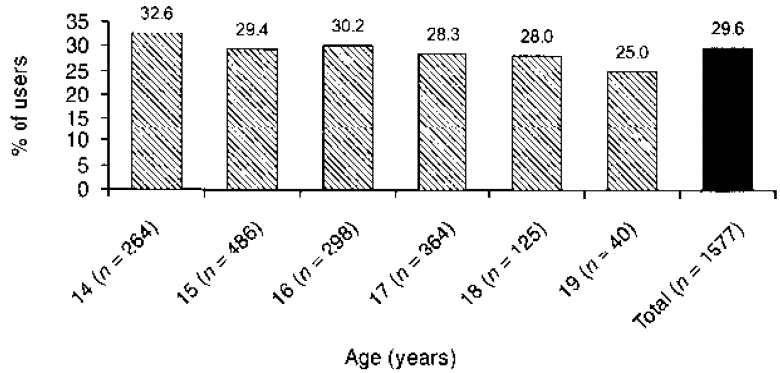

Figure 1 **Distribution of frequent users of health services by age (*n* = total number of respondents in each age group)**

Table 2 **Association of depressive and aggressive symptoms with adolescents' history of emotional disturbance in the year before the study**

Symptoms	History of emotional disturbance				Total	
	Yes		No			
	No.	%	No.	%	No.	%
Depression						
No	158	10.5	1350	89.5	1508	100.0
Yes	32	46.4	37	53.6	69	100.0
	Mantel–Haenszel $\chi^2 = 3.99$, $P = 0.046$					
Aggression						
No	175	11.7	1324	88.3	1499	100.0
Yes	15	19.2	63	80.8	78	100.0
	Mantel–Haenszel $\chi^2 = 80.19$, $P < 0.001$					
Total	190	12.0	1387	88.0	1577	100.0

history of organic illness in the year before the study (odds ratio (OR) = 1.80), having depressive symptoms (OR = 2.93) and having aggressive symptoms (OR = 5.53). Birth order (oldest child), history of dropping a class at school and history of emotional disturbance were significant negatively associated variables. Age, sex, social class score, satisfaction with school, number of friends, and family coherence were not significantly associated with frequent usage.

Discussion

The association between adolescents' psychiatric symptoms and the pattern of utilization of health services has to our knowledge never been studied before in Alexandria. However, the study relied only on self-report questionnaires that, despite being easy to apply, might yield inaccurate responses. The study should therefore be viewed as preliminary.

The results of the current study noted under-utilization of mental health services by adolescents. Cohen and Hesselbart mentioned that the under-use of services of middle income and rural children might reasonably be ascribed to access problems [*12*]. Kulka et al. indicated that people of lower socioeconomic status have a lower rate of mental health service utilization [*13*]. This is inconsistent with our findings, which showed no significant association between utilization of all types of health facility and the social class of the study sample. We also showed that there was no significant association between frequency of utilization of health facilities and the adolescents' sex, which is similar to what Thompson et al. found in their study [*14*]. Adolescents' age also had no association with utilization. Ryan et al. mentioned that age and sex were more important in predicting use of illness-related care than routine use of medical care [*15*].

The under-utilization of mental health services could be a resort to avoid the stigma of being mentally ill, lack of confidentiality on visiting the crowded general hospitals or because health insurance for

Table 3 **Independent variables associated with frequent use of health services (4+ visits/year) by 467 adolescents in stepwise logistic regression analysis**

Variable	OR	95% CI	*P*-value
Aggressive symptoms	5.53	3.33–9.19	< 0.001
Depressive symptoms	2.94	1.71–5.06	< 0.001
History of organic disease	1.80	1.18–2.77	< 0.001
Higher birth order	0.92	0.85–0.99	0.02
History of dropping a class	0.70	0.51–0.97	0.03
History of emotional disturbance	0.60	0.41–0.89	< 0.001

OR = odds ratio.
CI = confidence interval.

adolescents is not extended to private clinics. Ziv et al. found that adolescents underutilized physician offices and they were more likely to be uninsured than other age groups [*16*]. Ryan et al. concluded that having a regular source of care and health insurance were strong predictors of using medical care [*15*].

School health clinics were the most highly utilized facility in the current study. Only 2.9% of the sample did not use this facility in the year before the study because all school students in Alexandria, Egypt, are medically insured within the school health clinics, school health polyclinics and school health hospitals. That was in comparison with 6.3% who had not used primary health centres, 83.2% not using mental health services, or 86.4% not using other health services. School-based clinics staffed by an interdisciplinary team of health care professionals are among the pioneering efforts that address both health and education of adolescents [*17*]. School health clinics can also increase students' health knowledge and access to health-related services [*18*].

Adolescents who reported more frequent use of primary health centres or school health clinics were more likely to have aggressive and depressive symptoms than others. Consistent evidence has shown that a substantial proportion of people with emotional problems and mental disorders are treated in the physical health sectors [*19,20*]. Alegria et al. found that subjects with high scores for psychiatric symptoms were also found to make more use of general health services [*21*]. Johnson et al. found that major depression and depressive symptoms were associated with increased service utilization [*6*]. Hansson et al. also concluded that patients in contact with psychiatric services were more frequent users of other medical services [*22*]. In contrast, Katerndahl and Realini concluded that subjects with panic symptoms reported higher rates of health care utiliza-

tion despite having less insurance coverage and experiencing barriers to access [*23*]. The high utilization pattern of general health facilities by adolescents with depressive or aggressive symptoms in the current study could be explained if adolescents increased their utilization of general heath services when psychiatric services utilization was reduced. It could also be assumed that somatization occurs in response to stress for the predisposed personality, which in turn would increase utilization of the general health services.

Conclusion and recommendations

Depressive or aggressive symptoms were associated with increased utilization of school health clinics and primary health centre facilities. To achieve the goal of mental health care for all Africans, psychiatry should be included in the primary health care programme. Physicians actually provide services to more patients with depressive symptoms than to patients with formally defined conditions of depressive disorders. For those working with adolescents, it is important to take into account all aspects of the individual's world. Cumulative life stress, friendship networks and self-esteem should be considered when working with non-clinical as well as clinical populations.

We recommend further study using standardized personal interviews to establish a psychiatric diagnosis for adolescents in addition to reviewing utilization patterns from health facility records instead of self-reported use of health services.

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Practice and awareness of health risk behaviour among Egyptian university students

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الوعي بالسلوك الصحي المحفوف بالمخاطر وممارسته لدى طلاب جامعيين في مصر

أماني رفعت

الخلاصة: يساهم السلوك الصحي المحفوف بالمخاطر بشكل واضح في الوفيات في الوقت الحاضر. وقد أجريت دراسة وصفية مستعرضة لتقييم الوعي في الوقت الراهن والممارسات للسلوك الصحي المحفوف بالمخاطر لدى طلاب جامعيين في مصر. ولم يكن السلوك المحفوف بالخطر ممارساً إلا لدى 121 طالباً (18٪)، وبدت العلاقة إيجابية مع تدخين التبغ وتعاطي الكحول والمخدرات واقتراف السلوكيات الجنسية المحفوفة بالمخاطر. وقد أظهر التحليل بالتحوف المتعدد أن المحددات الرئيسية للسلوك المحفوف بالمخاطر هي الجنس المذكر، والتقدم في العمر، والتمتع بمخصصات مالية كبيرة، وعدم الاكتراث بالمخاطر. وكانت المعلومات حول الإيدز مفقودة لدى 30٪ من الطلاب، كما أن معظم الطلاب الذين اقترفوا ممارسات جنسية لم يستعملوا موانع الحمل أو أي وسيلة للوقاية من العدوى المنقولة جنسياً. وكان المصدر الرئيسي للمعارف هو وسائل الإعلام (38٪) والرملاء (30٪).

ABSTRACT Health risk behaviour contributes markedly to today's major killers. A descriptive cross-sectional study was conducted to assess current awareness and practice of health risk behaviour among Egyptian university students. Only 121 students (18%) were practising risky behaviour. Tobacco use, alcohol and drugs use and risky sexual behaviour were positively correlated. Multiple regression analysis revealed that the main determinants of risky behaviour were being a male, of older age, having a high allowance and having no attention to danger. About 30% of students lacked adequate knowledge on AIDS. Most of those who had sexual relationships did not use contraceptives or any method of protection from sexually transmitted infection. Main sources of knowledge were the media (38%) then peers (30%).

Pratique et connaissance des comportements à risque pour la santé chez les étudiants égyptiens

RESUME Les comportements à risque pour la santé contribuent sensiblement aux causes principales de décès actuelles. Une étude transversale descriptive a été réalisée pour évaluer la connaissance et la pratique actuelles des comportements à risque pour la santé chez les étudiants égyptiens. Seuls 121 étudiants (18 %) avaient un comportement à risque. La consommation de tabac, d'alcool et de drogues et le comportement sexuel à risque étaient corrélés positivement. L'analyse de régression multiple a révélé que les principaux déterminants des comportements à risque étaient le fait d'être un garçon, d'être plus âgé, d'avoir une allocation d'études élevée et de ne porter aucune attention au danger. Environ 30 % des étudiants n'avaient pas les connaissances suffisantes sur le SIDA. La plupart de ceux qui avaient des relations sexuelles n'utilisaient pas de contraceptifs ou d'autres moyens de protection contre les infections sexuellement transmissibles. Les médias (38 %) et les pairs (30 %) constituaient les principales sources de connaissances.

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Introduction

Today, the health of young people—and the adults they will become—is critically linked to the health-related behaviour they choose to adopt.

Young adults aged 15–24 constituted about 20% of the Egyptian population in 2000 [*1*], and university students, whose health and productivity are determined by their current behaviour, are the future highly educated work force. This study aims to evaluate the awareness and practice of health risk behaviour among Egyptian university students, focusing on the use of tobacco, alcohol and drugs and unsafe sexual practices.

Surveys among youth and young adults (10–24 years) in the United States of America during 1991–1999 show the trends for health risk behaviour. The improved behaviour types included sexual practices. On the other hand, tobacco, alcohol and drug use worsened [*2*].

Kann et al. showed that 35% of high school students in the United States of America had smoked cigarettes during the 30 days preceding the survey [*3*].

In the Middle East region, however, studies have given a different picture. In Egypt, a study done on students at Suez Canal University found that prevalence of current smoking was much lower, 12.7% in general, and commoner in males and those of older age. Most smokers lived in urban areas, lived with smokers, and used their pocket money to buy cigarettes [*4*]. About one third smoked the water pipe too. A study done in Syria reported the prevalence of current smoking among high school adolescents to be 16% for boys and 7% for girls. Smoking was strongly associated with parental and sibling smoking; high school students from families with parents and/or siblings who smoked were 4.4 times more likely to be current smokers than those from non-smoking families [*5*]. In a Turkish study there was evidence for the effects of school type, a smoking-related attitude, presence of a stepmother, father's use of alcohol, sister and brother who smoke, student's alcohol use, and participation in art activities as determinants of smoking among middle and high school students in Ankara [*6*].

Methods

A descriptive cross-sectional study was carried out on students at Suez Canal University main campus in Ismailia. The schools were stratified according to type of study: health, mathematical, scientific and human studies. One school was randomly selected from each study type. For the purposes of future intervention, students were randomly allocated from student lists of the first and second years of the 4 selected schools: medicine, computer and informatics, agriculture and education.

A questionnaire was designed, adapted from the questionnaire of the Centers for Disease Control and Prevention 1999 risk behaviour survey [*7*] with cultural modifications. Questions were added regarding source of knowledge and awareness of the danger of any of the types of risky behaviour. Awareness of the dangers of each practice was indicated on a 5-point scale (0–4), where 0 indicated not at all and 4 indicated strongly aware.

The questionnaire was tested on 87 volunteer students to check its validity and to estimate the sample size. Necessary changes were made after testing.

There were 30 063 students enrolled for the academic year. The sample size at 95% CI, was estimated using *Epi-Info* (version 6.4d) to be 655 students (721 stu-

dents adjusting for drop-out of 10%). Questionnaires were printed, enveloped and distributed via the junior faculty and students' affairs staff of the selected schools according to their weighting: 180 for medicine, 240 for agriculture, 250 for education and 51 for computers and informatics.

Epi-Info was used primarily for data entry. Data was analysed using *SPSS*, version 9.0. Sociodemographic characteristics were described. Risky behaviour in each domain was identified as the following:

- Tobacco use: current smokers of cigarettes or water pipes or others,
- Alcohol and substance use: current users of alcohol or other substances,
- Unsafe sexual behaviour: having multiple partners, or sex without contraceptives or protection from sexually transmitted infection (STI).

The different types of behaviour were analysed in relation to the students' sociodemographic characteristics, attention to risky behaviour and source of information using test of significance and odds ratio. A new variable was computed from practice of risky behaviour and examined by multiple regression analysis to identify its determinants.

As the study deals with young adults, certain ethical steps were implemented:

- Formal approval was obtained from the vice president of the university for education and student affairs.
- Approval and support from deans and vice deans of the selected schools were also obtained.
- Junior faculty and students' affairs employees who shared in distributing the questionnaires were introduced to the aim of the study and the importance of confidentiality for students who responded to the questionnaire.
- All the questionnaires were enveloped to preserve the confidentiality of the respondents
- The questionnaire began with a description of the study and a clear explanation of its purpose.

Results

Of the 721 questionnaires sent out, 687 were returned (95% response rate), 172 (25.0%) from the school of medicine, 48 (7.0%) from informatics, 237 (34.5%) from education and 230 (33.9%) from agriculture.

Sociodemographic characteristics

The median age of the students was 18 years; 59.0% were female, 71.0% urban residents and 98.8% never married. There were 3 formally married female students, while 5 male students stated that they were *orfi* married (not officially registered). Many of the students' parents were educated up to university degree level (50.6 % of fathers and 34.5% of mothers) while only 12.1% of fathers and 25.7% of mothers were uneducated. The median monthly allowance was 90 Egyptian pounds (mode 100 Egyptian pounds) and 121 students (17.7%) were working in addition to their study (Table 1).

The majority (58.0%) of students had a moderate awareness of the dangers of risky health behaviour while 25.9% had no awareness at all. The main source of information was the media (37.5%) followed by friends (29.8%).

Tobacco use

One third of the students in the study had ever smoked cigarettes with a median age of starting smoking of 15 years. Current smokers constituted 12.2% of the stu-

Table 1 **Sociodemographic characteristics of students practising different types of behaviour**

Characteristic	Tobacco use (n = 108)		Alcohol and drugs (n = 36)		Sexual behaviour (n = 27)		Total (N = 687)
	%	OR	%	OR	%	OR	%
Age (years)							
≤ 18	27.8	1.0	28.9	1.0	25.9	1.0	54.7
≥ 19	72.2**	3.9	71.1*	3.5	74.1*	3.6	45.3
Sex							
Female	11.1	1.0	18.8	1.0	18.5	1.0	59.0
Male	88.9**	17.0	81.2*	6.5	81.5**	6.8	41.0
Residence							
Rural	25.9	NA	22.3	NA	15.4	NA	29.0
Urban	74.1	NA	77.7	NA	84.6	NA	71.0
Marital status							
Single	99.1	NA	98.9	NA	92.6	1.0	98.8
Married	0.9	NA	1.1	NA	7.4*	8.7	1.2
Work							
No	62.9	1.0	57.6	1.0	46.2	1.0	82.3
Yes	37.1**	3.6	42.4**	3.7	53.8**	6.0	17.7
Allowance (Egyptian pounds/month)							
≤ 100	50.0	1.0	52.8	1.0	59.3	NA	73.1
> 100	50.0**	3.4	47.2*	2.6	40.7	NA	26.9
Father's education							
Uneducated	8.6	NA	12.5	NA	11.1	NA	12.1
Primary	9.5	NA	5.7	NA	7.4	NA	13.4
Preparatory	8.6	NA	6.8	NA	7.4	NA	6.1
Secondary	16.2	NA	14.8	NA	18.5	NA	17.9
University	57.1	NA	60.2	NA	55.6	NA	50.6
Mother's education							
Uneducated	23.6	NA	22.0	NA	11.1	NA	25.7
Primary	8.5	NA	7.7	NA	22.2	NA	10.3
Preparatory	6.6	NA	7.7	NA	3.7	NA	5.6
Secondary	26.4	NA	24.2	NA	33.3	NA	23.9
University	34.9	NA	38.5	NA	29.6	NA	34.5
School of study							
Medicine	20.4	1.0	22.2	1.0	7.4	1.0	25.0
Informatics	4.6	0.8	5.6	0.9	3.7	1.8	7.0
Education	13.9	0.5	5.6	0.2	7.4	0.7	34.5
Agriculture	61.1**	2.7	66.7**	2.4	81.5**	9.0	33.5
Attention to risk							
None (0)	35.5*	1.5	36.1	NA	37.0	NA	25.9
Moderate (1–2)	48.6	1.0	55.6	NA	48.1	NA	58.0
High (3–4)	15.9	1.0	8.3	NA	14.8	NA	16.1

Table 1 **Sociodemographic characteristics of students practising different types of behaviour** *(concluded)*

Characteristic	Tobacco use (*n* = 108)		Alcohol and drugs (*n* = 36)		Sexual behaviour (*n* = 27)		Total (*N* = 687)
	%	OR	%	OR	%	OR	%
Source of knowledge							
Family	6.3	1.0	0.0	NA	8.7	NA	19.1
Study	12.5	3.1	10.0	NA	0.0	NA	13.6
Friends	48.8**	6.2	46.7	NA	47.8	NA	29.8
Media	32.5	3.0	43.3	NA	43.5	NA	37.5
Total	100.0	15.7	36.0	5.2	100.0	3.9	100.0

**Significant at P < 0.05.*
***Significant at P < 0.001.*
OR = odds ratio.
NA = not available.

dents, with those smoking daily consuming 600 cigarettes per month (mode). The majority of smokers had tried to quit. About one fifth (20.4%) had ever tried smoking water pipes while only 10.9% were currently smoking, mode 1 time in the previous month. Water pipe use in cigarette smokers was 72%. About 3% had tried other tobacco products, mainly the pipe, mode 1 time in the previous month. The students had high awareness of the dangers of smoking both cigarettes and water pipes (Table 2).

Alcohol and substance use

Alcohol was ever tried by 14.4% of students questioned, median age 17 years for first use. Currently only 4.1% were drinking alcohol, mode 1 time in the previous month. Awareness of the dangers was very high.

Marijuana was ever tried by 6.8% of subjects, with median age 18 years for first use. At the time of the study, only 2.5% were using it, mode 1 time in the previous month. Seven students (1.0%) ever tried heroin at median age for first use of 13 years. Only 4 were still using it, mode 2 times in the previous month. Only 8 students ever used intravenous drug injection at median age of 13 years for first use and only 3 students were still using it daily. Ever using stimulating drugs was reported by 9.3%, while 18.2% ever sniffed substances such as benzene, paints and sprays. There was very high awareness regarding the danger of all drugs among students (Table 3).

Unsafe sexual practices

About 10% of the subjects ever had sex, median age for starting sex 16 years. Only 4.8% had sex in the previous 3 months, mode 1 partner. One third of them did not use any contraception and 61.5% did nothing to protect themselves from STI. A quarter of the students who had sex in the previous 3 months used coitus interruptus as a method of contraception. About one third did not have enough knowledge on AIDS. Attention to the danger of having multiple partners or unprotected sex was very high (Table 4).

Table 2 **Health risk behaviour in regard to tobacco use, *N* = 687**

Practice	No.	%
Ever smoked cigarettes	209	30.4
Current smokers	84	12.2
Ever smoked water pipe	140	20.4
Current smokers of water pipe	75	10.9
Current smokers of both cigarettes and water pipes	54	7.9
Tried to quit smoking (n = 84)	59	70.2
Where cigarettes were obtained (n = 80)		
Bought them	54	67.5
Another person gave them to me	21	26.3
Another person bought them for me	1	1.3
Took them from a person without his notice	4	5.0
Other tobacco-related practices		
Snuff	9	1.3
Pipe	10	1.5
Cigar	1	0.1

	Median	Mode
Smoking prevalence		
Age of starting smoking (years)	15	17
Days cigarettes smoked (in the previous month)	30	30
Number of cigarettes smoked (in the previous month)	90	600
Days water pipe smoked (in the previous month)	4	1
Days of other tobacco use (in the previous month)	2	1
Awareness of the dangers of:		
Smoking	4	4
Water pipe	4	4

Table 3 **Health risk behaviour in regard to alcohol and substances use, *N* = 687**

Practice	No.	%
Ever tried alcohol	99	14.4
Current alcohol consumer	28	4.1
Ever tried marijuana	47	6.8
Current user of marijuana	17	2.5
Ever tried heroin	7	1.0
Current heroin user	4	0.6
Ever use of intravenous drug injection	8	1.2
Current use of intravenous drug injection	3	0.4
Ever used stimulating drugs	63	9.2
Ever sniffed spray, benzene or paint	125	18.2
Ever driven a car or motorcycle under alcohol or substance influence (n = 450)	16	3.6

	Median	Mode
Age of first drinking alcohol (years)	17	18
Days of alcohol consumption[a]	2	1
Age of first marijuana use (years)	18	18
Days of marijuana use[a]	3	1
Age of first heroin use (years)	13	13
Days of heroin use[a]	2	2
Age of first use of intravenous drugs (years)	13	13
Days of intravenous drugs use[a]	30	30
Awareness of the dangers of:		
Alcohol	4	4
Marijuana	4	4
Heroin	4	4
Intravenous drug injection	4	4
Stimulants	4	4

N = *687.*
[a] *In previous month*

Table 4 **Health risk behaviour in regard to sexual practices, *N* = 687**

Practice	No.	%
Ever had sex	68	9.9
Had sexual relations in the previous 3 months	33	4.8
Had alcohol or drugs before sex the last time (*n* = 33)	10	30.3
Has knowledge on AIDS	454	66.1
Used contraceptive in the last sexual encounter (n = 32)		
Nothing	12	37.5
Pills	3	9.4
Condom	5	15.6
Cream/gel	2	6.3
Coitus interruptus	8	25.0
Others	1	3.1
Not sure	1	3.1
Used protection against sexually transmitted infection in the last sexual relationship (n = 26)		
Nothing	16	61.5
Condom	5	19.2
Other	4	15.4
Not sure	1	3.8
	Median	**Mode**
Age of starting sex (years)	16	17
Number of sexual partners in previous 3 months	2	1
Awareness of the dangers of:		
Having multiple sex partners	4	4
Having sex without protection	4	4

Risky behaviour

The risky behaviour is summarized in Table 1, which shows that:

- Tobacco current users constituted 16% of the students. Tobacco use was higher among older students (OR: 3.9, 95% CI: 2.5–6.0); males (OR: 17.0, 95% CI: 9.0–31.5); those with higher monthly allowance (OR: 3.4, 95% CI 2.2–5.2); those who were working (OR: 3.6, 95% CI: 2.3–5.6); students of agriculture (OR: 2.7, 95% CI: 1.6–4.8); those paying no attention to risky health behaviour (OR: 1.8, 95% CI: 1.1–3.0) and those getting their information from friends (OR: 6.3, 95% CI: 2.3–18.9).
- Current users of alcohol and other drugs comprised 5% of the students, more among the older students (OR: 3.5, 95% CI: 1.6–7.9); males (OR: 6.5, 95% CI: 2.7–16.6); those having higher allowance (OR: 6.2, 95% CI: 1.7–23.1); those who were working (OR: 3.7, 95% CI: 1.7–8.1); and students of agriculture (OR: 2.4, 95% CI: 1.1–6.0).
- Sexual risky behaviour was reported by only 4% of the students. It was more prevalent in older students (OR: 3.6, 95% CI: 1.5–8.7); male students (OR: 6.8, 95% CI: 2.5–18.1); *orfi* married (OR: 8.7, 95% CI: 1.7–45.4); and students of agriculture (OR: 9.0, 95% CI: 2.0–56.0).

As shown in Table 5, there is a statistically significant correlation between the 3 types of risky behaviour. Only 121 subjects (17.6%) were practising risky behaviour. Multiple regression analysis (Table 6) revealed that the main determinants of risky behaviour were being male (OR: 9.7), older in age (OR: 2.3), having a high allowance (OR: 1.7) and paying no attention to danger (OR: 1.5).

Discussion

Health risk behaviour, which can be a contributing factor in the leading causes of mortality and morbidity among youth and adults [3], is often established during youth and extends into adulthood. The different types of behaviour are interrelated and are

Table 5 **Correlation between the three types of risky behaviour, Pearson correlation**

Risk behaviour	Tobacco use	Alcohol and drug use	Sexual behaviour
Sexual behaviour	0.345**	0.457**	1.000
Alcohol and drug use	0.419**	1.000	–
Tobacco use	1.000	–	–

***Significant at P < 0.001.*

preventable. These include tobacco use, alcohol and other drug use, and risky sexual behaviour that can result in unintended pregnancy and STI, including HIV.

Two thirds of all deaths among persons aged > 25 years result from only 2 causes—cardiovascular disease and cancer. The commonest type of risk behaviour associated with these 2 causes of death is linked to tobacco use, which is initiated during adolescence [*3*]. The results of the present study confirm the results of a previous study [*4*] and show that tobacco users were mostly male, older in age, working or with high allowance, paying no attention to risks and highly influenced by peers and the media. The cigarette smoking rate has been stable since that study over all years, however, prevalence of smoking of water pipes increased dramatically (72% of smokers in the current study compared with 30% in the previous one). This is probably a reflection of the new trend among the youth in Egypt for smoking water pipes in cafés. Despite the banning of cigarette advertising in the Egyptian media, the depiction of water pipe smokers is widespread in TV serials and movies and is associated with pleasure. In addition, advertisements for water pipe tobacco products are common on Arab satellite channels. The effect of this habit on pulmonary function in comparison to cigarette smokers and non-smokers was investigated [*8*] and the results showed that the detrimental effects of water pipe smoking are not as great as those of cigarette smoking; lung function parameters were higher in water pipe smokers, especially the parameters for small airways. Water pipe smoking, however, has adverse effects on general health; it may predispose to oral cancer and is associated with a statistically increased incidence of squamous cell carcinoma and keratoacanthoma of the lips. [*9*]

Table 6 **Determinants of risky behaviour, multiple regression analysis**

Variable	B	*P*	OR	95% CI
Male	2.2743	<0.0001	9.7	5.5–17.0
Older age	0.8324	0.0009	2.3	1.4–3.8
High allowance	0.5494	0.0248	1.7	1.1–2.8
No attention to risk	0.3941	0.0346	1.5	1.1–2.1

Model significant at P < 0.001.
OR = odds ratio.
CI = confidence interval.

Intensive and sustained efforts to counter-market tobacco among youth are necessary to negate the "friendly familiarity" created by tobacco advertising and to communicate the true health and social costs of tobacco use [*10*].

Although the main risky behaviour of the students in this study was tobacco use, 4% of them practise unsafe sex risky behaviour and 5% practise substance use. As they get their knowledge mainly from media that project drug use and extra-marital sex as not culturally accepted, they were highly aware of their risks and minimally practising them.

In addition, 18.2% of the students in the study had ever sniffed substances such as petrol products, paints and sprays. Petrol sniffing as a specific form of substance abuse is associated with dysfunctions that range in severity from subtle cognitive impairment to encephalopathy and death. Petrol sniffing causes a progressive decline of cognitive function that eventually leads to permanent neurological changes *11*].

Only 70% of the subjects had enough knowledge on AIDS. Most of those who had sexual relationships did not use any method for protection from STI nor did they use contraceptives. Friends, the social culture at university, and the interaction of the two with the developmental characteristics of the period between adolescence and adulthood are more important influences on sexual relationships than parents or high-school sex education classes. How and whether friends talked about sex and practised safe sex were strong normative influences in predicting safer sex among individuals [*12*]. Adolescents have not changed their behaviour in response to the pandemic, despite being well informed about HIV/AIDS and having positive attitudes toward HIV/AIDS prevention [*13*]. Those AIDS-prevention programmes which, rather than merely providing information, focus on helping youths perceive HIV as a problem, motivating them to act safely and implement safe acts have successfully reduced adolescents' risk acts.

The majority of the students in this study got their information on AIDS and sex from friends and the media. The mass media in Egypt does not offer enough information on AIDS. In a previous study it was found that only 7% of female and 62% of male medical students approved doing tests for the detection of AIDS, reflecting cultural sensitivity (A. Refaat. Knowledge *and attitude of medical students towards premarital examination program*. Paper presented at the 1st international conference of behavioural medicine, Cairo, Egypt, November 1994).

The present study showed the 3 types of risky behaviour were correlated and that students started them before university age and in sequence: smoking at age 15, sex at 16 then alcohol and drugs at age 17. In the United States of America also, it was found that adolescents engage in multiple health-risk behaviour according to age and that many adolescents engage in these kinds of behaviour serially rather than at the same time [*14*].

Conclusions and recommendations

Less than one fifth of the students in this study practise risky behaviour, mainly as tobacco use. They paid moderate attention to risky behaviour, and got their information from the media and peers. The study revealed certain types of risky health behaviour that are not usually stressed in health communication programmes, such as smoking of water pipes, sniffing of petroleum products and lack of information on AIDS.

Based on the results of this study, it is recommended that a health communication programme be designed targeting Egyptian youth and adolescents that addresses all the types of risky behaviour identified. Counter-marketing campaigns should highlight a tobacco-free lifestyle as the majority lifestyle of diverse and interesting individuals.

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Opioid use in patients presenting with pain in Zahedan, Islamic Republic of Iran

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استخدام الأدوية الأفيونية المفعول في المرضى المتألمين في زاهدان، إيران

فافا رحيمي — موفاغار، فاطمة راخشاني، مهدي محمدي، عفارين رحيمي موفاغار

الخلاصة: لدراسة معدل انتشار استخدام الأدوية الأفيونية المفعول لمعالجة الألم والعوامل المصاحبة له، أجريت دراسة شملت 480 من المرضى المتعاقبين الذين كان الألم هو الشكوى الرئيسية لديهم، وذلك باستكمال استمارة أثناء المقابلة في عشر عيادات في زاهدان. واستُخدم كل من كي مربع واختبار t و التحوف اللوجستي لتحليل استخدام الأدوية الأفيونية المفعول بالمقارنة مع 18 من العوامل المرافقة المحتملة. لقد كان معدل انتشار استخدام الأدوية الأفيونية المفعول 28.5٪ من بين المرضى الذين كانت شكواهم الرئيسية هي الألم. ولم تكن هناك علاقة قوية بين استخدام الأدوية الأفيونية المفعول والألم المزمن (الذي يعود لفترة أطول من ستة أشهر)، ولكن كانت هناك علاقة مع العوامل الخمسة التالية: سوابق استخدام الأدوية الأفيونية المفعول مع الأصدقاء (72.9٪ بالمقارنة مع 20.4٪ بدون استخدامها مع الأصدقاء) والمهنة (58.5٪ لدى رجال أعمال مقابل 17.4٪ لدى سيدات منزل) تدخين السجائر (60.8٪ مدخنين مقابل 21.8٪ من غير المدخنين)، الاستشارة بسبب مشكلات نفسية (38.3٪ مقابل 23.3٪ بدون تلك الاستشارة)، وموت الزوج (60٪ مقابل 26.1٪ بدون موت الزوج).

ABSTRACT To study the prevalence and factors associated with opioid use in pain, 480 consecutive patients with a chief complaint of pain were interviewed at 10 clinics in Zahedan. The data were analysed in relation to 18 possible associated factors. The prevalence of opioid use was 28.5% in patients presenting with pain. There was no significant relation between opioid use and chronic pain (≥ 6 months), but there was a relationship with the following 5 factors: previous opioid use by friends (72.9% versus 20.4% without friends using), occupation (58.5% private sector employees/self-employed versus 17.4% housewives), cigarette smoking (60.8% versus 21.8% not smoking), consultation for a psychological problem (38.3% versus 23.3% without), and death of a spouse (60.0% versus 26.1% without).

L'utilisation des opioïdes chez les patients souffrant de douleurs à Zahedan (République islamique d'Iran)

RESUME Afin d'étudier la prévalence et les facteurs associés à l'utilisation d'opioïdes pour les douleurs, 480 patients consécutifs qui se plaignaient principalement de douleurs ont été interrogés dans dix centres de santé à Zahedan. Les données ont été analysées en relation avec 18 facteurs associés possibles. La prévalence de l'utilisation des opioïdes était de 28,5 % chez les patients souffrant de douleurs. Il n'y avait aucune relation significative entre l'utilisation des opioïdes et les douleurs chroniques (≥ 6 mois), mais il y avait une relation avec les cinq facteurs suivants : l'utilisation d'opioïdes précédemment par des amis (72,9 % contre 20,4 % sans utilisation par des amis), la profession (58,5 % employés du secteur privé/ travailleurs indépendants contre 17,4 % femmes au foyer), le tabagisme (60,8 % contre 21,8 % non-fumeurs), la consultation pour un problème psychologique (38,3 % contre 23,3 %), et le décès du conjoint (60,0 % contre 26,1 %).

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Introduction

Opium is one of many drugs that decrease pain [*1*]. Little is know about whether chronic pain leads to opioid misuse; studies have shown that from 3% to 23% of patients with chronic pain are addicted to opioids [*2,3*]. Some believe chronic pain leads to opioid use, more hospitalization, reduced physical efficiency and more psychiatric symptoms [*4*]. Others argue that it is the biopsychosocial nature of humans, rather than the pain itself, that predisposes certain individuals to opioid use [*5*].

Between 9% and 27% of white male opioid addicts reported that their addiction started with a prescription from a doctor for an opioid analgesic to treat a painful condition [*2,3*]. In a similar recent study of 58 patients, the authors warn physicians that severe oral opioid dependency occurs more frequently than previously recognized [*6*]. In a national study of opium addicts in the Islamic Republic of Iran, pain was mentioned by 17.5% as a trigger to opioid use [*7*].

There are multiple factors that have been documented as associated with opioid use, including demographic characteristics, personality, genetics and environment. Some of these include: younger age, being divorced/widowed/separated, having a family member who uses drugs, encouragement to use by friends or others [*8–10*], failure in education, severe economic deprivation, social isolation (separation from family) [*10*], illiterate or poorly literate father or mother [*9,11*], unemployment [*11,12*], cheap price of opioids [*13*], not knowing the harmful effects of opioids or believing they are useful [*9,10,12*], cigarette smoking [*8*], alcohol use [*11,14,15*], history of drug use [*16*], psychosocial problems such as depression [*9,10,17,18*], HIV positive, history of sexual abuse [*19*], past history of consultation in opioid clinic and/or treatment during 12 months before being in prison [*20*].

As far as we know, there has been no evaluation of the prevalence of opioid use in patients suffering from pain in the Islamic Republic of Iran. Our study was done in the south-east of the Islamic Republic of Iran, an area which is the main gateway of opium transit from Afghanistan and Pakistan to Europe and where opioids may be easily accessible. Sistan va Baluchestan province is also socioeconomically a very deprived province. We studied opioid use in patients with pain attending clinics in Zahedan, the main city of the province. The aims of the study were to ascertain: whether chronic pain causes opioid use; the prevalence of opioid use in patients presenting with pain; and the predisposing factors for opioid misuse (i.e. why one patient consumes opioids and another does not).

Methods

A cross-sectional study was conducted on 480 consecutive patients who presented with pain at the study clinics in Zahedan. There is no pain clinic in Zahedan, so patients with pain are referred to various clinics within the city. The 10 clinics selected were those where specialists agreed to participate in the study: 3 neurosurgery, 3 psychiatry, 1 preoperative anaesthesia, 1 physiotherapy, 1 emergency and 1 neurology clinic. Around one-third of consecutive patients attended the neurosurgery clinic, one-third the psychiatry clinic and one-third the other clinics. The study was conducted over 2 months in March and April 2001. The inclusion criteria were all patients aged over 16 years old with a chief complaint of pain.

A questionnaire was devised to collect data on sociodemographic variables, characteristics of pain and opioid use (types of opioids taken, amounts taken and routes of administration). In our study, chronic pain was defined as frequent or constant pain for a duration of 6 months or more. Acute pain was defined as pain frequently (always/often) for 1 month or less. Opioid use was defined as occasional or regular use of opium, opium residue (*shireh*), heroin, morphine or codeine in the previous year. The questions covered 18 factors that have been associated with opioid use: age, male sex, unemployment, illiteracy, urban residency, divorce, death of spouse, location of pain, duration of pain, frequency of pain, opioid use recommended by friends or physician, cigarette smoking, alcohol drinking, history of opioid use in family, history of opioid use in friends, decreased economic situation, death of friend or family member and poor psychological health. Our practical definition of poor psychological health was the patient's report of opioid use for relief of psychological stress, history of consultation for a psychological problem or history of psychological treatment. The questionnaire was prepared at the pain clinic of the Medical University of Iran following a literature review and was modified in Zahedan University of Medical Sciences. Four other psychiatrists reviewed the questionnaire for face and content validity.

The specialists at the clinics delivered the questionnaire to patients by interview to minimize bias between literate or illiterate patients. The specialist and assistant in each clinic were trained in how to deliver the questionnaire.

Comparisons of patients with and without opioid use and associated factors were made with chi-squared, Student *t*-test and logistic regression analysis. *SPSS*, version 6 was used for the statistical calculations.

Results

Of the 480 patients with pain, 57.5% were female. The mean age of pain patients was 36.0 ± SD 13.9 years. A total of 81.0% were married and 6.0% had experienced the death of a spouse. Only 7.0% of patients were unemployed; 77.0% of female pain patients were housekeepers. There were 46.7% illiterate patients (62.0% of the women and 25.1% of the men) and 32.1% had attended only guidance school.

The pain was said to be located in the head, lower back or the whole body by 75.6% of patients. The duration of pain was chronic (≥ 6 months) in 64.4% of patients. The frequency of pain was described as 'always' or 'often' by 71.5%.

The overall prevalence of opioid use was 28.5% (137/480) in patients presenting with pain. Opium and opium residue (*shireh*) was used by 65.9% and 15.9% of users respectively. Most users (92.7%) had consumed for more than 2 months duration. Consumption was 1 or more times per day for 70.1% of users. The most common method of consumption was inhalation, used in 59.1% of cases. There were 5 patients using cannabis (*hashish*).

Opioid users claimed pain as an origin of their opioid use in 79.6% of cases. Although opioid use was more prevalent in chronic pain than acute pain (26.3% versus 15.9%), the result was not statistically significant ($P > 0.05$). On the other hand, statistical analysis did not show that pain preceded opioid use ($P = 0.074$).

In the first stage of analysis, 11 of the 18 factors included were associated with opioid use: age, sex, occupation, education, death of spouse, consultation for a psychological problem, cigarette smoking, alcohol, opioid use in the family, opioid use in friends, and worsened economic situation. The number of alcohol drinkers was low.

In the second step, a logistic regression test was performed to eliminate confounding factors, leaving 5 factors that remained significant (Table 1): previous opioid use by friends, type of occupation, cigarette smoking, neuropsychiatric consultation and death of spouse. Among those whose friends were opioid users, 72.9% were users compared with 20.4% without friends using. People working in the private sector or self-employed were the highest opioid users (58.5%) and housewives the lowest (17.4%); unemployed people were 29.4%. Among cigarette smokers, 60.8% were opioid users compared with 21.8% among non-smokers. Among patients needing neuropsychiatric consultation, opioid use was 38.3% compared with 23.3% among those who did not. Opioid use in those who had experienced the death of a spouse was 60.0% compared with 26.1% for those who had not.

Table 1 **Factors associated with opioid use among 480 patients presenting with pain**

Variable	Total	Opioid users		Non-users		*P* value
	No.	No.	%	No.	%	
Opioid use in friends						< 0.001
Yes	70	51	72.9	19	27.1	
No	333	68	20.4	265	79.6	
Total	403	119	29.5	284	70.5	
Occupation						0.023
Manual/semiskilled worker/farmer	56	18	32.1	38	67.9	
Government employee	79	23	29.1	56	70.9	
Private sector employee/ self-employed	65	38	58.5	27	41.5	
Housewife	213	37	17.4	176	82.6	
Unemployed	34	10	29.4	24	70.6	
Total	447	126	28.2	321	71.8	
Cigarette smoking						0.005
Yes	102	62	60.8	40	39.2	
No	312	68	21.8	244	78.2	
Total	414	130	31.4	284	68.6	
Neuropsychiatric consultation						0.013
Yes	175	67	38.3	108	61.7	
No	292	68	23.3	224	76.7	
Total	467	135	28.9	332	71.1	
Death of spouse						0.034
Yes	30	18	60.0	12	40.0	
No	426	111	26.1	315	73.9	
Total	456	129	28.3	327	71.7	

Discussion

Opioid use was 28.5% among patients presenting with pain to clinics in this city in the Islamic Republic of Iran. Availability of drugs is a potent predisposing factor to opioid use [*21*]. The nearby countries of Afghanistan and Pakistan are 2 of the major production areas in the world, named the 'golden crescent'. When opioids are more readily available, the stigma in society is reduced and if they are cheap, the vicious cycle of dependency and crime is less [*2*].

Our study found no significant association between opioid use and chronic duration of pain. Millions of patients worldwide are regularly exposed to opioid analgesics for the treatment of pain. The documented incidence of iatrogenic addiction from the treatment of acute pain or cancer pain is extremely low [*22*]. A prospective study in a community family practice clinic comparing a group of patients with chronic severe low back pain with a control group attending for other reasons found no significant difference in the prevalence of substance use disorder between these 2 groups [*23*]. Patients with chronic pain are simply representative of the general population rather than having a higher prevalence rate of substance use disorder [*5*]. Current use of opioids in the Islamic Republic of Iran has been reported to be 5.85% in males and females ≥ 15 years [*24*]. The study of opioid addiction in Vietnam War veterans showed that the overall prevalence of opioid addiction in the study group had dropped to that of the general United States population after veterans had been back home for 3 years [*25*]. It seems that opioid use for the treatment of pain is no different from opioid misuse in the stressful environment of war.

In a previous study, 81% of codeine-dependent patients said that their codeine use started with a chronic pain problem [*14*]. In our study, a similar proportion of opioid users (79.7%) told us the same. However, we found no statistically significant relationship between the presence of chronic pain and opioid use in pain patients.

The identification of an addictive disorder in a patient with chronic pain does not necessarily preclude the use of opioids as a component of pain management, unless maladaptive patterns of behaviour develop which define addiction. These are adverse consequences such as persistent oversedation, loss of control over use and preoccupation with using opioids despite adequate analgesia [*26*]. Diagnosing addiction and drug-seeking behaviour is important in chronic pain patients [*27*].

It should be emphasized that pain is not the main culprit in opioid misuse; biopsychosocial risk factors are especially important for addiction disorder. The Screening Instrument for Substance Abuse Potential (SISAP) [*28*] helps the clinician categorize patients into lower or higher risk of abusing prescribed opioids. Patients who are at higher risk for substance abuse include those whose exceed 3–4 alcoholic drinks per day, those who admit to marijuana or cannabis use in the past year and patients under 40 years old who smoke cigarettes [*15*].

We found 5 risk factors associated with opioid use in pain patients: previous opioid use by friends, occupation, cigarette smoking, neuropsychiatric consultation and death of a spouse. A cross-sectional non-concurrent cohort study among young males in south-west China using multivariate analysis identified the following significant risk factors for drug use: being divorced/widowed/separated, having been encouraged by friends/others to try drugs, smoking cigarettes, and having a family

member who used drugs [*8*]. In our study, smoking cigarettes and/or being widowed were also significantly associated with opioid use, but being divorced/separated, having been encouraged by friends/others to try drugs, and having a family member who used drugs were not significantly associated with opioid use. In a European multicentre study of drug injecting in prison, friends injecting opioid substances were identified as a risk factor [*20*]. The role of friends was the most important associated factor in our study. It seems friends have more influence than families in this particular situation.

There was a higher level of consultation for a psychological problem among opioid users than non-users in our study. Another study has shown a 23% depression rate and 21% anxiety disorder rate among codeine-dependent patients [*14*].

Predisposing factors for illicit drug use have been shown to be unemployment and poor education [*12*]. In our study, however, people working in business were the most likely to use opioids. Greater spending power for luxury goods and increased leisure time over recent decades have been linked to increased drug abuse in society [*21*].

In an analysis comparing addicts and non-addicts in the Islamic Republic of Iran, cigarette smoking and alcohol were associated with opioid use [*11*]. Opium is the most commonly misused substance in our country [*7,11*]. These agree with our study showing an association between cigarette smoking and opioid use particularly opium.

In other studies [*7,11*], there were more men than women using opioids, but in our study sex was excluded by the logistic regression analysis. Historical anecdotes suggest that more addicts were women 200 years ago [*29*], but it seems that nowadays men have more opportunities to misuse opioids [*30*].

There is a high prevalence of opioid use in patients who complain of pain in Zahedan. Opioid use in friends was the most important associated factor for opioid use in pain and further research into this would help parents, teachers, trainers and leaders of society to know how to deal with this. Clinicians with pain patients should be aware of the other factors associated with opioid use, such as cigarette smoking and asking for psychiatric support from neuroconsultants. More support for widows and widowers, who are a small but vulnerable group, may be needed. Pain provides a guilt-free reason for opioid use and more research is needed into opioid use in society generally, not just small groups such as students, and the biopsychosocial factors involved.

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Right heart haemodynamic values and respiratory function test parameters in chronic smokers

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القيم الدينامية الدموية في القلب الأيمن ومتثابتات اختبارات الوظيفة التنفسية لدى المدخنين المزمنين

مورات غول باران، تولين كاغاتاي، توفيق غومن، بنبي كاغاتاي

الخلاصة: أخذت قياسات الوظيفة القلبية أثناء إجراء تصوير الأوعية الإكليلية لدى 24 من المدخنين المزمنين المصابين بمرض القلب الإكليلي، ثم ربطت مع اختبارات الوظيفة التنفسية. وقد كان لدى 14 من المرضى بيّنات على مرض رئوي انسدادي مزمن، وكان لنتاج القلب ارتباط مباشر مع السعة الحيوية، ومع السعة الحيوية القسرية، ومع الحجم الزفيري القسري في الثانية الأولى ومع سرعة الجريان للهواء في 25٪ من السعة الحيوية القسرية. وكانت مقاومة الشريان الرئوي ذات علاقة سلبية مع حصيلة النسبة بين الحجم الزفيري القسري في الثانية الأولى والسعة الحيوية القسرية، كما كان إشباع الأكسجين في الشريان الرئوي ذا علاقة ضعيفة مع مقاومة الشريان الرئوي وذا علاقة متوسطة مع الضغوط في الأذين الأيمن وفي البطين الأيمن. إن التشخيص الباكر والمعالجة الباكرة للمرض الرئوي الانسدادي المزمن لدى المدخنين ممكنان بدون اللجوء إلى طرائق باضعة.

ABSTRACT During coronary angiography in 24 chronic smokers with coronary heart disease, cardiac function measurements were taken and correlated with respiratory function tests. Fourteen patients had evidence of chronic obstructive pulmonary disease. Cardiac output had a direct correlation with vital capacity, forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and velocity at 25% of FVC (Vmax25). Pulmonary artery resistance was inversely correlated with FEV_1/FVC, while pulmonary artery oxygen saturation weakly correlated with FEV_1 and Vmax25. The pulmonary artery pressure had a weak correlation with the pulmonary artery resistance and an intermediate correlation with the right atrium and the right ventricular pressures. Early diagnosis and therapy of chronic obstructive pulmonary disease in smokers may be possible without using invasive methods.

Valeurs hémodynamiques du coeur droit et paramètres du test de la fonction respiratoire chez des fumeurs chroniques

RESUME Lors d'une angiographie coronaire réalisée chez 24 fumeurs chroniques atteints de coronaropathie, des mesures de la fonction cardiaque ont été effectuées et corrélées aux tests de la fonction respiratoire. Quatorze patients présentaient des signes de maladie pulmonaire obstructive chronique. Le débit cardiaque avait une corrélation directe avec la capacité vitale, la capacité vitale forcée (CVF), le volume expiratoire maximum/seconde (VEMS) et le débit expiratoire maximum à 25 % de la capacité vitale forcée (Vmax25). La résistance artérielle pulmonaire était inversement corrélée au rapport de Tiffeneau (VEMS/CVF), tandis que la saturation en oxygène du sang artériel au niveau de l'artère pulmonaire était faiblement corrélée au VEMS et au Vmax25. La pression artérielle pulmonaire était faiblement corrélée à la résistance artérielle pulmonaire et il y avait une corrélation moyenne avec les pressions de l'oreillette droite et du ventricule droit. Le diagnostic précoce et le traitement rapide de la maladie pulmonaire obstructive chronique chez les fumeurs peuvent être possibles sans avoir recours à des méthodes invasives.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by chronic impediment of air circulation in the lungs, which usually has a slow progression and is mostly irreversible. COPD is a complex condition, influenced by multiple genetic and environmental risk factors [*1,2*].

Smoking is one of the most common environmental causes of COPD; it increases 10-fold the risk for the disease compared with non-smokers. The dose–response relationship between cigarette smoking and pulmonary function is well-established [*1*]. Respiratory function tests rapidly worsen over time in smokers. The adverse effect of smoking primarily works by attacking the larger airways and then the small arterioles. The diagnosis of COPD is then easily made after these changes have become irreversible and airways obstruction has occurred [*1*].

COPD is a major cause of morbidity and mortality worldwide. Thus, early diagnosis and cure of the disease is very important. The gradual increase of airways resistance causes some changes in pulmonary haemodynamics, such as pulmonary hypertension and pathological changes in the right heart. The pulmonary vasoconstriction and hypertension that occurs through alveolar hypoxia leads to deterioration of right heart function. Alveolar hypoxia does not need to be continuous; right ventricular hypertrophy develops with as little as 2 hours a day of hypoxia and abnormal pulmonary haemodynamics have been reported in patients with only nocturnal oxyhaemoglobin desaturation secondary to sleep apnoea [*3*].

COPD is defined by a reduction of the maximal expiratory flow rate that is progressive and irreversible. Intermediate or high level COPD is determined through the forced expiratory volume in the first second (FEV_1). Dividing FEV_1 by the forced vital capacity (FVC) is a sensitive test for mild-degree COPD [*1*].

Pulmonary hypertension is the most important factor in determining the prognosis of patients with COPD and the measurement is most accurately made during right-heart catheterization [*4*]. In COPD patients, the right ventricular ejection fraction (RVEF) is often reduced [*5*]. COPD, combined with deterioration of right ventricular function and pulmonary hypertension, limits peripheral oxygen utilization, reduces exercise capability and increases mortality [*6*]. Thus, it is necessary to recognize and control pulmonary hypertension as early as possible. The structural and functional adaptations of the right ventricle to the increases in afterload posed by disorders of the respiratory system are commonly referred to as cor pulmonale.

Detailed history taking, physical and X-ray examinations and electrocardiography may be helpful in diagnosing pulmonary hypertension and cor pulmonale, but due to anatomical differences of the thoracic structure it is not always possible to obtain accurate results. In addition, specific examinations such as arterial blood gas measurements, echocardiography, angiography and right-heart catheterization should be undertaken [*1,7*].

The aim of the study was to investigate whether pulmonary function tests can predict right heart pressure without the need to perform catheterization. It is hoped this will lead to better early diagnosis of deterioration in pulmonary function as well as haemodynamic functions caused by chronic smoking.

Methods

The study was carried out in the Institute of Cardiology of the Cerrahpasa Medical

Faculty at the University of Istanbul. The participants were 24 patients (3 women and 21 men) with known coronary heart disease who were referred for coronary angiography between February and May of 2002. Considering the risk and the cost of the catheterization procedure, we only intervened in patients who were chronic smokers and in need of cardiac catheterization for their coronary problems.

The evaluation of COPD was made by the Department of Bronchopneumology. COPD was evaluated in the patients through detailed history, clinical examinations, chest X-ray and pulmonary function tests. Pulmonary function tests were undertaken with a computerized spirometer (Vitalograph). The following were calculated: peak expiratory flow rate (PEF), vital capacity (VC), FVC, FEV_1, and flow rates at 25%, 50% and 75% lung of FVC (Vmax25, Vmax50 and Vmax75).

After obtaining informed consent from the patients, right-heart catheterization was performed with a 6 F (French) multipurpose catheter via the vena femoralis using the vacuum puncture method. The pressure was measured via liquid filled pressure monitoring sets with an external pressure transducer. Left heart as well as right heart pressures were taken. The following were measured: pulmonary artery pressure (PAP), pulmonary artery resistance (PAR), pulmonary capillary wedge pressure (PCWP), right atrium pressure (RAP) and right ventricle pressure (RVP).

For measurement of arterial partial oxygen pressure (PaO_2) and haemoglobin levels, blood samples (2 mL) were taken from the main pulmonary artery and the aorta using heparinized syringes. PaO_2 was measured through a gas analyser, which calculates oxygen saturation using the Severinghaus equation. The procedure was terminated after removal of the arterial and venous sheaths and after control of bleeding.

The respiratory function data was linked to the data obtained through catheterization. Cardiac output (CO) was calculated with the direct Fick method [*8*] and then placed in the PAR formula [*9*]. The normal range for PAR was taken as 0.9 ± 0.38 mmHg min/L for the 30–49 years age group and 1.0 ± 0.5 mmHg min/L for those aged over 49 years [*10*].

Statistical evaluation of the data was performed using Pearson correlation and regression analysis.

Results

The mean ± SD age of the participants was 52.1 ± 10.7 years. All the patients were chronic smokers; 21 had smoked a packet of cigarettes (20 singles) daily for more than 15 years and 3 had smoked a packet daily for about 11–14 years. Of the 24 patients, 14 were diagnosed with mild COPD; none were in the acute exacerbation phase of their disease and none had any cardiac valve disease. The remaining 10 patients did not meet the COPD criteria. Haemoglobin values for the group were 13–16 g/dL (mean 14.1 g/dL).

Cardiac output showed significant ($P < 0.05$) direct correlations with VC ($r = 0.50$), FVC ($r = 0.50$), FEV_1, ($r = 0.47$), Vmax25 ($r = 0.45$), Vmax50 ($r = 0.48$) and PEF ($r = 0.47$).

A weak and direct correlation was found between PAP and PAR ($r = 0.44$, $P < 0.05$). A strong and direct correlation was found between PAP and PCWP ($r = 0.84$, $P < 0.05$), and a medium and direct correlation between PAP and RAP ($r = 0.68$, $P < 0.05$) and RVP ($r = 0.65$, $P < 0.05$) (Table 1).

Table1 **Correlations of cardiac and respiratory function parameters in 24 patients with chronic obstructive pulmonary disease**

Parameters compared	Correlation *r*	*P*-value
CO and FEV_1	0.48	< 0.05
CO and PEF	0.47	< 0.05
CO and VC	0.50	< 0.05
CO and FVC	0.50	< 0.05
CO and Vmax25	0.45	< 0.05
CO and Vmax50	0.48	< 0.05
CO and PAR	–0.46	< 0.05
PAP and PAR	0.44	< 0.05
PAP and PCWP	0.84	< 0.05
PAP and RAP	0.68	< 0.05
PAP and RVP	0.65	< 0.05

CO = cardiac output; FEV_1 = forced expiratory volume in 1 second; PEF = peak expiratory flow rate; Vmax25 = 25% of forced vital capacity; Vmax50 = 50% of forced vital capacity. PAP = pulmonary artery pressure; PAR = pulmonary artery resistance; PCWP = pulmonary capillary wedge pressure; RAPPP = right atrium pressure; RVP = right ventricle pressure.

There was a very weak and direct correlation between PaO_2 and Vmax25 (r = 0.40, $P < 0.05$) and FEV_1 (r = 0.37, $P < 0.05$).

A very weak and inverse correlation was found in all patients between PAR and the FEV_1/FVC ratio (r = –0.35, $P < 0.05$). In the COPD group of 14 patients, there was a medium strong inverse correlation between PAR and FEV_1/FVC (r = –0.54, $P < 0.05$).

Discussion

The pulmonary circulation is a dynamic system that is affected by mechanical, neural and biochemical factors. Beginning from the response of the pulmonary circulation to these stimuli, researchers are attempting to reverse pulmonary hypertension. Oxygen inhalation, acetylcholine and tolazoline infusions have been tested for their ability to overcome the reflex pulmonary vasoconstriction. A serious pulmonary vasoconstriction may develop in elderly COPD patients with left heart insufficiency due to alveolar hypoventilation and the resultant hypoxia. Inhalation of 100% oxygen in these patients causes PAP and vascular resistance to drop [*1*].

In this study, we determined oxygen saturation of the aorta and the pulmonary artery as well as PCWP, PAP, RVP and RAP, cardiac output and especially PAR. PAR gives the resistance pressures of the main pulmonary artery, the arterioles and the precapillary and pulmonary capillary beds. In comparison with PAP, PAR is a better way to determine the presence and the severity of pulmonary vascular disease [*11*]. We therefore investigated the relation between PAR and the pulmonary function test parameters, and revealed that there was an inverse correlation between PAR and FEV_1/FVC.

In the study of Wolf et al. with krypton-81m perfusion, the RVEF was investigated in COPD patients and in healthy individuals [*12*]. The RVEF was distinctly low in COPD patients and there was a linear inverse correlation between PAP and RVEF. In COPD patients with pulmonary hypertension, RVEF was clearly lower than in COPD patients without pulmonary hypertension. Concerning RVEF, they again found a weak positive correlation with the PaO_2 and FEV_1, but no correlation with partial CO_2 pressure, pulmonary hypertension or FEV_1/FVC.

The data of Wright et al. show that PAP and pulmonary artery wedge pressures and cardiac output were normal at rest [*13*].

However, patients with more severe disease showed greater increases in PAP and pulmonary artery wedge pressures with exercise than did patients with minimal or no disease. Oxygen breathing had no effect at rest but it lowered PAP and PCWP during exercise in the patients with more severe disease. Histological studies showed that patients with moderate obstructive lung disease have structural changes in the pulmonary arteries consistent with pulmonary hypertension when compared with patients with minimal or no disease. Zhang et al. showed that exercise testing can identify the early phase of latent pulmonary hypertension and that in cor pulmonale patients PAP rises after exercise [*14*].

The criteria for pulmonary hypertension are a mean PAP greater than 20 mmHg resting and greater than 30 mmHg on exercise. The RVP increase cannot be determined for these criteria. PAR is more sensitive in showing the right ventricular load in conjunction with PAP [*9*]. In 14 of our patients, PAP was greater than 20 mmHg. There was a direct correlation between PAR and PAP.

According to the Fick formula, cardiac output is equal to the right ventricular flow, assuming that there are no intracardiac shunts [*8*]. It is thus useful to note that we found a direct correlation between cardiac output and FEV_1, PEF, VC, FVC and the Vmax25. As COPD develops, the parameters above and the right ventricular output deteriorate. Furthermore, it seems consistent that there is an inverse correlation between the cardiac output and PAR and a direct correlation between the PAP and the PCWP and the RAP as well. PEF is a useful measure of pulmonary health status. In a study of van Helden et al., a trend towards reduced peak flow was already evident in teenagers who smoked and whose parents were smoking [*15*]. In a similar study, the results showed that, even for younger people, cigarette smoking is associated with significant detrimental effects on cardiopulmonary function and exercise tolerance. Objective evidence of an effect of smoking on cardiopulmonary function and exercise tolerance in this age group may assist educators and health care professionals in convincing teenagers to quit smoking [*16*].

Pulmonary function testing, at least the measurement of FEV_1, in all middle-aged smokers has been recommended. The smokers with abnormal FEV_1 should be advised to quit smoking [*17*]. Further investigations are needed to understand the relationship of oxygen saturation to Vmax25 and FEV_1 values, although we found a correlation between these parameters and PaO_2.

Finally, we could have obtained results that are more reliable if we had focused on COPD patients and compared their right heart pressures according to disease severity. Our study evaluated right heart function for patients who primarily came for coronary angiography and then we looked for COPD. Since we had no severe COPD patients, right heart function did not show deterioration. Invasive diagnostic procedures are usually unnecessary if non-invasive studies indicate mild to moderate pulmonary hypertension in chronically hypoxaemic patients with severe airways obstruction. We should keep in mind that there is an inverse correlation between the FEV_1/FVC ratio and PAR, and a direct correlation between the Vmax25 and FEV_1, which are indicators of a mild to moderate degree of COPD. We believe that this approach may be useful for estimating deterioration of right heart parameters without using invasive methods and thus improving the early diagnosis and therapy of chronic obstructive pulmonary disease in smokers.

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Detection of cotinine in neonate meconium as a marker for nicotine exposure *in utero*

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كشف الكوتينين في عِقْي الولدان كواسم للتعرض للنيكوتين داخل الرحم

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الخلاصة: تم قياس مستوى الكوتينين في عِقْي الولدان كواسم يدلُّ على تعرضهم قبل ولادتهم للنيكوتين من تدخين أمهاتهم للتبغ. نقد قسمت الوالدات اللواتي أدخلن إلى مستشفى الولادة في الإسكندرية، مصر إلى ثلاث مجموعات: 10 مدخنات فاعلات، 10 مدخنات بالتعرض لدخان المدخنين الاخرين، 10 لم يتعرضن للتبغ أثناء حملهن. وقد جمعت عينات من لعاب وبول الأمهات وعِقْي ولدانهن في اليوم الأول. وقد وجد أن المستوى الوسطي للكوتينين في بول الأمهات يختلف لدى المجموعات الثلاث لدى قياسه بالمقايسة المناعية الإشعاعية. كما وجد مثل هذا الاختلاف في المستوى الوسطي للكوتينين في اللعاب والعقي. وكان هناك ارتباط إيجابي هام بين مستويات الكوتينين في العقي وكل من مستويات الكوتينين في لعاب وبول الأمهات. وهكذا يعد العِقْي واسماً بيولوجيا مثالياً لاختبار التعرض المباشر للجنين لدخان التبغ في فترة حياة الوُلدان.

ABSTRACT Neonate meconium cotinine level was evaluated as a marker of prenatal exposure to nicotine from tobacco smoking by mothers. Mothers admitted to a maternity hospital in Alexandria, Egypt, were divided into 3 groups: 10 active smokers, 10 passive smokers and 10 with no tobacco exposure during pregnancy. Urine and saliva samples were collected from mothers and first-day meconium samples from their neonates. Mean maternal urinary cotinine levels, measured using radioimmunoassay, differed significantly between the 3 groups, as did mean salivary cotinine and mean cotinine levels in meconium. There was a significant positive correlation between cotinine levels in meconium and both maternal urinary and salivary cotinine levels. Meconium is an ideal biological marker for testing direct fetal exposure to tobacco smoke in the neonatal period.

Détection de la cotinine dans le méconium du nouveau-né comme marqueur de l'exposition à la nicotine *in utero*

RESUME Le taux de cotinine dans le méconium du nouveau-né a été évalué en tant que marqueur de l'exposition prénatale à la nicotine du fait du tabagisme de la mère. Les mères admises dans une maternité à Alexandrie (Egypte) ont été réparties en trois groupes : 10 fumeuses actives, 10 fumeuses passives et 10 femmes qui n'avaient pas été exposées au tabac pendant la grossesse. Des échantillons d'urine et de salive ont été recueillis chez les mères et des prélèvements de méconium du premier jour ont été effectués chez leurs nouveau-nés. Le taux moyen de cotinine urinaire de la mère, mesuré par radio-immunodosage, différait significativement entre les trois groupes, tout comme le taux moyen de cotinine salivaire, ainsi que le taux moyen de cotinine dans le méconium. Il y avait une corrélation positive significative entre le taux de cotinine dans le méconium et le taux de cotinine salivaire et urinaire chez la mère. Le méconium est un marqueur biologique idéal pour déceler l'exposition directe du fœtus au tabac dans la période néonatale.

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Introduction

There is no doubt that smoking during pregnancy is hazardous to both the mother and baby. The harmful effects of maternal smoking have been well documented. The relation between maternal cigarette smoking and adverse pregnancy outcomes include abruptio placenta, bleeding during pregnancy, premature rupture of membranes, fetal death, neonatal mortality, and deficits in growth, intellectual and emotional development and in behaviour [*1*]. The majority of epidemiological studies have found no association between cigarette smoking and congenital malformations [*2*]. However, one study suggested that mothers aged 35 years and older who smoke may have a higher risk of delivering infants with minor malformations [*3*].

The risk of passive smoking or environmental exposure to tobacco smoke has been a major concern in the past two decades. It has been suggested that passive maternal smoking may be associated with lower score on tests of neurodevelopment as a result of long-term neurotoxicity [*4*]. This could be explained by the fact that fetal and neonatal levels of carboxyhaemoglobin are generally higher than maternal concentrations because of a higher affinity of fetal haemoglobin for carbon monoxide [*4*].

Cotinine is a more specific indicator for the inhalation of tobacco smoke, i.e. nicotine, compared with other indicators such as carboxyhaemoglobin. Carboxyhaemoglobin is a non-specific indicator as it is formed on exposure to carbon monoxide, which is widely distributed as an air pollutant and is also endogenously produced. Cotinine is the major metabolite of nicotine [*5*]. It is considered a better marker of long-term exposure to tobacco smoke than the parent alkaloid, since it is endogenously produced only through an oxidative metabolism of nicotine in the body [*6*]. It is also more easily identified, since its concentrations are higher in both blood and urine. This is due to its longer plasma half-life (30 hours) as well as a more protracted excretion rate [*7,8*].

The aims of the study were to: evaluate the use of neonate meconium as a marker of prenatal exposure to nicotine from tobacco smoking by the mother; to study the correlation between maternal urinary and salivary cotinine levels and the levels found in the neonate meconium; and to study the effect of nicotine on birth weight.

Methods

The study was carried out from 1 July 1999 to 31 January 2000 on 30 consecutive mothers and their neonates admitted to El-Shatby Maternity University Hospital. All mothers were interviewed and a full history was taken with emphasis on history of smoking during the last pregnancy. Neonates were weighed and clinically examined for any congenital anomalies. The mothers were subdivided into 3 subgroups according to smoking habits: 10 mothers with a history of active smoking during the pregnancy; 10 mothers with a history of passive smoking during the pregnancy; and 10 mothers with no exposure to smoking during the pregnancy.

Urine and saliva were collected from mothers as well as first day meconium from their neonates. Consent was obtained from mothers before urine collection. Urine was collected from mothers at the time of delivery by a catheter to avoid contamination of urine with blood. A 10–20 mL sample of urine was placed in a plastic container and frozen at –15 °C to –20 °C until the time of assay. A saliva sample of 2–3 mL was collected (unstimulated) from mothers shortly after labour and placed in

plastic test-tubes and deep frozen until the time of assay. First-day meconium was collected straight from the diaper of the neonates using a spatula and deep frozen in plastic tubes until the time of extraction.

Cotinine extraction and assay

The urine and saliva samples of the mothers were centrifuged and the supernatants were assayed directly by radioimmunoassay (RIA) using a double antibody nicotine metabolite procedure (Diagnostic Products Corporation, Los Angeles, USA) [*9*]. The procedure is a liquid-phase RIA in which cotinine labelled with iodine 125 competes for antibody sites for a fixed time with cotinine (and other nicotine metabolites) in the patient's sample. After incubation for a fixed time, separation of bound from free parts is achieved by the polyethylene glycol (PEG)-accelerated double antibody method. Finally, the antibody-bound fraction is precipitated and counted. The patient sample concentration is read from a calibration curve.

The reproducibility of this method was examined on urinary samples selected to represent a range of 3 different cotinine levels. Results revealed a coefficient of variation of 5.3%–9.4% for intra-assay (within-run) and 4.3%–6.8% for inter-assay (run-to-run) results.

After thawing, meconium samples of 0.5 to 1.0 g were weighed and vortexed rigorously in 5 mL of methanol. After centrifugation at 1500 × g, the supernatant was dried in a 40 °C water bath using a stream of nitrogen [*10*]. The sample was then reconstituted with 1.0 mL of methanol and 50 µL was taken off and assayed using the same RIA method [*9*].

Statistical analysis

Statistical analysis was made using *SPSS*, version 8 [*11*]. Statistical tests used were the mean and standard deviation (SD), *F*-test (ANOVA), Pearson correlation coefficient, Spearman correlation coefficient and Scheffe test. A *P*-value of 5% was used as the level of significance.

Results

Maternal data

The age of the active smoker group ranged from 20 to 32 years with a mean of 27.8 ± 3.7 years. The age of passive smokers group was lower, ranging from 18 to 35 years with a mean of 26.3 ± 5.4 years. In the non-exposed group, the age ranged from 20 to 29 years and the mean was 25.0 ± 2.9 years. There was no significant difference between the mean ages of the 3 groups ($F = 1.15$).

The number of cigarettes smoked per day by the mothers of the active smokers group ranged from 2–18 cigarettes with a mean of 11.7 ± 5.3 cigarettes.

Neonatal data

Using the Scheffe test, neonates whose mothers were active smokers had a lower mean birth weight than those whose mothers were passively exposed to smoke (mean difference 200.0 ± 82.5 g). This difference was not significant, however. Neonates whose mothers smoked had a significantly lower weight than those with non-smoking mothers (mean difference 490.0 ± 82.5 g) ($P < 0.001$) (Table 1). The mean birth weight of neonates in the passive smoking group was less than those in the non-smoking group (mean difference 290.0 ± 82.5 g), and this was also significant ($P < 0.05$).

Laboratory data

The present study revealed an overlap between the levels of maternal urinary cotinine in active and passive smokers. The highest level was detected in the active

Table 1 **Mean difference in birth weight in relation to maternal smoking habit**

Groups compared	Difference in birth weight (g) Mean	SE	*P*-value
Active (n = 10) versus passive (n = 10) smokers	–200.0	82.5	0.07
Active (n = 10) versus non-smokers (n = 10)	–490.0	82.5	< 0.001
Passive (n = 10) versus non-smokers (n = 10)	–290.0	82.5	0.006

SE = standard error.

smoking group, ranging from 1390 to 22 300 ng/mL, with a mean of 16 110 ± 10 851 ng/mL (Table 2). In passive smoking mothers, the cotinine level ranged from 79.3 to 14 385 ng/mL, with a mean level of 3096 ± 5783 ng/mL. The lowest level was detected in the urine of non-smoking mothers where it ranged from 0 ng/mL (non-detectable) to 122.5 ng/mL with a mean of 75.1 ± 42.2 ng/mL. There was a significant difference in the urinary cotinine levels across the 3 groups (F = 14.41, $P < 0.01$).

There was a significant difference in the mean cotinine levels in mothers' saliva among the 3 groups (91.8 ± 101.6 ng/mL, 13.7 ± 19.2 ng/mL and 1.1 ± 2.0 ng/mL in active, passive and non-smokers respectively) (F = 6.79, P = 0.01) (Table 3).

There was a significant difference between the mean cotinine levels in the saliva of active smokers and passive smokers using the Scheffe test (mean difference 78.2 ± 26.7 ng/mL). There was also a significant mean difference between the salivary cotinine levels of active smokers and non-smokers (mean difference 90.7 ± 26.7 ng/mL) (Table 4).

The Pearson correlation test showed a positive correlation between the cotinine levels in mothers' saliva and mothers' urine (r = 0.582, where P = 0.01) (Figure 1).

Table 2 **Maternal urinary cotinine levels in relation to maternal smoking habit**

Smoking group	Urinary cotinine level (ng/mL) Range	Mean	SD
Active smokers (n = 10)	1390–22 300	16 110.0	10 851.3
Passive smokers (n = 10)	79.3–14 385	3095.9	5782.8
Non-smokers (n = 10)	0–122.5	75.1	42.2
F-test			F=14.41, P< 0.01

SD = standard deviation.

Table 3 **Maternal salivary cotinine levels in relation to maternal smoking habit**

Smoking group	Salivary cotinine level (ng/mL) Range	Mean	SD
Active smokers (n = 10)	1.7–250.0	91.8	101.6
Passive smokers (n = 10)	0–65.0	13.7	19.2
Non-smokers (n = 10)	0–6.5	1.1	2.0
F-test		F = 6.79, P < 0.01	

SD = standard deviation.

Table 5 shows that in the active smoker group, the cotinine level in meconium ranged from 232 to 700 ng/mL with a mean of 367.2 ± 143.7 ng/mL. In the passive smokers group, it ranged from 148 to 350 ng/mL with a mean of 263.4 ± 52.5 ng/mL, while in the non-smokers group it ranged from 153 to 213 ng/mL with a mean of 185.0 ± 24.2 ng/mL. Within the 3 groups, there was a significant difference in the cotinine levels in meconium (F = 10.45, P = 0.01).

There was a positive correlation between the cotinine levels in meconium and in mothers' urine (r = 0.688, P = 0.01) (Figure 2). There was also a significant positive correlation between the cotinine levels in meconium and in mothers' saliva (r = 0.784, P = 0.01) (Figure 3).

The Pearson correlation test showed a significant negative correlation between the cotinine levels in maternal urine and the neonatal birth weight (r = 0.546, P = 0.01) (Figure 4). The study also showed a significant negative correlation between the cotinine level in meconium and birth weight (r = 0.497, P = 0.01) (Figure 5).

Discussion

Cigarettes are the most common non-medicinal drugs used by pregnant women [*12*]. The present study was carried out to evaluate meconium as an alternative biological marker for the detection of the nicotine metabolite cotinine.

Thirty mothers and their neonates were chosen for this study. The small sample size is due to the small number of mothers who confessed that they smoke, as smoking by women is culturally unacceptable in Egypt. The babies were all full term, as gestationally premature babies were ex-

Table 4 **Mean difference in maternal salivary cotinine levels in relation to maternal smoking habit**

Groups compared	Difference in salivary cotinine level (ng/mL) Mean	SE	P-value
Active (n = 10) versus passive (n = 10) smokers	78.15	26.7	0.024
Active (n = 10) versus non-smokers (n = 10)	90.74	26.7	0.008
Passive (n = 10) versus non-smokers (n = 10)	12.59	26.7	0.895

SE = standard error.

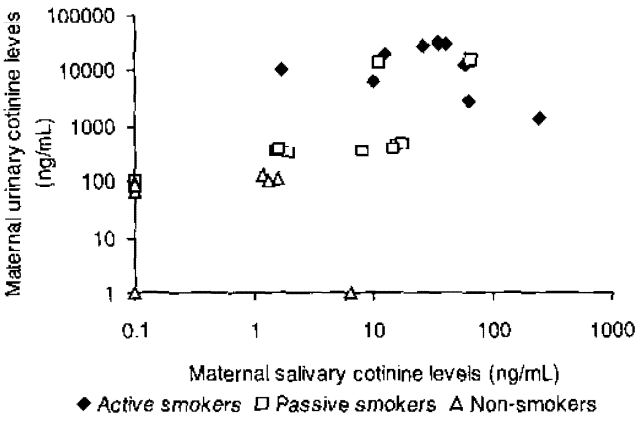

Figure 1 **Correlation between cotinine levels in maternal saliva and maternal urine**

cluded from the study. This was to limit any confusion between the effects of tobacco smoking by the mother during pregnancy and the effects of prematurity.

The maternal data showed that there was no significant difference between the mean age of active smokers, passive smokers and non-smokers. That both smoking and exposure to passive smoking are not confined to a specific age group highlights the general lack of awareness regarding the effect of smoking during pregnancy.

The mean number of cigarettes smoked by the active smoker group was 11.7 per day. This is almost identical to the study of Eliopoulos et al. who found the mean number of cigarettes smoked per day by pregnant smokers was 11.8 [*13*].

In the present study using the Scheffe test, the lowest birth weight was recorded in neonates of active smoking mothers. This is to be expected, as it has been reported that for every 10 cigarettes smoked by mothers, the risk of delivering a low birth weight for gestational age infant increases by a factor of 1.51. The effect is dose dependent, and is not the result of a shortened gestation period, but is due to fetal hypoxia arising from decreased uteroplacental perfusion [*14*]. Neonates of passive smoking mothers also had a significantly lower birth weight than babies of non-smokers in our study, indicating the dangers of passive smoking to neonates.

Table 5 **Cotinine levels in neonate meconium in relation to maternal smoking habit**

Smoking group	Meconium cotinine level (ng/mL)		
	Range	Mean	SD
Active smokers ($n = 10$)	232–700	367.2	143.7
Passive smokers ($n = 10$)	148–350	263.4	52.5
Non-smokers ($n = 10$)	153–213	185.0	24.2
F-test		$F = 10.45$, $P = 0.01$	

SD – standard deviation.

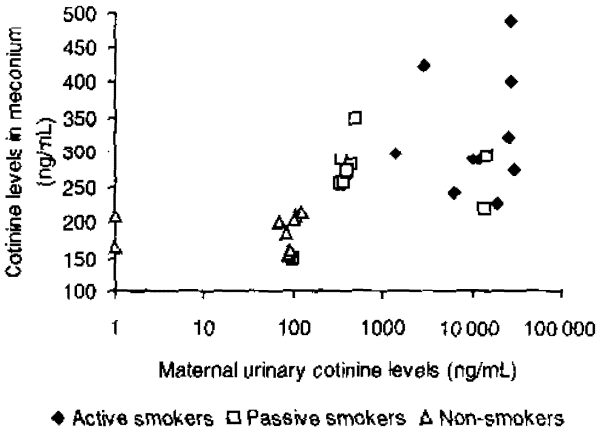

Figure 2 **Correlation between cotinine levels in maternal urine and neonate meconium**

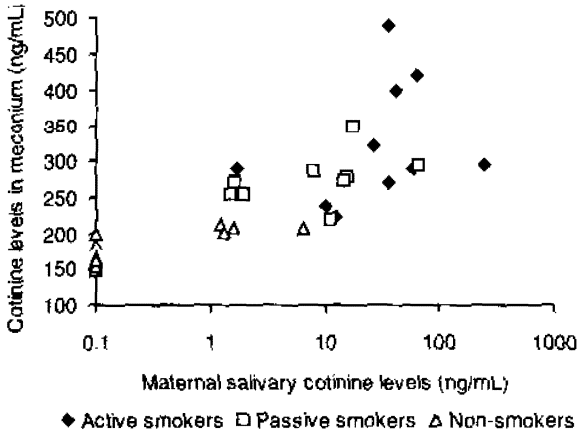

Figure 3 **Correlation between cotinine levels in neonate meconium and maternal saliva**

Cotinine was chosen for detection of smoking as it is the main metabolite of nicotine (70%) [*15,16*]. Its long plasma half-life (30 hours) and protracted excretion rate mean that concentrations in both blood and urine are high [*7,8*]. The present study showed that cotinine was detected in the urine and saliva of active, passive and non-smoking groups, ranging from a mean of 16 110 ng/mL in active smoking mothers to

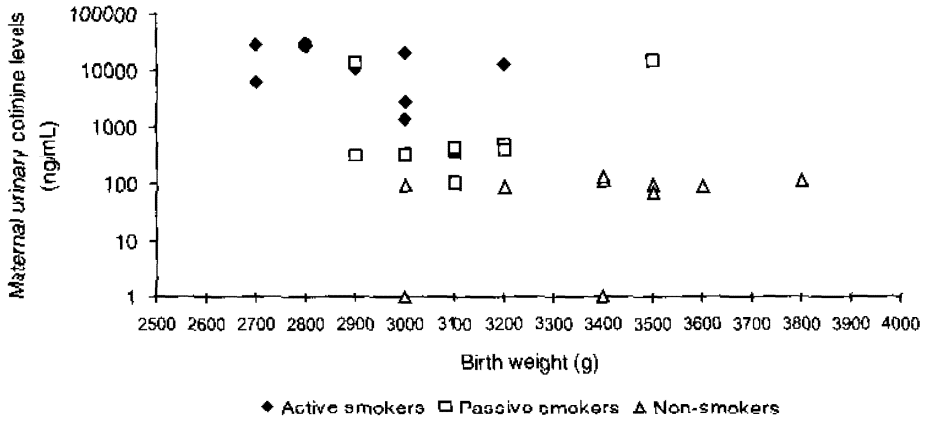

Figure 4 **Correlation between cotinine levels *in maternal urine* and birth weight**

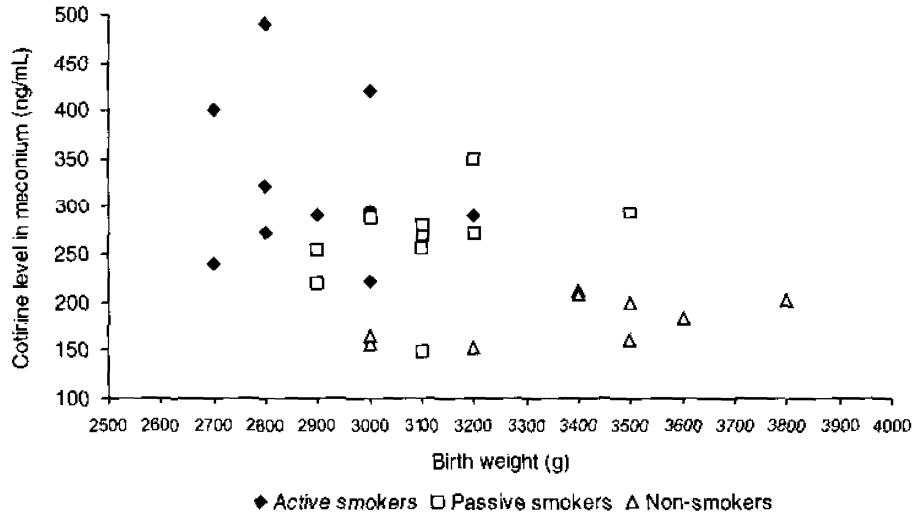

Figure 5 **Correlation between cotinine levels in neonate meconium and birth weight**

3096 ng/mL and 75.1 ng/mL in passive and non-smoking mothers respectively. There was an overlap between the range of maternal urinary cotinine levels in active and passive smokers, which agrees with the study of Wald et al. [*17*]. This may be attributed to variations in exposure, as an active smoking mother may be a light smoker

while a passive smoking mother may be heavily exposed to smoking. The study also revealed a slight overlap between urinary cotinine levels of passive and non-smokers. This is expected if the exposure is slight in the passive group. The presence of cotinine in the urine of non-smokers is not surprising as complete avoidance of cigarette smoke in the environment is almost impossible. Also, there are small amounts of nicotine in common foods such as potatoes, eggplant and tea [*18*].

There were significant differences between the 3 groups in both the mean cotinine levels in mothers' urine and the mean cotinine levels in mothers' saliva. Using the Pearson correlation test, we found a significant positive correlation between cotinine levels in urine and saliva ($r = 0.582$, $P = 0.01$). So mother's saliva could serve as an alternative to urine as a marker for detection of tobacco smoking. It is easier to obtain and difficult to adulterate.

The presence of cotinine in meconium was used as a direct indicator of fetal exposure to tobacco smoking in the neonatal period as cotinine is mainly deposited in meconium through bile secretion and to a lesser extent by fetal swallowing of amniotic fluid containing fetal urine [*15,16*]. The study revealed a significant difference in the cotinine levels of meconium between the 3 groups ($F = 10.45$, $P = 0.01$).

The mean concentration of cotinine in the meconium of neonates of active smokers was significantly higher than in neonates of passive and of non-smokers. But the mean concentration of cotinine in the meconium of neonates of passive smokers was not significantly higher than that of neonates of non-smokers.

Ostrea et al. used RIA to measure nicotine metabolites in the meconium which was extracted by vortex-mixing (0.5–0.6 g) meconium with 10 mL distilled water and 1 mL concentrated HCl, then filtering the homogenate through glass wool and using the centrifuged (9770 × g for 10 minutes) supernatant for the assay. They reported much lower cotinine levels ranging from 10.9 ng/mL in neonates of non-smokers to 54.6 ng/mL in neonates of heavy smokers [*19*], whereas the range of meconium cotinine levels in the present study were from 153 ng/mL in neonates of non-smokers to 700 ng/mL in neonates of active smokers. These differences in the levels could be attributed to either the difference in the extraction method used, as the extraction procedure substantially affects the outcome of the analysis [*20*], or to environmental tobacco smoke exposure in the present study.

As regards babies' birth weight, the Pearson correlation test showed a significant negative correlation between both maternal urinary cotinine levels and neonatal meconium cotinine levels and babies' birth weight. This confirms other studies showing that tobacco smoking leads to low birth weight and that fetal hypoxia increases with increasing exposure of the mother to tobacco smoke [*14*].

Conclusion

Cotinine was detected in maternal urine and saliva and these two biological fluids are good markers for tobacco smoking. Similarly, its presence in meconium proved that meconium is an ideal biological marker for testing direct fetal exposure to tobacco smoking in the neonatal period. This is important as it may throw light on the cause of a neonate with low birth weight. Added to this, collection of meconium is easy and non-invasive.

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Growth charts of Egyptian children with Down syndrome (0–36 months)

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بطاقات نمو الأطفال المصريين المصابين بمتلازمة داون: دراسة للحالات والشواهد

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الخلاصة: تصف هذه الدراسة للحالات والشواهد، منحنيات للنمو وسرعة النمو والطول ومحيط الرأس لـ 350 من الأطفال المصريين المصابين بمتلازمة داون (188 من الذكور و162 من الإناث) ممّن تتراوح أعمارهم بين ساعة الولادة و36 شهراً. وقد لوحظ لدى المصابين بمتلازمة داون متغيرات نمو أقل مما لدى الأطفال الأسوياء الشواهد خلال السنوات الثلاث الأولى من حياتهم. ويعاني الأطفال المصابون بمتلازمة داون من نقص أكبر بوضوح في الوزن ولاسيّما لدى الإناث منهم إذا ترافق ذلك بمرض قلبي خلقي (لدى 90 حالة) وذلك بالمقارنة بأقرانهم من غير المصابين بمرض قلبي خلقي. وخلال السنتين الأوليين كانت سرعة النمو بالنسبة للوزن ومحيط الرأس أعلى لدى الذكور. لقد كان لدى الذكور المصابين بمتلازمة داون سرعة في الطول أعلى بقليل مما لدى الشواهد خلال السنوات الثلاث الأولى من العمر.

ABSTRACT A study established growth and growth velocity curves for weight, length and head circumference in 350 Egyptian Down syndrome children (188 males and 162 females) from 0–36 months. Down syndrome children had poorer growth variables than normal healthy children through the first 3 years of life. Down syndrome children with associated congenital heart disease (90 cases) had significantly lower weight, especially in girls, compared with those without heart disease. In the first 2 years, growth velocity for weight and head circumference were higher in Down syndrome females than males, while growth velocity for length was higher in males. Down syndrome boys had slightly higher velocity of length than normal children in the first 3 years of life.

Les courbes de croissance des enfants égyptiens atteints du syndrome de Down (âgés de 0 à 6 mois)

RESUME Une étude a permis d'établir les courbes de croissance et de vitesse de croissance pour le poids, la taille et le périmètre crânien chez 350 enfants égyptiens, âgés de 0 à 36 mois, qui étaient atteints du syndrome de Down (188 garçons et 162 filles). Ces enfants avaient des variables de croissance inférieures à celles des enfants normaux en bonne santé pendant les trois premières années de la vie. Les enfants atteints du syndrome de Down qui présentaient une cardiopathie congénitale associée (90 cas) avaient un poids significativement plus faible, particulièrement les filles, que ceux ne présentant pas de cardiopathie. Au cours des deux premières années, la vitesse de croissance pour le poids et le périmètre crânien était plus élevée chez les filles atteintes du syndrome de Down que chez les garçons, tandis que la vitesse de croissance staturale était plus élevée chez les garçons. Les garçons atteints du syndrome de Down avaient une vitesse de croissance staturale légèrement plus élevée que les en enfants normaux au cours des trois premières années de la vie.

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Introduction

Down syndrome (trisomy 21) is the most prevalent unbalanced chromosomal aberration seen in live births, with an incidence of 1 per 600 live births or 1 per 150 conceptions [1]. It results in moderate to severe mental retardation, high risk of congenital heart disease and other multiple malformations and/or various medical problems. These congenital malformations have a consistent effect on physical development [2]. Growth retardation is one of the cardinal features of Down syndrome, characterized by deficient prenatal growth, i.e. reduced by 0.5–1.5 standard deviations (SD) from normal control mean values, and extending postnatally through the end of growing at 3–5 years of age [3]. Progressive de-institutionalization of Down syndrome children and their integration into the community, with improved medical and psychological follow-up, have greatly changed their lives and lengthened their life span [4,5].

Although growth is influenced by biological and environmental factors, racial variations certainly have a major role. The publication of growth charts specifically for children with Down syndrome in various populations, e.g. American, Sicilian, Dutch and French [3,6–8], has drawn attention to the importance of constructing growth charts for Egyptian Down syndrome children. The potential benefits of growth charts include: growth monitoring to detect any deviation in growth patterns, evaluating the efficacy of measures aimed at promoting growth, providing reassurance to parents, evaluating the results of clinical research or intervention for individual patients and, finally, comparing their growth with that of the normal population.

This study aimed to establish growth curves for Egyptian children with Down syndrome aged 0–36 months to investigate and characterize their size, monitor their growth and evaluate the effect of congenital heart disease on their growth pattern. The present study provides reference data (growth charts) for weight, length and head circumference for Egyptian children with Down syndrome covering the age range 0–36 months based on cross-sectional and longitudinal data.

Methods

The study was carried out between January 1999 and July 2001. The data for this study were based on 1700 observations of 350 Down syndrome children: 188 boys (53.7%) and 162 girls (46.3%) aged 0–36 months with free trisomy 21. All were cases referred to the Human Genetics Clinic at the National Research Centre for diagnosis, genetic counselling and/or attendance at early intervention and stimulation programmes. The data represent an unselected, therefore presumably unbiased, sample of children with Down syndrome in Egypt. The Down syndrome children were divided into 2 groups: group 1 was 260 children without congenital heart disease (143 males and 117 females) and group 2 was 90 children with congenital heart disease (45 males and 45 females).

Each patient underwent pedigree analysis, meticulous clinical examination and basic anthropometric measurements. Chromosomal analysis by G-banding technique, complete thyroid profile and electrocardiographic (ECG) examination were done in all patients. Patients with mosaic 21 or translocation and those with malabsorption, hypothyroidism or severe congenital heart disease were excluded. Down syndrome patients with associated mild and moderate congenital heart disease were included in

the study. Children with mild congenital heart disease had a single cardiac defect without pulmonary vascular involvement and did not require medication or surgery. Those with moderate congenital heart disease had more complex cardiac defects, often requiring digitalis, while severe congenital heart disease consisted of serious anatomic heart lesions, e.g. tetralogy of Fallot. All studied Down syndrome children lived with their family members.

Growth measurements (weight, length, head circumference) were taken at 3-monthly intervals. Measurements were taken by trained physicians and a second person assisted in alignment and immobilization of the child during the measurements. Length was measured to the nearest millimetre using a recumbent length board infant measuring table. The weight was assessed by a sensitive balance scale to the nearest gram. Head circumference was measured to the nearest millimetre by a non-stretchable plastic tape taking the maximum occipitofrontal diameter. The recorded measurements for weight, length and head circumference represent a combination of cross-sectional and longitudinal data.

The data for Down syndrome children were compared with data obtained from measurements on normal healthy Egyptian children (2735 girls and 3315 boys) from birth to 36 months visiting Cairo hospitals over 1996–2000 (Egyptian growth charts, published by the Faculty of Medicine, Cairo University and the National Research Centre, Cairo, Egypt).

Growth curves for weight, length and head circumference comparing male and female Down syndrome cases (group 1) with normal children were plotted using polynomial curves. Baker and his co-workers found that polynomial curves adequately represent the growth pattern [9].

Growth velocities for weight, length and head circumference were calculated for 6-monthly intervals for group 1. The mean value for each age interval was subtracted from that for the subsequent age interval. Growth velocities for Down syndrome cases were compared with the growth velocities of normal children.

Statistical analysis of data was carried out, using *SPSS* software, version 6. Based on age and sex, the data were divided into monthly intervals since birth until 36 months. Descriptive statistics and percentiles (3rd, 50th, 97th) were estimated for weight, length and head circumference for each sex for group 1. Means and standard deviations were calculated for group 2. Student *t*-test was used to compare the mean of weight, length and head circumference at all age intervals between the 2 sexes, and between the 2 groups and the normal population. P values < 0.05 were considered significant.

Results

At birth, all Down syndrome children in the study showed lower mean anthropometric values than that of the normal children. For weight, length and head circumference the values were: –1.6 SD, –2.2 SD and –1.8 SD for boys, and –1.7 SD, –2.8 SD and –3.5 SD for girls respectively (Table 1).

Of the 90 Down syndrome patients with associated congenital heart disease (group 2), common atrioventricular canal was the most common congenital heart disease seen in 34.4% of our sample (31 cases), followed by ventricular septal defect in 26.7% (24 cases) and multiple cardiac anomalies in 13.3% (12 cases). These Down syndrome patients with congenital heart disease had significantly lower weight, especially in girls, than those with-

Table 1 **Mean anthropometric measurements for Down syndrome children at birth compared with normal children**

Variable	Males			Females		
	Down syndrome Mean	SD	Normal Mean	Down syndrome Mean	SD	Normal Mean
Weight (kg)	2.97	–1.6	3.90	2.86	–1.7	3.80
Length (cm)	49.3	–2.2	54.0	48.1	–2.8	53.3
Head circumference (cm)	34.8	–1.8	36.6	32.7	–3.5	36.5

n = *total number of children.*

out congenital heart disease ($P = 0.02$) (Table 2).

In comparison with the normal population, patients with Down syndrome in group 1 and group 2 showed lower values of weight, length and head circumference. In boys of group 1, the mean weight, length and head circumference were reduced by 1.5 SD, 1.6 SD and 1.8 SD below the mean of the normal boys respectively. The girls showed a reduction of 1.6 SD, 1.7 SD and 1.8 SD below the mean of normal girls for weight, length and head circumference. Down syndrome boys of group 2 showed mean values reduced by 2.8 SD, 2.2 SD and 1.8 SD for weight, length and head circumference respectively; the girls had reductions of 2.8 SD, 2.9 SD and 1.9 SD respectively.

Males and females of group 2 had significantly lower mean weight versus group 1 patients ($P = 0.03$ and $P = 0.02$ respectively). They also had lower values for mean length and head circumference but these were not statistically significant compared with group 1 (Table 2).

In all age stages, Down syndrome boys of group 1 and group 2 showed higher val-

Table 2 **Mean anthropometric measurements for male and female Down syndrome children with and without congenital heart disease**

Variable	Down syndrome males			Down syndrome females		
	No heart disease ($n = 143$)	Heart disease ($n = 45$)	*P* value	No heart disease ($n = 117$)	Heart disease ($n = 45$)	*P* value
Weight (kg)	9.3	7.8	0.03*	8.6	7.1	0.02*
Length (cm)	73.8	71.9	0.50	70.8	68.5	0.30
Head circumference (cm)	43.7	42.0	0.06	42.1	41.3	0.40

n = *total number of children.*
*$P < 0.05$.

ues for weight, length and head circumference when compared with girls (Table 2). However, these values did not reach statistical significance.

Growth charts at the 3rd, 50th, and 97th percentiles from 0–36 months for male and female Down syndrome patients (group 1) were constructed and compared

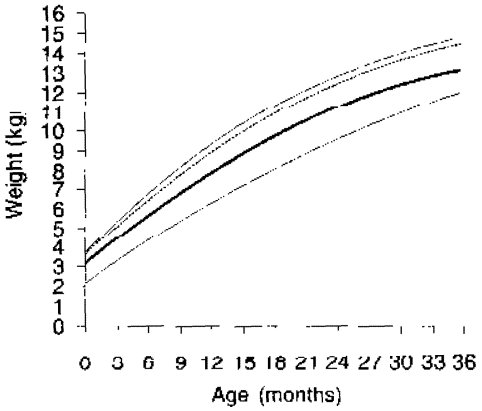

Figure 1 **Weight growth charts (0–36 months) for males with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian males (dotted line)**

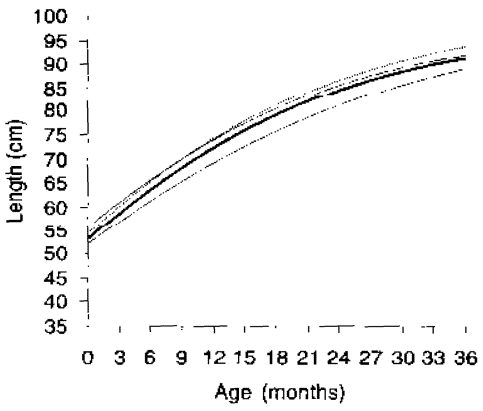

Figure 3 **Length growth charts (0–36 months) for males with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian males (dotted line)**

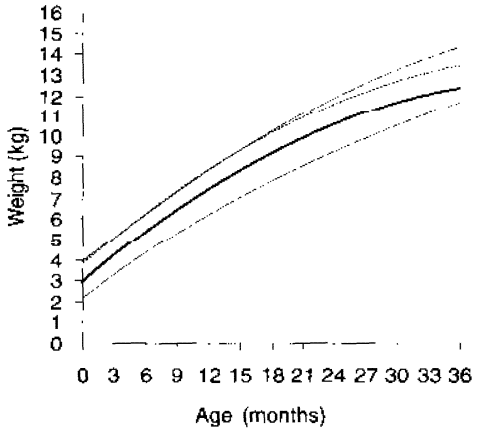

Figure 2 **Weight growth charts (0–36 months) for females with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian females (dotted line)**

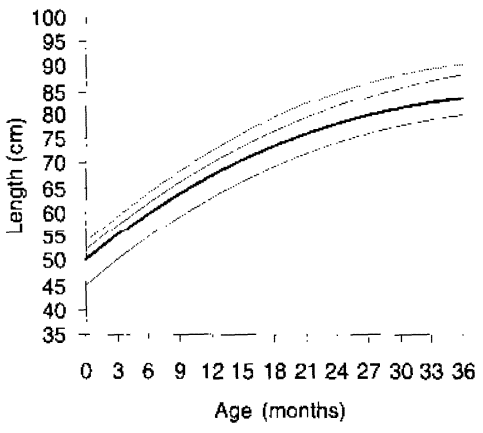

Figure 4 **Length growth charts (0–36 months) for females with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian females (dotted line)**

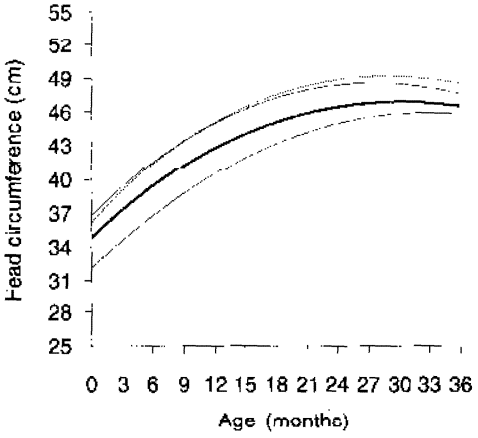

Figure 5 **Head circumference growth charts (0–36 months) for males with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian males (dotted line)**

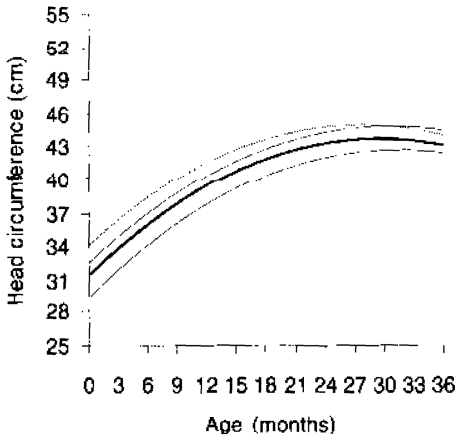

Figure 6 **Head circumference charts (0–36 months) for females with Down syndrome (solid lines) compared with 50th percentile of normal Egyptian females (dotted line)**

with the 50th percentile for the normal group (Figures 1–6).

Growth velocity for Down syndrome girls (calculated for 6 monthly intervals) showed higher values of weight and head circumference versus Down syndrome boys. However, both sexes reached the same values by the end of the third year. Growth velocity of weight for Down syndrome cases was higher than the normal children until the 12 and 18 months of life in girls and boys respectively. Then it became lower until 24 and 33 months for girls and boys respectively (Figures 7 and 8).

Velocity of length for Down syndrome girls was lower than the normal group; however, the greatest deficiency was between 15 and 33 months (Figure 10). On the other hand, Down syndrome boys showed velocity of length slightly higher than the normal population at all age intervals (Figure 9).

Velocity of head circumference for Down syndrome girls showed higher values than the normal group until age 12 months. Then it was lower until 24 months and, after that it showed higher values than the normal population. A similar trend was seen in Down syndrome boys (Figures 11 and 12).

Discussion

We have produced the first growth charts relevant to children with Down syndrome in Egypt from birth until 3 years old in both sexes. Current data indicate that growth patterns of children with Down syndrome differ from those of normal children during the first 3 years of life.

At birth, the growth pattern of Egyptian children with Down syndrome ranged from −1.6 to −3.5 below the mean of the normal population. The same tendency was reported in French babies with Down syndrome at birth [*8*]. On the other hand, growth reduction by 0.5–1.5 SD from nor-

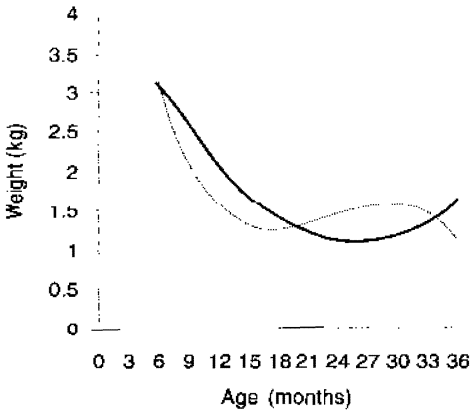

Figure 7 **Weight velocity curves for males with Down syndrome (solid line) compared with normal Egyptian males (dotted line)**

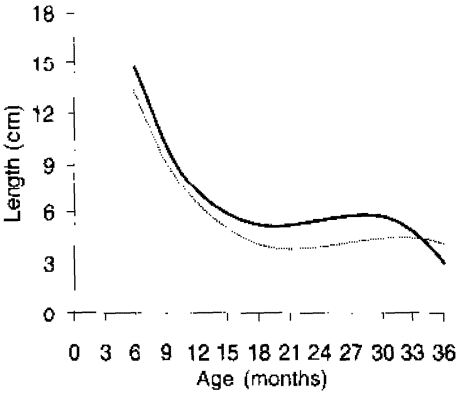

Figure 9 **Length velocity curve for males with Down syndrome (solid line) compared with normal Egyptian males (dotted line)**

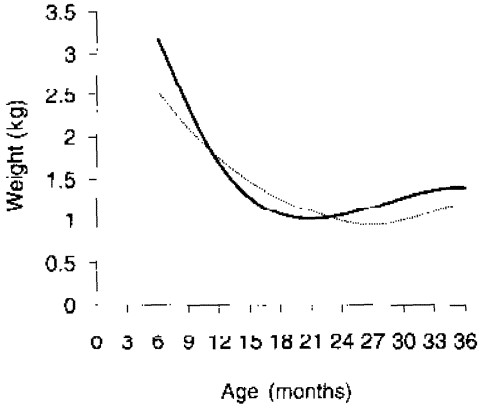

Figure 8 **Weight velocity curve for females with Down syndrome (solid line) compared with normal Egyptian females (dotted line)**

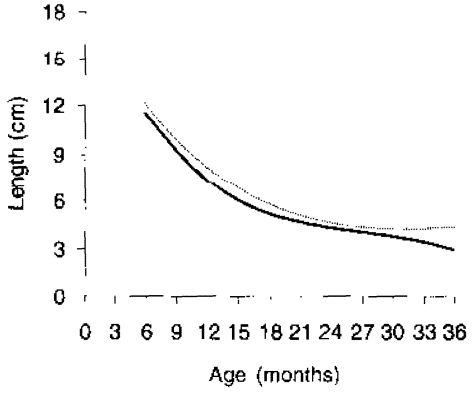

Figure 10 **Length velocity curve for females with Down syndrome (solid line) compared with normal Egyptian females (dotted line)**

mal control means was reported in American children [*3*]. This could be due to racial variations. However, our results confirm that the growth retardation started prenatally and was more severe in cases with Down syndrome with congenital heart disease, which was the same observation of Wessels et al. [*10*].

All through the first 3 years of life, growth charts showed that male and fe-

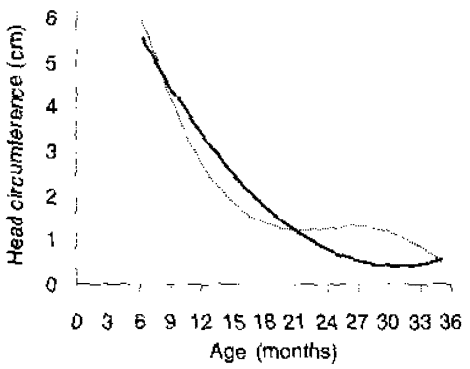

Figure 11 **Head circumference velocity curve for males with Down syndrome (solid line) compared with normal Egyptian males (dotted line)**

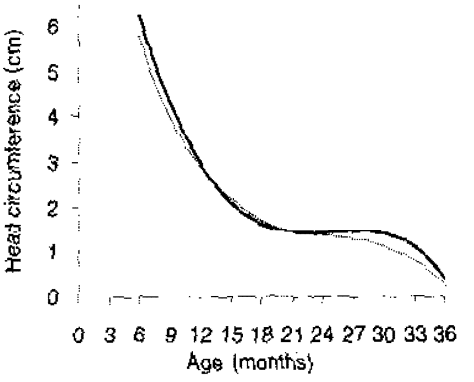

Figure 12 **Head circumference velocity curve for females with Down syndrome (solid line) compared with normal Egyptian females (dotted line)**

male patients with Down syndrome had lower growth parameters compared with normal children. Similar results have been documented by other researchers [*3,6-8,11*], which demonstrate the growth retardation effect of trisomy 21. Further analysis of the current growth charts revealed that growth impairment in Down syndrome is not severe and that children with Down syndrome are less likely than normal children to remain at a given percentile level.

The growth retardation ranged from −1.5 to −1.8 SD in Down syndrome children without congenital heart disease and −1.8 to −2.9 SD in Down syndrome children with congenital heart disease compared with a group of normal children from 1 to 36 months. These results demonstrate a trend toward better growth in Down syndrome children without cardiac anomaly and add weight to the effect of mild to moderate congenital heart disease on the growth retardation seen in Down syndrome patients having cardiac anomalies. Both boys and girls with congenital heart disease showed significant weight reduction compared with Down syndrome children without cardiac anomalies ($P = 0.03$ and $P = 0.02$ respectively). However, values of length and head circumference did not reach the statistical significance. It seems likely, therefore, that the influence of congenital heart disease in our sample affected weight more than the other parameters. These results are in concordance with those reported before [*3,12*].

At all age stages, Down syndrome boys, either with or without congenital heart disease, showed higher growth parameters than girls. However, no statistical significance between the 2 sexes was noted. This is in agreement with the American, Sicilian, Swedish and English Down syndrome growth charts showing significantly higher length and head circumference in boys versus girls [*3,4,6,13,14*].

Most published data showed deficient growth velocity all through the first 3 years of life but these authors did not comment on sex differences in growth velocity [*3,8,12*]. Interestingly, our results demonstrated higher growth velocity in children with Down syndrome versus normal children during the first 12–18 month of life, showing higher values in girls than boys (except for length). While during the 2nd year of life reduced growth velocity was equal in both sexes, by the 36th month of life growth velocity of Down syndrome patients reached values slightly lower than the normal population. The increased growth velocity, especially during the 1st year of life, could be due to the great care that our patients were receiving from their families, and the proper nutritional and medical consultations applied to their lifestyle. All our patients lived with their families and most of them were breastfed during the first 12–18 months of their life. This finding highlights the important role of home care and breastfeeding and their effect on growth. Leonard and his co-workers attributed a similar observation to emotional support of the family and increased nutritional adequacy [*15*].

In conclusion, growth charts at all percentiles showed reduced patterns of growth in Down syndrome compared with normal children. The correct use of growth charts specific for Down syndrome can help highlight a physical developmental delay and suggest the need to look for concomitant diseases affecting growth. In addition, comparing our growth velocity with other results emphasizes the importance of breastfeeding and de-institutionalization during the early years of life. Our data also confirm the negative influence of congenital heart disease on growth patterns, especially on weight gain.

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The Work of WHO in the Eastern Mediterranean Region: Annual Report of the Regional Director 1 January–31 December 2003

This report (published in both Arabic and English) describes the areas of work carried by the WHO Regional Office for the Eastern Mediterranean for the year 2003. It can be obtained from: Distribution and Sales, World Health Organization Regional Office for the Eastern Mediterranean, Abdul Razzak Al Sanhouri Street, PO Box 7608, Nasr City, Cairo 11371, Egypt. Telephone: (202) 670 25 35; Fax: (202) 670 24 92/4. Both the English and Arabic versions are available free on line at: http://www.emro.who.int/rd/annualreports/2003/index.htm and http://www.emro.who.int/rd/annualreports/2003/arabic/index.htm

Fragile X syndrome: a clinico-genetic study of mentally retarded patients in Kuwait

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متلازمة الصبغي X الهش: دراسة سريرية وراثية على مرضى متخلّفين عقلياً في الكويت

ليلى بستكي، فاطمة حجازي، مها الهنيدي، ناديا تركي، أيمن عرب، كمال نجيب

الخلاصة: أجريت في الكويت دراسة استباقية للتحري، شملت 182 من الذكور المصابين بالتأخر العقلي الذين استوفوا خمساً أو أكثر من المعايير السريرية لتشخيص متلازمة الصبغي X الهش، وذلك باستخدام اختبار التفاعل السلسلي للبوليميراز. وقد وجد أن عشرين مريضاً (11٪) على درجة عالية من الشك بأنهم مصابون بمتلازمة الصبغي X الهش الناجمة عن طفرة في موقع FRAXE، ولم يكن لدى أي من المدروسين طفرة في الموقع FRAXE. ومن بين العشرين كان 11 منهم (55٪) ممن تأكد أن لديه متلازمة الصبغي X الهش بتطبيق كل من أسلوب التفاعل السلسلي للبوليميراز والأسلوب الخلوي الوراثي. وقد كانت أهم الملامح السريرية تكرراً: تبارز الجبهة (100٪) وضخامة الأذنين (90٪) وتبارز الفك السفلي (90٪) وفرط الحركة (85٪). أما الملامح الأقل شيوعاً فتجنب التواصل بالعينين (45٪)، الذاتوية (45٪) والاختلاجات (30٪)، وكانت الخصيتان ضخمتين في 55٪ من الحالات. وكانت المعايير السريرية في فترة ما قبل البلوغ وما بعده مختلفة.

ABSTRACT In a prospective study in Kuwait, 182 mentally retarded male patients who fulfilled 5 or more clinical criteria of fragile X syndrome were screened using polymerase chain reaction (PCR) testing. Twenty patients (11%) were highly suspected of having fragile X syndrome due to mutation at the *FRAXA* locus; none had mutation at the *FRAXE* locus. Of these, 11 (55%) were confirmed fragile-X-positive by both cytogenetic and PCR techniques. The most frequent clinical features were: prominent forehead, high arched palate, hyperextensible joints, long ears, prominent jaw, height > 10th centile and attention-deficit hyperactivity. Less common were avoidance of eye contact (45%), autism (45%) and seizures (30%). Large testes were found in 55% of cases. Pre-pubertal and post-pubertal clinical criteria were different.

Syndrome du chromosome X fragile: étude clinico-génétique chez des patients présentant un retard mental au Koweït

RESUME Dans une étude prospective au Koweït, 182 patients de sexe masculin présentant un retard mental qui remplissaient 5 ou plus des critères cliniques du syndrome de l'X fragile ont fait l'objet d'un examen PCR (amplification en chaîne par polymérase). Vingt patients (11 %) étaient fortement suspectés d'être atteints de ce syndrome du fait d'une mutation au niveau du locus *FRAXA* ; aucun patient n'avait de mutation au niveau du locus *FRAXE*. Les techniques de cytogénétique et de PCR ont permis de confirmer que 11 (55 %) de ces patients étaient X-fragiles. Les caractéristiques cliniques les plus courantes étaient un front proéminent, un palais ogival, un relachement des articulations, de grandes oreilles, une mâchoire proéminente, une taille superiente a cilli correspondant au 10e contile et une hyperactivité avec troubles de l'attention. Le fait d'éviter le contact visuel (45 %), l'autisme (45 %) et les convulsions (30 %) étaient moins courants. On a observé dans 55 % des cas une macro-orchidie. Les critères cliniques prépubertaires et postpubertaires étaient différents.

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Introduction

Fragile X syndrome is the second most common cause of inherited mental retardation with an estimated prevalence of 0.4–0.8 per 1000 males and 0.2–0.6 per 1000 females [*1*]. More recent studies using molecular genetic testing of the gene for fragile X have estimated a prevalence of 16:100 000 to 25:100 000 males affected with the syndrome [*2–4*]. The syndrome is mainly characterized by a variable degree of mental retardation, typical long and narrow facial appearance, large ears and large testes [*5,6*]. It is inherited as an X-linked dominant trait with reduced penetrance, i.e. only 80% of carrier males and 30% of carrier females are affected [*7*]. The responsible gene was identified in 1991 and was designated as 'fragile X mental retardation gene 1' (*FMR-1*) [*8*]. The fragile site was located at Xq27.3 and designated as *FRAXA*, which can be observed in the metaphase chromosome following selective culture conditions. Three other fragile sites, 1 proximal and 2 distal to *FRAXA*, have been cloned and termed *FRAXD*, *FRAXE* and *FRAXF* respectively.

Chromosome analysis using modified culture technique to induce fragile sites is no longer used due to its low sensitivity and increased costs compared with DNA-based techniques. Direct analysis of the CGG expansion mutation by Southern blotting has begun to replace cytogenetic analysis for the laboratory diagnosis of fragile X syndrome as it detects all the repeat expansion mutations including both full and premutation. However, blotting is a relatively expensive and labour-intensive procedure, particularly in the context of screening routine referrals.

The non-radioactive polymerase chain reaction (PCR) method specific for *FMR1* gene mutation detection is a very rapid test and has high sensitivity for normal and lower premutation repeat size. However, potential misdiagnosis from false negatives is rare due to cellular mosaicism.

The aim of the present study was to apply PCR testing for the first time in Kuwait and use it as a screening tool for detection of fragile X syndrome among a group of mentally retarded male patients who had clinical signs of the syndrome.

Methods

This prospective study in the Kuwait Medical Genetics Centre, Kuwait, started in January 2000 and lasted for 30 months.

Clinical study

The participants were 182 male patients referred with mental retardation of unknown etiology for clinico-genetic evaluation and diagnosis. A preconstructed sheet was used to record the following: nationality, age, parental age at patient's birth, consanguinity, birth weight height, occipto-frontal circumferance, craniofacial features, dermatological findings, skeletal findings, neurological and psychological features, speech, hyperactivity and the external genitalia. Associated anomalies and pedigree study were included too. Neurological and psychometric evaluations were conducted on each patient. Cognitive ability was assessed using the Wechsler Intelligence Scales (for Children or Adults). The severity of mental retardation was categorized into one of 3 groups according to the intelligence quotient [IQ] score: mild (50–70), moderate (35–50) and severe (20–35).

Patients were selected for the study if they fulfilled 5 or more criteria out of the most common 10 criteria associated with fragile X syndrome: mental retardation of unknown cause, family history of mental

retardation, large ears (ear length > 7.0 cm), large testes (testicular volume > 25 mL), long narrow face (inner canthal distance < 3.5 cm), prominent ears/jaws, high arched palate, calluses on hand, hyperactivity, avoidance of eye contact.

After informing the parents about the purpose of the study, peripheral vein blood samples (5 mL) were taken from each patient and stored in tubes with EDTA anticoagulant. Cytogenetic analysis was performed on blood samples cultured for 96 hours in folate-deficient tissue culture medium 199 with 5% fetal bovine serum.

Laboratory testing

Blood samples were obtained from healthy individuals for calibration of the test. The deoxyribonucleic acid (DNA) of the patients and control subjects was extracted from blood samples. The concentration and purity of DNA were measured in a PCR reaction before use. Two sets of primers were used for mutation detection of the *FRAXA* (*FXD1* and *FXE*) and *FRAXE* (598 and 603) loci. The primers were synthesized locally in our laboratory using 391 DNA synthesizers.

For amplification of the triplet repeat sequences at the *FRAXA* and *FRAXE* loci, the total volume of PCR mix was 25 µL, containing 100 mg of DNA mixed with 20 pmol of *FXD1* and *FXE* and 35 pmol of 598 and 603 primers to amplify *FRAXA* CCG and *FRAXE* CCG repeats respectively. It also contained: 2.5 µL of 10X polymerase buffer (Taq, BioCarta, San Diego, California, USA); 2.5 µL dimethyl sulfoxide; 200 µmol/L from each of dATP (deoxyadenosine 5'-triphosphate), dCTP (deoxycytidine 5'-triphosphate), dTTP (deoxythymidine 5'-triphosphate), 100 µmol of dGTP (deoxyguanosine 5'-triphosphate), 100 µmol 7-deaza-2-dGTP; and 0.25 µL (1.25 unit) of DNA polymerase enzyme (AmpliTaq Gold, BioCarta, San Diego, California, USA). The amplification was carried out using the Gene Amp PCR System 9700 (Applied Biosystems, Foster City, California, USA). The PCR was started by DNA denaturation for 10 min at 95 °C followed by 40 cycles of 95 °C for 1 min, 65 °C for 1.30 min and 72 °C for 2 min with a final extension for 7 min at 72 °C.

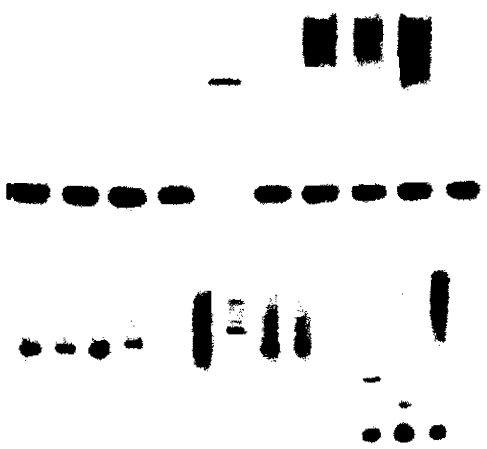
A total of 15 µL of PCR product were analysed by electrophoresis using 2% agarose gel, 1% agarose and 1% low melting agarose gel (Nusieve GTG, Cambrex, East Rutherford, New Jersey, USA) containing 0.5 µg/mL ethidium bromide.

Results

Out of 182 mentally retarded patients, 20 patients proved to be positive for fragile X syndrome by the PCR technique, giving an incidence of 11%. Figure 1 shows the amplification products of FRAXA and FRAXE.

Table 1 shows the clinical features of the fragile-X-positive patients. The frequency of siblings (85%) and relatives (70%) affected with fragile X syndrome was high. The most frequent clinical features among our patients were: mental retardation (100%), prominent forehead (100%), hyperextensible joints (100%), high arched palate (100%), large ears (90%), prominent jaw (90%), height > 10th centile (90%), attention-deficit hyperactivity (85%), stereotyped speech (85%) and biting hand movements (85%). Large testes (55%), avoidance of eye contact (45%), autistic-like behaviour (45%) and seizures (30%) were recorded less frequently (Table 1).

There were some differences between pre- or post-pubertal patients. All post-pu-



Lanes 1,4,6 and 7 are negative DNA samples of PCR products within normal range of repeat size (up to 224 bp)

Lanes 2,3,5 and 8 are suspected to be positive DNA samples with *FRAXA* mutation showing failure of amplification due to high repeat expansion.

Lanes 10 to 17 are amplification products of *FRAXE* locus of the same DNA samples showing that all cases are negative for *FRAXE* mutation

Lanes 19 to 22 are PCR amplification products of *FRAXA* locus using C7d GTP-For more confirmation of positive DNA samples

Lanes 9 and 18 are negative controls without DNA

Figure 1 Amplification products of *FRAXA* and *FRAXE*

Table 1 **Characteristic features of mentally retarded patients positive for fragile X syndrome by PCR**

Features	No. of patients	%
Stage of puberty		
Post-pubertal	11	55
Pre-pubertal	9	45
Family		
Siblings with fragile X	17	85
Relatives with fragile X	14	70
Behavioural characteristics		
Mental retardation	20	100
Attention-deficit hyperactivity	17	85
Stereotyped speech	17	70
Biting hand movements	17	70
Autistic behaviour	9	45
Avoidance of eye contact	9	45
Seizures	6	30
Physical characteristics		
Prominent forehead	20	100
High arched palate	20	100
Hyperextensible joints	20	100
Ear length > 75th centile	18	90
Prominent jaw	18	90
Height > 10th centile	18	90
Birth weight > 3 kg	17	85
Dry skin	15	75
Head circumference > 50th centile	13	65
Large testes	11	55
Flat feet	6	30
Gynaecomastia	5	25

No cases were found of abnormal heart, pectus excavatum or kyphosis.
n = *number of patients.*

bertal patients had macro-orchidism (100% of 11). The most prominent features among the 9 pre-pubertal fragile-X-positive patients were: prominent forehead, 100%), hyperactivity (100%), hyperextensible joints (89%), large ears (89%), high arched palate (89%), prominent jaw (78%), avoidance of eye contact (56%), stereotype speech (56%), autistic behaviour (33%) and seizures (22%). There was no difference in the severity of mental retardation among pre-pubertal and post-pubertal groups. Mild and severe mental retardation were equally found (around

45% in both groups), while moderate mental retardation was found in 1 patient in each group (around 10%).

There were major differences between the percentage frequency of the criteria among the positive fragile X patients and mentally retarded patients negative for fragile X (Table 2).

Cytogenetic analysis detected only 11 cases of fragile X syndrome (55% of the cases positive by PCR), an incidence of 6% among mentally retarded patients.

Discussion

Fragile X syndrome is the second most common cause of inherited mental retardation and is characterized by relative macrocephaly or normocephaly, variable degree of mental retardation, typical long and narrow facial appearance, large ears and large testes [*1,5,6*]. The dysmorphic features are seldom severe and many males in the past were referred purely with mental retardation.

The population prevalence of fragile X syndrome has been reported to vary from 0.4–0.8 per 1000 in males and 0.2–0.6 per 1000 in females [*3,9,10*]. More recent studies using molecular genetic testing of *FMR-1* have estimated a prevalence of 16:100 000 to 25:100 000 males affected with fragile X syndrome [*2–4*]. The prevalence of females affected with fragile X syndrome is presumed to be approximately one-half of the male prevalence. A population based prevalence study of affected African–American males revealed a higher estimate, 39:100 000 to 78:100 000 at 95% confidence interval [*11*].

Among mentally retarded patients, the incidence of fragile X syndrome varies from 3.5% to 8% [*12,13*], an incidence lower than that reported here (11%). Our higher incidence may be due to selection of the patients based on the most prominent criteria of fragile X syndrome, which increases the likelihood of finding fragile X positives. Alternatively, the PCR technique might increase the detection rate: we found 11% of mentally retarded patients were

Table 2 **Frequency of fragile X traits in fragile-X-positive and -negative mentally retarded patients**

Clinical sign	Fragile-X-positive ($n = 20$) %	Fragile-X-negative ($n = 162$) %	*P*-value
Long narrow face	100	38	< 0.0001
High arched palate	100	21	< 0.0001
Large ears	90	29	< 0.0001
Hyperactivity	85	18	< 0.0001
Large testes	55	10	< 0.0001
Avoidance of eye contact	45	7	< 0.0001

n = *number of patients.*

positive using PCR compared with 6% using cytogenetic analysis. The symptoms and signs of mental retardation are variable, and hyperactivity and seizures are common features. The degree of mental retardation varies from mild to severe, depending on the age group of the selected cases [*14–16*].

Brain scans in fragile X syndrome are usually normal. However, Mostofosky et al. studied 32 males with magnetic resonance imaging (MRI) and found the size of the cerebral posterior vermis was decreased, the hippocampus enlarged and the fourth ventricle increased [*17*]. A low frequency of fragile X cases (0.5%) was found among males with unexplained learning difficulties and language delay [*2,18,19*].

There are some specific features associated with fragile X syndrome. These are not necessarily found in all patients at different age groups and the frequency of each feature is age dependent. The most suggestive criteria for the diagnosis of fragile X syndrome found in this and other studies were: mental retardation, a family history of mental retardation, large or prominent ears, an enlarged face, attention deficit hyperactivity disorder and autistic-like behaviour. If a patient had 5 of these features then no case of fragile X would have been missed.

The frequency of macrocephaly (circumference > 50th centile) in our study was low (65%) compared with other reports [*20*] that the single most useful clinical criterion is head circumference above the 90th centile.

Macro-orchidism is difficult to identify early in life and it is frequently absent in the pre-pubertal period. The frequency of enlarged testes in our study was 55% overall, 100% in post-pubertal patients. This finding is consistent with other studies [*1,5,6,13*]. Accordingly, the presence of macro-orchidism is not necessary for the diagnosis of fragile X syndrome in the prepubertal child. The frequency of macro-orchidism in fragile X syndrome varies from 11% to 20% [*1,21*].

Other studies have suggested a relationship between autism and fragile X syndrome. However, a molecular study of 141 patients showed no association of autism with fragile X syndrome and the Xq27 region is not a candidate gene for autism [*22*]. Nevertheless, the present study showed a high incidence (45%) of patients who had autistic-like behaviour and most of them were in the post-pubertal stage. Other authors have reported a lower incidence of autism in fragile X syndrome (10.7%) [*1*].

Familial cases of fragile X have been reported before [23] but were not as high as reported in our study (85% and 70% of patients had affected siblings and relatives respectively). This incidence represents the frequency of fragile X syndrome among the siblings and relatives of fragile X patients themselves and not among the mentally retarded patients. Genetically, all mothers of isolated male cases of fragile X must be assumed to be carriers of a permutation or full mutation and about one-third of carrier females are retarded [24]. However, around 70% of females with a full mutation have below average IQ (less than 85%) [*25*]. Premutation was found to be behind the phenomenon of phenotypically normal transmitting males with no fragile sites [*21,26*]. This was confirmed by the discovery of an unstable CCG trinucleotide repeat sequence in the gene (*FMR-1*) [*27,28*]. Repeat length appears to be an important but not sufficient condition leading to instability of the *FMR-1* gene [*29,30*]. It has been suggested that expansion of the CCG trinucleotide repeat occurs during

early development and not during meiosis [*31,32*]. In males carrying full mutation, only sperm carried the premutation. However Malter et al. looked at the gonads of the fetuses carrying the full mutation and showed that full expansion alleles were detected in oocytes and in the testes of 13-week-old males [*33*].

Screening of fragile X syndrome can be carried out by different techniques: cytogenetics, molecular or antibody testing for *FMR-1* protein (using blood, chorionic villus or hair root samples) [*34–38*]. DNA testing is a cost-effective alternative to cytogenetic analysis, while antibody testing for *FMR-1* protein is rapid but of limited use (false positive results are high) and needs to be used in conjunction with DNA methods.

In conclusion, the criteria needed for diagnosis of fragile X syndrome will depend on the age of the patient. The laboratory diagnosis should depend on molecular studies rather than cytogenetic ones. PCR is the most suitable screening tool and should be confirmed by Southern blotting.

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Some facts on genetics

- 7 million children around the world are born annually with severe genetic disorders or birth defects.
- 90% of infants born with genetic disorders are found in developing countries, contributing significantly to global child mortality.
- Mutations have been characterized for most major single-gene disorders, and there is a growing understanding of the role of genes in complex diseases such as cancer, cardiovascular disease, diabetes and asthma.
- The final version of the entire Human Genome sequence was unveiled in April, 2003.
- Prevention and management of genetic disorders are published health priorities in some developing countries, for which the WHO Human Genetics Programme (HGN) is developing significant capacity building initiatives and normative and regulatory guidance.
- The top ten biotechnologies for improving health in developing countries have been identified by a WHO Human Genetics Collaborating Centre.

Source: WHO Fact sheets: Genomic Resource Centre; genetics and health (http://www.who.int/genomics/about/en/E_grc_final.pdf) and (http://www.who.int/genomics/en/E_hgn-_final.pdf)

Apolipoprotein B gene polymorphisms in people in the East Mediterranean area of Turkey

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تعدد أشكال جين الأبوليبوبروتين – ب لدى الأتراك في المنطقة المُطلّة على شرق المتوسط

لوتوفر تامر، كهرمان تانريفردي، باهادير اركان، علي أونلو، نهير سوكو، حسن بكدمير، أوغور عتيق

الخلاصة: قد تزيد الطفرات النقطية في المجال الرابط للمستقبلات في الليبوبروتين المنخفض الكثافة من مستويات الكوليسترول في الدم. وقد أدت ثلاث طفرات في أبوليبوبروتين ب – 100 إلى عيب في الربط على الشكل التالي: (الأرجينين 3500 ← غليسين، أرجينين 3500 ← تريبتوفان والأرجينين 3531 ← سيستين). وقد قدرنا معدل تكرار الطفرات النقطية للأبوليبوبروتين – ب (الرامزة 3500) C9774T (الأرجينين 3500 ← تريبتوفان) وG9775A (الأرجينين 3500 ← غليسين)، وذلك في 179 من المصابين بالتصلب العصيدي و145 من المصابين بفرط شحميات الدم و272 من الأصحاء في المناطق التركية المُطلّة على شرق المتوسط. وقد تم قياس مستويات الشحوم والبروتينيات الشحمية بجهاز اعتيادي للقياس الطيي البيولوجي، فيما تم كشف طفرة الأبوليبوبروتين – ب باستخدام التفاعل التسلسلي للبوليميراز ذي الزمن الحقيقي، ولم يمكن العثور بأي من هذه الوسيلتين على الطفرة. لذا فإن هذه المنطقة تندر فيها الطفرات النقطية للأبوليبوبروتين ب – 100 وقد لا تعود أسباب فرط شحميات الدم والتصلب العصيدي لتلك الطفرات.

ABSTRACT Point mutations in the receptor binding domain of low density lipoprotein may increase cholesterol levels in blood. Three mutations of Apo B-100 protein result in defective binding (Arg 3500 Gln, Arg 3500 Trp and Arg 3531 Cys). We estimated the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500 Trp) and G9775A (Arg 3500 Gln) in 179 atherosclerotic, 145 hyperlipidaemic individuals and 272 healthy individuals in the east Mediterranean region of Turkey. Lipid and lipoprotein levels were measured with routine biochemical analyser and Apo B mutation was detected using real-time PCR. Neither mutation was found. In this region, Apo B-100 protein mutations are rare and causes of hyperlipidaemia and atherosclerosis may therefore be unrelated to them.

Les polymorphismes du gŁne de l apolipoprotØine B dans la population de la rØgion est-mØditerranØenne en Turquie

RESUME Les mutations ponctuelles sur le site de liaison du rØcepteur des lipoprotØines de basse densitØ peuvent faire augmenter le taux de cholestØrol sanguin. Trois mutations de la protØine apo B-100 entra nent une liaison dØfectueuse (Arg 3500 Gln, Arg 3500 Trp and Arg 3531 Cys). Nous avons estimØ la frØquence des mutations ponctuelles de l apo B (codon 3500) C9774T (Arg 3500 Trp) et G9775A (Arg 3500 Gln) chez 179 patients athØrosclØrotiques, 145 sujets hyperlipidØmiques et 272 *sujets en bonne* santØ de la rØgion est-mØditerranØenne en Turquie. Le taux de lipides et de lipoprotØines a ØtØ mesurØ l aide d un analyseur biochimique et la mutation de l apo B a ØtØ *recherchØe en utilisant la PCR en temps rØel.* Aucune mutation n a ØtØ trouvØe. Dans cette rØgion, les mutations de la protØine apo B-100 sont rares et les causes de l hyperlipidØmie et de l athØrosclØrose peuvent *donc ne pas Œtre liØes ces mutations.*

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Introduction

Nearly two-thirds of all circulating cholesterol is transported by low density lipoprotein (LDL) particles. The plasma concentration of cholesterol is regulated by which circulating LDL is taken primarily into the hepatocytes. Apolipoprotein B-100 (Apo B-100) is the major protein associated with the LDL particle and contains the ligand that binds LDL to its receptor [*1*]. Human Apo B-100 is a large, hydrophobic protein of 4536 amino acids and a molecular weight of approximately 540.000 Da. It is synthesized in the liver [*2*]. Gene mutations in the LDL receptor or in the receptor-binding zone of the Apo B-100 can disrupt binding and impair removal of circulating LDL. More than 150 mutations have been identified in the LDL receptor gene, associated with familial hypercholesterolaemia (FH), an autosomal dominant inherited disorder characterized by severe hypercholesterolaemia, frequent presence of tendon xanthomas, and premature coronary heart disease [*3*].

To date, several point mutations of the putative receptor binding domain of Apo B-100 have been identified [*4,5*]. Only 3 of these mutations have been shown to produce binding-defective Apo B-100 by appropriate genetic and functional investigations [*6,7*]. The first substitution to be discovered, and apparently the most frequent one, is Apo B-100 (Arg 3500→Gln). The other 2 substitutions are Apo B-100 (Arg 3500→Trp) and Apo B-100 (Arg 3531 >Cys) and occur less frequently [*1,5,6*]. Compared with Apo B-100 (Arg 3500→Gln), B-100 (Arg 3531→Cys) is associated with a smaller increase in LDL. Consistently, LDL that contained Apo B 100 (Arg 3531→Cys) exhibits less reduction of LDL receptor binding *in vitro* than did LDL endowed with Apo B-100 (Arg 3500→Gln) [*6,8*].

In the present study, we aimed to estimate the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500→Trp) and G9775A (Arg 3500→Gln) in 179 patients with atherosclerosis and compare this with 272 healthy subjects and 185 hypercholesterolaemic patients in the people living on the east Mediterranean coast of Turkey.

Methods

Participants

A total of 596 people were included in the study from the people living on the East Mediterranean coast of Turkey. The study sample included 272 healthy controls (133 female, 139 male), 145 controls with hypercholesterolaemia (117 female, 28 male) and 179 patients with atherosclerotic coronary artery disease (69 female and 110 male). All participants were randomly recruited from 2 hospitals in Turkey: university hospitals in Mersin and Adana. The participants were aged between 40 and 60 years. Patients with coronary artery disease were classified according to their coronary angiographic evidence of ≥ 70% stenosis of a major coronary artery. They were attending the cardiology clinic and scheduled to undergo coronary by-pass. In all, 124 patients with atherosclerosis (69%) had a history of myocardial infarction and 90 patients (50%) had smoking history. Patients with diabetes were excluded from the study. The control group was selected from clinically healthy individuals whose lipid parameters were in the normal reference range. They had no history of coronary heart disease, diabetes or hypertension; 115 individuals (42 %) had a smoking

history in the control group. People who had cholesterol levels of 200 239 mg/dL were considered as having borderline high blood cholesterol and those ≥ 240 mg/dL cholesterol levels were considered as having high blood cholesterol [9]. Since both levels are undesirable for blood cholesterol, we considered that individuals with cholesterol levels ≥ 200 mg/dL had hypercholesterolaemia; 75 (52%) subjects had a smoking history in the group with hypercholesterolaemia. Whole blood (EDTA-anticoagulated) and serum were collected after an overnight fast.

DNA isolation

DNA samples were isolated from blood samples, collected by venepuncture in sterile siliconized EDTA 2-mL Vacutainer tubes, using High Pure PCR Template Preparation Kit and MagNA Pure LC DNA Isolation Kit I by MagNa Pure LC automated DNA isolation instrument (Roche Molecular Biochemicals, Mannheim, Germany).

Detection of Apo B mutation

The determination of Apo B mutation was done using Real-time PCR and the Apo B mutation detection kit (Roche Diagnostics, GmbH, Mannheim, Germany).

Real Time PCR Principle: a 207 bp fragment of the apolipoprotein B gene was amplified with specific primers from human genomic DNA. The amplicon was detected by fluorescence using a specific pair of hybridization probes. The hybridization probes consist of 2 different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle. If a mutation is present, the mismatches of the mutation probe with the target destabilize the hybrid. With a wild type genotype, mismatches do not occur, and the hybrid has a higher T_M. The temperature is slowly increased and when the mutation probe melts off and the 2 fluorescent dyes are no longer in close proximity, the fluorescence decreases. For mutated genotypes, this occurs at lower temperatures than for the wild type genotype.

In addition, we performed DNA sequencing of 5 randomly selected patients, 5 controls and 5 individuals with hypercholesterolaemia.

Measurement of lipids and lipoproteins

Apolipoprotein A (Apo A) and Apolipoprotein B (Apo B) were determined by immunoturbidometric methods. Triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL) were analysed by GPO/PAP enzymatic colorimetric, CHOD/PAP enzymatic colorimetric and direct COHD/PAP enzymatic colorimetric methods, respectively. LDL content was calculated from the primary measurements using the empirical equation of Friedewald et al. [10]. All these parameters were determined by Cobas Integra 700 biochemical analyser (Roche Diagnostics, GmbH, Mannheim, Germany). The results were expressed in terms of arithmetic means ± standard deviation.

Results

The levels of TC, HDL, LDL, VLDL, TG, Apo A and Apo B are given in Table 1. Our results show that there was an increase in the levels of TC, LDL, VLDL, TG and Apo B and a decrease in Apo A levels in individuals with hypercholesterolaemia and patients with atherosclerosis compared to normolipidaemic controls. On the other hand, neither C9774T (Arg 3500→Trp) nor G9775A (Arg 3500→Gln) mutations for Apo B codon 3500 was detected in the individuals by Real-Time PCR.

Table 1 **Mean cholesterol, triglyceride, lipoprotein and apoprotein levels of individuals with atherosclerosis, hypercholesterolaemia and normolipidaemia**

Individuals with:	TC (ref < 200 mg/dL)	HDL (ref > 65 mg/dL)	LDL (ref < 130 mg/dL)	VLDL (ref < 40 mg/dL)	TG (ref < 200 mg/dL)	APO A (ref 180–225 mg/dL)	APO B (ref 60–133 mg/dL)
Hypercholesterolaemia (n = 185)	263.3 ± 27.0	43.8 ± 10.2	183.1 ± 4.3	35.3 ± 17.4	187.4 ± 55	144.1 ± 18.7	147.5 ± 23.1
Normolipidaemia (n = 272)	182.3 ± 28.0	47.5 ± 15.2	113.6 ± 26.0	22.5 ± 12.4	105.9 ± 42	153.3 ± 29.2	96.9 ± 22.2
Atherosclerosis (n = 179)	202.2 ± 45.6	37.5 ± 10.8	119.0 ± 36.6	30.1 ± 19	150.3 ± 45	114.9 ± 20.2	101.83 ± 26.4

Results expressed as the arithmetic mean ± standard deviation.
n = number of sample.

Discussion

The Apo B arginine-to-glutamine change at codon 3500 by been established as a cause of the failure of the LDL particle to bind to its receptor and the consequent hypercholesterolaemia of familial defective Apo B-100 [*5*]. The Apo B (Arg 3500→Gln) mutation has been identified in the USA, Denmark, Germany, Italy, France, Austria, Australia, the Netherlands, South Africa, Switzerland and indirectly in Norway and Sweden since some of the probands from the Oregan study were of Norwegian and Swedish descent [*2*]. However, the mutation has not been detected in either Finland or former Soviet Union republics. The Apo B-100 (Arg 3500→Gln) mutation has been observed with an approximate frequency of 1 in 500 to 1 in 700 in populations of European (Caucasian) origin [*2,8*].

Several point mutations of the putative receptor binding domain of Apo B-100 have been identified. Only 3 of these mutations have been shown to produce defective binding Apo B-100 by appropriate genetic and functional investigations [*3–6,10,12*]. The first substitution to be discovered, and apparently the most frequent one, was Apo B-100 (Arg 3500→Gln). The other 2 substitutions, Apo B-100 (Arg 3500→Trp) and Apo B-100 (Arg 3531→Cys), occur less frequently [*8,13*].

Teng et al. showed that the prevalence of heterozygote in 373 Chinese individuals with hyperlipidaemia was 0.3% for the Arg 3500→Gln mutation and 2.4 % for the Arg 3500→Trp mutation [*13*]. When 373 unrelated individuals with hyperlipidaemia were screened for the presence of Apo B-100 mutations, Tai et al. found 9 Arg 3500→Trp index cases, 7 classified as having type IIa and 2 as having type IIb hyperlipidaemia [*14*]. The authors reported that one of them was of Scottish descent

and the others were of Asian descent. They suggested that Arg 3500→Trp alleles were inherited from a common ancestor in Asian populations.

Fisher et al. investigated 297 consecutive individuals with LDL concentrations > 155 mg/dL and triglycerides < 200 mg/dL for Apo B mutation. Apo B-100 (Arg 3500→Trp) was described in just 1 family of European origin, in 2 families with a mixed Chinese and Malayan descent, in 1 family of Asian descent living in the Glasgow region and in another 9 unrelated individuals from Taiwan [*15*].

In our study we investigated the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500→Trp) and G9775A (Arg 3500→Gln) in 596 individuals (272 healthy controls, 185 controls with hypercholesterolaemia and 179 patients with atherosclerosis). Neither C9774T (Arg 3500→Trp) nor G9775A (Arg 3500→Gln) mutation was found in the people living on the East Mediterranean coast of Turkey. As can be seen in our study, the mutations of Apo B-100 protein are very rare. As regards this population, they were not observed in our sample.

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A practical guide for health researchers

A practical guide for health researchers, by Mahmoud F. Fathalla and Mohamed M.F. Fathalla, is intended for health researchers, who are not limited to scientists pursuing a research career. They include health professionals, administrators, policy-makers and nongovernmental organizations, among others, who can and should use the scientific method to guide their work for improving the health of individuals and communities. This comprehensive guide covers , among others, the areas of ethics in research, choice of research, preparing for research, conducting research, analysing and interpreting results, disseminating research and writing a scientific paper. It is highly readable and easy to understand. The guide can be obtained from: Distribution and Sales, World Health Organization Regional Office for the Eastern Mediterranean, Abdul Razzak Al Sanhouri Street, PO Box 7608, Nasr City, Cairo 11371, Egypt. Telephone: (202) 670 25 35; Fax: (202) 670 24 92/4. It is also available free on line at: http://www.emro.who.int/publications/pdf/healthresearchers_guide.pdf

Improved serum HDL cholesterol profile among Bangladeshi male students during Ramadan fasting

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تحسن مرتسم الكوليسترول للبروتين الشحمي المرتفع الكثافة في المصل لدى الطلاب الذكور في بنغلاديش أثناء صيام رمضان

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الخلاصة: تم تقييم أثر صيام رمضان على شحميات المصل في عشرين من الذكور الأصحاء في بنغلاديش. وتم قياس المتغيرات للمقياسات البشرية وشحميات الدم قبل يوم واحد من رمضان وفي اليوم السادس والعشرين منه وبعد شهر من انقضاء رمضان. وقد انخفض وزن الجسم ومنسب كتلة الجسم انخفاضاً ملحوظاً أثناء شهر رمضان بالمقارنة مع قياسات الفترة السابقة له واللاحقة به. وقد كان استهلاك الدهنيات أعلى أثناء شهر رمضان مقارنة بما بعده. وقد ازداد كوليسترول البروتين الشحمي المرتفع الكثافة زيادة ملحوظة أثناء شهر رمضان، في حين لم يكن هناك تغير ملحوظ في الكولسترول الكلي، وكولسترول البروتين الشحمي المنخفض الكثافة وثلاثي الغليسريدات. وتشير التحليلات المتعددة التحوُّف لمستويات كولسترول البروتين الشحمي الرفيع الكثافة في رمضان وجود علاقة إيجابية مع سرعة النبض واستهلاك الدسم مع علاقة سلبية مع ضغط الدم الانقباضي وخسارة الوزن. وتشير الموجودات إلى تحسن مرتسمات كولسترول البروتين الشحمي المرتفع الكثافة أثناء صيام رمضان.

ABSTRACT Effects of Ramadan fasting on *serum lipids of 20 healthy* males in Bangladesh were assessed. Anthropometric parameters and blood lipids were measured 1 day before Ramadan, day 26 of Ramadan and 1 month after Ramadan. *Body weight and* body mass index decreased significantly during Ramadan compared with before and after Ramadan. Fat intake was significantly higher during Ramadan than after. High-*density lipoprotein* (HDL) cholesterol increased significantly during Ramadan. Other lipids were not significantly different. Regression analysis of Ramadan HDL cholesterol levels indicated positive association with pulse rate and fat intake and negative association with systolic blood pressure and weight loss. The findings indicate improved HDL cholesterol profiles during Ramadan.

Amélioration du profil du cholestérol HDL sérique chez les étudiants bangladais pendant le jeûne du mois de ramadan

RESUME Les effets du jeûne du mois de ramadan sur les lipides sériques chez 20 sujets de sexe masculin en bonne santé au Bangladesh ont été évalués. Les paramètres anthropométriques et les lipides sanguins *ont été mesurés un jour* avant le ramadan, au 26e jour du ramadan et un mois après le ramadan. Le poids corporel et l'indice de masse corporelle ont considérablement diminué pendant le ramadan par rapport aux mesures avant et après le ramadan. L'apport lipidique était significativement plus élevé pendant le ramadan qu'après. Le cholestérol des lipoprotéines de haute densité (HDL) augmentait de *manière significative* pendant le ramadan. Les autres lipides n'étaient pas significativement différents. L'analyse de régression pour les taux de cholestérol HDL pendant le ramadan indiquait une *association positive avec la fréquence du* pouls et l'apport lipidique et une association négative avec la pression artérielle systolique et la perte de poids. Ces résultats montrent une amélioration des profils du cholestérol HDL durant le ramadan.

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Introduction

Ramadan fasting is one of the 5 pillars of Islam and one of the most significant *ibadat* (worships) of Islam [*1*]. Throughout the world, millions of Muslims fast during Ramadan to fulfil this religious obligation. Because the lunar calendar determines the month of Ramadan and is about 11 days shorter than the solar year, Ramadan is not fixed to any season. The timing of daily fasting varies from country to country and with the season in which the month of Ramadan falls. Thus, depending upon the season and the geographical position of the country, the length of the fast varies from 12 to 19 hours per day [*2*].

During Ramadan, Muslims abstain from food and drink from dawn until sunset. Traditionally the practice is to eat 2 meals, 1 before dawn, *sahri*, and 1 just after sunset, *iftar*. Often Muslims eat a greater variety of foods in their meals during Ramadan than in other months. As a result, the Ramadan fast provides an excellent opportunity to study the effects of various diets on the human body and can serve as an excellent research model for metabolic and behavioural studies [*3*].

Ramadan fasting and starvation are not synonymous. Many physiological and psychological changes take place during Ramadan, most probably due to the changes in eating patterns, eating frequency and sleep patterns [*4*]. Some studies in the eastern Mediterranean area have indicated improved high-density lipoprotein (HDL) cholesterol during Ramadan fasting [*5,6*].

The objective of our study was to investigate the serum lipid profile as influenced by diet patterns and other parameters during Ramadan.

Methods

The study was conducted in Dhaka, Bangladesh, with 20 healthy male volunteers residing in the hostel of the National Institute of Preventive and Social Medicine (NIPSOM). Prior to selection, written consent was obtained from each. Clearance was obtained from the Ethical Committee of NIPSOM. Data were collected at 3 intervals: before Ramadan (1 day before the start of Ramadan), during Ramadan (day 26 of Ramadan) and after Ramadan (1 month after Ramadan). Data collection started in December 1998 and ended in March 1999. During the month of Ramadan the average duration of fasting was approximately 12 hours and the maximum ambient temperature ranged from 11 °C to 29 °C [*7*]. The mean age ± standard deviation of the volunteers was 38.27 ± 4.07 years. All volunteers were in good health and none were using any medication. All were engaged in light physical activities and the nature of their work was more or less similar. Body weight and mid-arm circumference were recorded before, during and after Ramadan.

Diet information was obtained during Ramadan and after Ramadan using 24-hour recall method for 3 consecutive days. No dietary intervention was provided to the volunteers and they were allowed to eat anything they wanted. Food intake was analysed for energy, protein, carbohydrate and fat. Diet composition was calculated with published and unpublished composition tables of the foods of Bangladesh (A. Haque, unpublished data) [*8*].

Blood was tested in the Department of Nutrition and Biochemistry of NIPSOM. Before and after Ramadan, blood was col-

lected in the morning after overnight fasting; during Ramadan it was taken just after breaking the fast with a glass of water. Total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was obtained by the formula:

LDL cholesterol = total cholesterol – HDL cholesterol - triglycerides/5

Determination of all biochemical parameters was done with a photoelectric colorimeter and reagent kits (manufactured by Human Gesellschaft fur Biochemica und Diagnostica GmbH, Weisbaden, Germany) [9].

Data were analysed by ANOVA for model-period (fixed effect) and subjects (random effect) using *StatSoft Statistica*, version 5. The mean values of the 3 test periods were compared for significant differences using Duncan's multiple range *t*-test at $P < 0.05$.

Results

Anthropometric measurements

The mean values of anthropometric measurements significantly changed before, during and after Ramadan (Table 1). All parameters at day 26 of Ramadan, i.e. body weight, body mass index (BMI), mid-arm circumference, pulse rate and systolic and diastolic blood pressure, were significantly lower than pre-Ramadan values ($P < 0.05$). One month after Ramadan fasting, body weight and other parameters had a trend to recoup to pre-Ramadan status; however, they were still significantly lower than the pre-Ramadan values ($P < 0.05$). When the post-Ramadan values were compared with the day 26 Ramadan values, body weight, BMI, mid-arm circumference and systolic blood pressure were significantly higher ($P < 0.05$). Pulse rate and diastolic blood pressure were not significantly different. This indicates longer lasting lowering effect of

Table 1 **Anthropometric and other measurements (mean ± standard deviation) of the 20 healthy male volunteers**

Measurements	Pre-Ramadan[a]	Day 26 of Ramadan	Post-Ramadan[b]	*F*	*P* value
Weight (kg)	64.05 ± 7.78[c]	62.07 ± 8.08[e]	63.05 ± 7.75[d]	14.1	< 0.001
Body mass index (kg/m²)	24.20 ± 2.48[c]	23.44 ± 2.52[e]	23.81 ± 2.37[d]	13.4	< 0.001
Mid-arm circumference (cm)	27.75 ± 1.88[c]	27.20 ± 1.88[e]	27.42 ± 1.91[d]	13.1	< 0.001
Pulse rate (per minute)	82.5 ± 8.9[c]	75.2 ± 7.8[d]	77.0 ± 8.0[d]	21.0	< 0.001
Systolic blood pressure (mmHg)	124.3 ± 13.9[c]	111.8 ± 10.8[e]	116.0 ± 9.9[d]	26.3	< 0.001
Diastolic blood pressure (mmHg)	82.3 ± 11.4[c]	77.3 ± 10.6[d]	78.8 ± 10.6[d]	9.04	< 0.001

[a] *One day before Ramadan.*
[b] *One month after Ramadan.*
Dissimilar superscripts indicate significant difference between 2 means; Duncan's multiple range t-test, $P < 0.05$.

Ramadan fasting on pulse rate and diastolic blood pressure.

Energy intake

Total daily energy, carbohydrate and protein intake were not significantly different between during and post-Ramadan periods (Table 2). However, there was a significant increase in fat intake ($P < 0.01$). Energy from fat as a percent of the total energy intake was 10.6% during Ramadan versus 7.6% after Ramadan. Significant decreases in blood glucose levels during Ramadan suggest energy intake as the limiting factor in our study (Table 3).

Table 2 **Mean energy intake (± standard deviation) of the 20 male volunteers during and after Ramadan**

Energy intake	During Ramadan	Percent of total energy intake	Post-Ramadan	Percent of total energy intake
Energy (kcal/day)	2113.8 ± 168.5	–	2134.1 ± 132.6	–
Carbohydrate (g/day)	406.3 ± 49.9	77.1 ± 5.2	433.3 ± 42.6	81.1 ± 5.0
Protein (g/day)	64.6 ± 20.5	12.3 ± 4.1	60.1 ± 21.5	11.3 ± 4.1
Fat (g/day)	24.7 ± 3**	10.6 ± 1.4	17.9 ± 3.61	7.6 ± 1.5

***Significant difference between 2 means at P < 0.01.*

Table 3 **Serum lipids and glucose measurements (mean ± standard deviation) of the 20 healthy male volunteers**

Measurement	Pre-Ramadan	Day 26 of Ramadan	Post-Ramadan	*F*	*P*-value
Total cholesterol (mg/dL)	166.4 ± 30.32	165.15 ± 24.24	174 ± 29.66	1.1	NS
HDL cholesterol (mg/dL)	38.14 ± 7.40	46.71 ± 14.33[a]	41.72 ± 7.70	4.1	< 0.05
LDL cholesterol (mg/dL)	103.92 ± 34.57	92.33 ± 23.40	99.68 ± 27.54	1.2	NS
Triglycerides (mg/dL)	146.66 ± 72.78	131.04 ± 41.47	152.71 ± 57.59	1.7	NS
Total cholesterol/LDL cholesterol	4.6 ± 1.3	3.7 ± 0.9[b]	4.3 ± 1.1	5.2	< 0.01
LDL/HDL cholesterol	2.9 ± 1.2	2.1 ± 0.8[a]	2.5 ± 0.8	4.3	< 0.05
Glucose (mg/dL)	95.3 ± 14.1[c]	85.6 ± 12.4	91.0 ± 11.6	4.8	< 0.05

Dissimilar superscripts indicate significant difference between 2 means; Duncan's multiple range t-test at P < 0.05.
LDL = low-density lipoprotein.
HDL = high-density lipoprotein.
NS = not significant at P < 0.05.

Serum lipids

Significant improvement in the HDL cholesterol profile (HDL cholesterol, total cholesterol/HDL cholesterol, LDL cholesterol/HDL cholesterol) was observed during the Ramadan fasting period compared with the pre-Ramadan period ($P < 0.05$) (Table 3). There were no significant differences in total cholesterol, LDL cholesterol and triglycerides between the 3 periods.

Discussion

Anthropometric and other measurements

The mean difference between pre-Ramadan and during Ramadan body weights was 1.97 kg. Significant reductions in body weight, BMI and mid-arm circumference during Ramadan suggest that the subjects in our study had a negative energy balance ($P < 0.05$). Similarly, many studies have reported weight loss during the month of Ramadan fasting [*3,10–16*]. In contrast to this, one Saudi Arabian study reported weight gain during Ramadan [*17*] and still others did not find any significant change in body weight [*2,18*]. In one study among healthy males, a significant reduction in skin fold thickness was reported during Ramadan fasting [*3*]. A study of Tunisian women suggested that increased fat oxidation during Ramadan fasting results in an adaptive mechanism for body weight maintenance [*18*]. Our observation of decreases in systolic and diastolic blood pressure is supported by the findings of Athar and Habib [*16*].

Energy intake

The mean energy intake during Ramadan (2113.8 ± 168.5 kcal/day) was somewhat below the average energy consumption of Bangladeshi people, which has been estimated at 2244 kcal/day [7]. Among tropical Asiatic males energy intake has been reported to be 2557 kcal/day on normal days with a reduction of 20%–25% during Ramadan fasting [*10*]. In contrast, Frost et al. reported increased energy intake during Ramadan (3680 kcal/day) compared with energy intake after Ramadan (2425 kcal/day) [*17*].

There were no significant differences in carbohydrate and protein intake during and after Ramadan in our study. The Bureau of Statistics of Bangladesh reports average protein consumption to be 65 g/day [7]. This is approximately in agreement with the reported protein intake of our subjects. Protein intake has been reported to increase during the month of Ramadan fasting among both Tunisian women and Moroccans [*4,18*].

Fat intake was significantly higher among our subjects during Ramadan ($P < 0.001$). This was similar to increases in fat intake during Ramadan among Tunisian women and among Moroccans [*4,18*]. Bangladeshi people prefer fried food items during *iftar*. This habit increases the intake of unsaturated fat (mainly soybean oil) that ultimately results in an increase in fat consumption during Ramadan. It may be noted that fat intake calories as a percentage of total energy intake during and after Ramadan in our study were far lower than is consumed in the affluent societies of some industrialized nations. Because low intake of fat rather than excess fat is a concern in the general population in developing countries like Bangladesh, it seems that during Ramadan fat intake slightly improved.

Serum lipids

It is well established that a high level of HDL cholesterol has an inverse relation with coronary heart disease [*19*]. The improved HDL cholesterol profile in our study is supported by many studies [*4–6,20,21*].

Streja et al. and Murphy et al. noted similar increases in HDL cholesterol profiles in 2 non-Ramadan studies [*22,23*]. Nonetheless, some studies have reported decreases [*3,24*]. In multiple regression analysis (r^2 = 0.74, $P < 0.001$, $n = 20$), HDL cholesterol was positively associated with pulse rate and fat intake and negatively with weight loss and higher systolic blood pressure. The prediction equation is:

HDL cholesterol = 1.4024 + 1.4032 pulse rate + 1.5642 fat intake – 0.9608 systolic blood pressure – 4.3295 weight change

where HDL cholesterol was measured in mg/dL, fat intake was measured in g/day and weight change was calculated as weight on Ramadan day 26 – weight before Ramadan measured in kg.

Improvement in HDL cholesterol profile with higher fat intake agrees with the findings of Nomani et al. [*1*].

In conclusion, Ramadan fasting contributed to better blood lipid profiles under the prevailing limited energy intake conditions of the study. One of the contributing factors may be higher fat intake. The findings may have an application in improving HDL cholesterol levels among subjects irrespective of religion under restricted energy intake conditions.

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Ischaemic stroke in Jordan: a 2-year hospital-based study of subtypes and risk factors

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السكتة الدماغية الإقفارية في الأردن: دراسة مرتكزة على المستشفيات لمدة سنتين حول الأنماط الفرعية وعوامل الاختطار

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الخلاصة: أجريت دراسة استعادية شملت 200 مريض متعاقب عانوا من سكتة دماغية لأول مرة في حياتهم، وأدخلوا في مستشفى الجامعة الأردنية خلال فترة سنتين. وقد كان العمر الوسطي 61.2 عاماً (متراوحاً بين 29 – 95 عاماً)، وكان أكثر الأنماط الفرعية شيوعاً هو الاحتشاء الجوبي (51.5٪) إلا أن تكرار السكتات الدماغية الناجمة عن صمّات قلبية المنشأ كان منخفضاً (8.0٪)، وكان أكثر عوامل الاختطار شيوعاً ارتفاع ضغط الدم والسكري والتدخين في السكتات الدماغية الناجمة عن التصلب العصيدي وليس عن صمات قلبية المنشأ. وكان الرجفان الأذيني المزمن أكثر عوامل الاختطار شيوعاً في السكتات الدماغية الناجمة عن صمات قلبية المنشأ. ولم يكن لدى أي من المرضى تضيق شديد في الشريان السباتي خارج القحف أو في الشريان الفقري (تضيق يزيد على 50٪). وتظهر السكتات الدماغية الجوبية بشكل رئيسي على شكل سكتة دماغية حركية صرفة (لدى 67 مريضاً من بين 103 مرضى) وقد تركزت بشكل رئيسي في المحفظة الداخلية (لدى 34 مريضاً من بين 103 مرضى). أما النتائج الحميدة المآل (85٪ من الحالات خرجت من المستشفى إلى المنزل، ومعظمها بعجز عصبي طفيف) فكانت ناجمة عن الاحتشاءات الجوبية التي تغلب حدوثها في أعمار صغيرة نسبياً.

ABSTRACT A retrospective study was made of 200 consecutive patients with first-ever ischaemic stroke, admitted to Jordan University Hospital over a 2-year period. The mean age was 61.2 years (range 29–95). The most common stroke subtype was lacunar infarct (51.5%), but frequency of cardioembolic stroke was low (8.0%). Hypertension, diabetes mellitus and smoking were the most common risk factors for atherosclerotic non-cardioembolic stroke. Chronic atrial fibrillation was the most common risk factor for cardioembolic stroke. No patient had severe extracranial carotid or vertebral artery stenosis (> 50% narrowing). Lacunar strokes presented predominantly as pure motor stroke (67/103) and were mainly in the internal capsule (34/103). The favourable outcome (85% discharged home) may be due to the relatively young age and the predominance of lacunar infarcts.

L'accident ischémique cérébral en Jordanie : étude hospitalière sur deux ans des sous-types et des facteurs de risque

RESUME Une étude rétrospective a été réalisée sur une période de deux ans chez 200 patients consécutivement admis à l'Hôpital universitaire de Jordanie pour un premier infarctus cérébral. L'âge moyen était de 61,2 ans (fourchette : 29-95 ans). Le sous-type d'accident cérébral le plus courant était l'infarctus lacunaire (51,5 %), mais la fréquence des embolies cérébrales d'origine cardiaque était faible (8,0 %). L'hypertension, le diabète sucré et le tabagisme étaient les facteurs de risque les plus courants pour les infarctus cérébraux non cardio-emboliques d'origine athéroscléreotique. La fibrillation auriculaire chronique était le facteur de risque le plus courant pour les embolies cérébrales d'origine cardiaque. Aucun patient n'avait de sténose sévère de l'artère vertébrale ou de la carotide extracrânienne (> 50 % de rétrécissement). Les infarctus lacunaires se présentaient principalement comme une hémiplégie motrice pure (67/103) et étaient localisés surtout dans la capsule interne (34/103). L'issue favorable (85 % des patients sont rentrés à leur domicile à la sortie de l'hôpital) peut être due à l'âge relativement jeune et à la prédominance des infarctus lacunaires.

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Introduction

Stroke is the third leading cause of death and among the leading causes of disability in the United States, Europe and many developing countries [1]. More recent studies demonstrate that different ethnic/racial populations may have different incidence rates and may be predisposed to different stroke subtypes. For instance, African Americans have a significantly higher incidence and mortality rate compared with white people [2]. Elderly Hispanic patients have a lower mortality rate secondary to stroke compared with white people. African-Americans and Native Americans also present more frequently with lacunar infarcts and intracranial haemorrhage [3]. Meanwhile, the difference in cardioembolic stroke rates between African-Americans and whites is not statistically significant. However, these rates are lower in Hispanic Americans [3].

Few studies have explored the risk factors and prevalence of stroke in the Arab population and no study could be found regarding stroke in Jordan [4–8]. A hospital-based study in Sudan demonstrated an earlier incidence of stroke but a similar risk factor profile to other populations [7]. In a hospital-based study in Qatar, patients also had earlier peak stroke rates as well as higher rates of hypertension and diabetes [8]. The largest Arab registry is in Saudi Arabia, which also demonstrated an earlier onset of stroke [4]. In Saudi Arabia, large infarcts (56%–67% of all strokes) were the most common stroke type; there was also a large proportion of lacunar infarcts (33%–46%) [4].

This case review of 200 Jordanian patients with ischaemic stroke was conducted to explore the age and sex distribution, stroke subtypes, risk factors, clinical and imaging findings, treatment and outcome.

Methods

The Jordan University Hospital is a 530-bed tertiary care teaching hospital providing health care to a large section of the middle-class Jordanian population. As the major university hospital in Jordan, it serves as a principal referral centre for the entire Jordanian population. The case notes of 200 consecutive patients with first-ever ischaemic stroke admitted to the hospital through the emergency department between January 2000 and December 2001 were reviewed.

We applied the definition of stroke given by the World Health Organization [9]: rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than vascular origin.

All patients had an initial computed tomography (CT) brain scan to exclude intracerebral haemorrhage followed after a few days by a second CT and/or magnetic resonance imaging (MRI) scan to confirm the infarct location and size. When clinically indicated and technically feasible, some patients had carotid Doppler ultrasound and/or magnetic resonance angiography (MRA) of the neck and brain vessels to rule out arterial stenosis or occlusion. Most patients had 2 D-transthoracic echocardiography to rule out a valvulopathy or left atrial/ventricular clot. Tests for hypercoagulable state or vasculitis were done when clinically indicated.

Each patient was assessed for risk factors for stroke. Hypertension, diabetes mellitus, and hyperlipidaemia were defined by standard guidelines [10–12]. Ischaemic heart disease, myocardial infarction and atrial fibrillation were confirmed by a 12-lead electrocardiogram (ECG) and with a cardiology consultation. History of prior

transient ischaemic attacks and smoking were also noted.

An assessment was made of the cause(s) of the stroke and these were classified into subtypes using the TOAST criteria [*13*] and according to whether the cause was determined or undetermined (2 or more possible causes or negative evaluation).

Management included treatment with antiplatelet drugs (aspirin 325 mg/day, ticlopidine 250 mg twice daily), anticoagulants (warfarin, maintaining an international normalized ratio of 2–3), physiotherapy, antioedema agents (mannitol), reduction of risk factors or supportive measures, each when indicated. None of the patients received thrombolytic drugs owing to the lack of supportive facilities at this hospital and late presentation to the emergency department (the mean time between onset of stroke and arrival at the emergency department was 21 hours, range 6–120 hours).

Outcome was classified broadly into: ambulatory with mild neurological deficit, moderate neurological deficit compromising activities of daily living (aphasia, hemiparesis, assistance in ambulation), and severe neurological deficit (hemiplegia) or vegetative state and death.

Results

There were 200 patients with ischaemic stroke, accounting for 3.5% of admissions to the medical ward/intensive care unit during the study period. The mean age was 61.2 years, range 29–95 years, comprising 112 males and 88 females (male to female ratio of 1.3). The age and sex distribution is shown in Table 1. Most of the patients (159/200) were between 51 and 80 years; however, there was a high proportion of patients with stroke aged under 55 years (41/200).

Table 1 **Age and sex distribution of 200 patients with first-ever ischaemic stroke**

Age (years)	Male No.	Female No.	Total No.
21–30	1	0	1
31–40	3	2	5
41–50	14	8	22
51–60	37	22	59
61–70	38	31	69
71–80	12	19	31
81–90	7	4	11
91–100	0	2	2
Total	112	88	200

The cause of stroke could be determined for 161 patients (80.5%); the majority of cases (51.5%) were lacunar infarcts (Table 2). Most of the etiologically undetermined cases, 30/39 (76.9%), had atherosclerotic and/or lacunar infarcts.

The risk factors for each stroke subtype are shown in Table 3. The most common risk overall was hypertension (76.0%) followed by diabetes mellitus (44.0%), smoking (35.0%), hyperlipidaemia (33.0%) and ischaemic heart disease (20.5%). Hypertension was also a major risk factor for lacunar infarcts in 87 out of 103 patients (84.5%) followed by diabetes mellitus in 49/103 (47.6%) and smoking in 30/103 (29.1%).

Among the 152 patients with hypertension, 100 had known hypertension, 23 were newly diagnosed and 29 had uncontrolled hypertension at the time of stroke. Among the 88 diabetic patients, 59 had known diabetes mellitus, 14 were newly diagnosed and 15 had uncontrolled diabetes mellitus at the time of stroke. Among the 66

Table 2 **Subtypes of ischaemic strokes among 200 patients with first-ever ischaemic stroke, classified using the TOAST criteria [13]**

Category	No. of patients (n = 200)	%
Cause determined		
Large artery atherosclerosis	40	20.0
Lacunes	103	51.5
Cardioembolism	16	8.0
Hypercoagulable state	2	1.0
Total	161	80.5
Cause undetermined		
Atherosclerosis and/or lacunes	30	15.0
Embolism with 2+ possible causes	3	2.0
Negative evaluation	6	3.0
Total	39	19.5
Total	200	100.0

n = *total number of patients.*

patients with hyperlipidaemia, 35 had known and 31 newly diagnosed hyperlipidaemia. Among the 70 smokers, 62 were males and 8 females. No risk factors except elderly age could be determined in 12 patients. Two patients had hypercoagulable state (primary antiphospholipid syndrome).

There were 16 patients (8.0%) with cardioembolic strokes. The majority (14/16) were female patients above the age of 50 years (7 above 70 years) with 12 out of 16 having chronic atrial fibrillation and at least 1 other risk factor (mainly hypertension and left ventricular hypertrophy). None of the patients with atrial fibrillation had therapeutic levels of warfarin before the stroke (2 patients discontinued warfarin, 4 were taking warfarin but were subtherapeutic, the remainder were not taking the medication).

Large artery atherosclerotic strokes (20.0% of cases) were predominantly in the carotid territory (22/37) as opposed to the vertebrobasilar region (15/37) and presented mainly with hemiparesis/hemiplegia with or without aphasia and lateral homonymous hemianopsia. MRI and/or CT brain scans showed infarcts located predominantly in branch vessels (13/22 in a branch of middle cerebral artery and 8/15 in the posterior cerebral artery). Strikingly, none of these patients had haemodynamically significant extracranial carotid or vertebral artery atherosclerosis (> 50% luminal narrowing) on carotid Doppler ultrasound and/or MRA of neck vessels. The most common clinical presentation of lacunar infarcts was pure motor stroke (67/103) followed by mixed sensorimotor stroke (26/103). The most common lacunar infarct location on CT and/or MRI brain was in the internal capsule (34/103) followed by multiple locations (25/103) and the periventricular area (17/103). Lacunes were shown on CT scan in 73 out of 103 patients (70.9%), but in 17/103 (16.5%) they were seen only on MRI and in 13/103 were shown on both scans.

Of the etiologically undetermined stroke subtypes, 30 patients had atherosclerosis and/or lacunes. Three patients had embolisms with 2 or more possible causes; 2 had atrial fibrillation and hypertension and the third cardiomyopathy and hypertension. There were 6 patients with a negative evaluation who had multiple risk factors (hypertension, diabetes mellitus, smoking).

A few treatment complications occurred. Four patients had bleeding duodenal ulcers due to aspirin and 2 had warfarin toxicity. With regard to outcome, most patients did well. After a mean follow-up of 18 months (range 3 to 24 months), 170 patients (85.0%) were discharged home. Among those discharged, 107 had mild

Table 3 **Risk factors for stroke among the 200 patients with first-ever ischaemic stroke according to subtype (most patients had more than one risk factor)**

Risk factor	Cause determined				Cause undetermined			Total (n = 200)	
	Large artery AS (n = 40) No.	Lacunes (n = 103) No.	Cardio-embolism (n = 16) No.	HC state (n = 2) No.	AS/ lacunes (n = 30) No.	Embolism plus[a] (n = 3) No.	Negative evaluation (n = 6) No.	No.	%
Hypertension	32	87	–	–	28	–	5	152	76.0
Diabetes mellitus	26	49	–	–	10	–	3	88	44.0
Smoking	16	37	–	–	14	–	3	70	35.0
Hyperlipidaemia	18	30	–	–	14	–	4	66	33.0
Ischaemic heart disease	10	22	–	–	9	–	–	41	20.5
Previous transient ischaemic attack	8	6	–	–	3	–	–	17	8.5
Obesity	3	3	–	–	–	–	–	6	3.0
Chronic atrial fibrillation[b]	–	–	12	–	–	3	–	15	7.5
Prosthetic valve	–	–	3	–	–	–	–	3	1.5
Myocardial infarct	–	–	1	–	–	–	–	1	0.5
Antiphospholipid syndrome	–	–	–	2	–	–	–	2	1.0
Nothing identified	–	5	–	–	6	–	1	12	6.0

AS = atherosclerosis.
HC = hypercoagulable.
n = *total number of patients.*
[a] *Embolism with 2+ possible causes.*
[b] *Associated with hypertension and/or diabetes mellitus.*

neurological deficit and 63 required assistance with ambulation. The latter group of patients had a prolonged stay in the hospital due to lack of a rehabilitation centre at Jordan University Hospital. Thirty patients (15.0%) died, 10 due to large infarcts in middle cerebral artery territory with herniation, 7 due to brainstem infarcts and 13 secondary to medical comorbidities (such as aspiration pneumonia, deep vein thrombosis with pulmonary embolism, sepsis, renal failure).

Discussion

Several points emerge from this retrospective study of 200 patients with first-ever ischaemic stroke admitted to Jordan University Hospital over a 2-year period. The stroke admission rate of 3.5% was similar to that from other Arab countries [*4–6,14*]. The mean age of our patients (61.2 years) was younger than in industrialized countries. For instance, in the Framingham study the mean age of stroke patients was 65.4 years for men and 66.1 years for women [*15*]. In the Northern Manhattan Stroke Study (NO-MASS) the mean age at ischaemic stroke was 70 years [*16*].

Concerning stroke subtypes, 161/200 (80.5%) of our patients had a stroke of determined cause, similar to the determined stroke rates 96/136 (70.5%) found by Conti [*17*]. The majority of determined strokes were lacunar infarcts (51.5%), which were more frequent compared with other Middle Eastern and Western studies [*2 8,14–18*]; the frequencies of lacunes in most populations ranged from 11% to 42%. Our higher percentage of lacunar infarcts may be due to the higher rate of hypertension in our patients compared with most populations. In addition, the proportion of large artery atherosclerotic strokes (20.0%) was similar to some studies [*17,19*] but less than in others (30% and 39.5%) [*18,31*]. Cardioembolic strokes in our series (8.0%) were less frequent in comparison with other studies (17.5% to 31%) [*17–19,27,31*]. The number of patients (3.0%) with a negative evaluation was less than that found by others [*17,31*] and furthermore these patients did not receive transesophageal echocardiography to rule out cardiogenic or aortic arch embolic sources. As demonstrated by Amarenco, as many as 38% of patients with no discernible cause for embolic strokes may have atherosclerotic plaques > 4 mm in diameter in the aortic arch [*20*].

The most frequent risk factor in our patients with non-cardioembolic strokes was hypertension (76.0%), followed by diabetes mellitus (44.0%). Hypertension was also a major risk factor for lacunar infarcts, as was diabetes mellitus and smoking. Similarly, in a population-based study in Minnesota, Sacco noted that hypertension was found in 81% of patients with lacunar infarcts [*21*]. Smoking and hyperlipidaemia were also important risk factors in our study, as in other Middle and Far Eastern studies [*14,26*].

Concerning cardioembolic strokes, the most common risk factor was chronic atrial fibrillation (12/16 patients) associated with other risk factors (diabetes mellitus, hypertension with left ventricular hypertrophy) occurring in women above the age of 50 years, which is in agreement with other studies [*22–25*]. Interestingly, we had few men with atrial fibrillation in this study.

With regard to clinical and imaging findings in non-cardioembolic strokes, there was a striking absence of severe extracranial carotid and/or vertebral atherosclerosis (> 50% narrowing) in all of our patients. This is similar to Qari's study, in which only 4 out of 71 Saudi Arabian patients had

severe carotid stenosis [*14*]. Other Middle Eastern reports [*4*] found a higher frequency of lacunar infarcts and a less frequent prevalence of extracranial large artery disease. On the other hand, pure motor stroke was the most common sign of lacunar infarct (67 of 103 patients, 65.0%), which is in accordance with other studies. [*16,26,27*]. The most frequent location was in the internal capsule (34/103). With respect to cardioembolic strokes, the most common clinical sign was hemiparesis/ hemiplegia with or without aphasia and all patients had cortical infarcts in accordance with Dulli et al. where the odds ratio for cortical stroke was highest for atrial fibrillation on ECG (OR = 4.77; CI: 2.08–10.94) [*28*].

In cardioembolic strokes, only 6 out of 16 of our patients were on warfarin prior to their stroke (2 had stopped recently prior to the stroke and 4 had sub-therapeutic levels). Thus 12 out of 16 patients (75%) were not properly treated with warfarin prior to stroke. The problem of undertreating patients with atrial fibrillation has been demonstrated by Ibrahim and Kwoh who found in a cohort of 2093 patients with heart failure and atrial fibrillation that only 414 patients (20%) received oral anticoagulants [*29*]. Old age was negatively associated with their use. Deplanque et al. also noted that among 137 patients with atrial fibrillation eligible for oral anticoagulation, 108 (78.8%) did not receive treatment [*30*].

With regard to outcome, after a mean follow-up of 18 months, 85% of patients were discharged home (two-thirds independent, one-third dependent) and 15% died. The mortality rate was in the lower range, similar to Vilalta and Arboix's Barcelona registry at 14% [*31*]. Ikebe et al. found among 315 stroke patients followed up for 1 year that 33% died, 13% were dependent and 54% independent in activities of daily living [*32*]. The relatively good outcome in our patients may be due to their relatively low mean age and the predominance of lacunar infarcts.

In conclusion, this study has shown, first, a younger age of stroke prevalence compared with developed countries; second, a distinctive predominance of lacunar infarcts with absence of severe extracranial atherosclerotic disease and a lower frequency of cardioembolic strokes; and, third, that the major risk factors for non-cardioembolic and cardioembolic strokes were hypertension and chronic atrial fibrillation respectively. Although this study has the limitations associated with being a small, retrospective, hospital-based study, our results suggest the morbidity and mortality of stroke would be greatly reduced by appropriate management of hypertension. The lacunar stroke and hypertension rate is higher than most regions, and should be a focus of public health in Jordan and the Arab world. Furthermore, more nursing homes and rehabilitation centres in Jordan may reduce the length of hospital stay. Future community-based stroke registry and clinical trials in the region would further elucidate the health needs of the population.

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Prevalence of atrial fibrillation in a primary health care centre in Fars province, Islamic Republic of Iran

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معدل انتشار الرجفان الأذيني في مراكز الرعاية الصحية الأولية في مقاطعة فارس في إيران

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الخلاصة: توضح هذه الدراسة معدل انتشار الرجفان الأذيني في مركز الرعاية الصحية الأولية في مقاطعة فارس في إيران. فقد تم تحري جميع المرضى المتجولين البالغين عمر خمسين سنة أو أكثر والذين زاروا مركز الرعاية الصحية الأولية في الفترة من نيسان/أبريل وتشرين الأول/أكتوبر من عام 2001 لكشف الرجفان الأذيني باستخدام التخطيط المعياري ذي الاتجاهات الاثني عشر. لقد كان العمر الوسطي للمشاركين في الدراسة 64.0 ± 8.9 سنة. ومن بين هؤلاء المشاركين الذين يبلغ تعدادهم 463 والذين تراوحت أعمارهم بين خمسين و79 عاماً كان لدى 13 منهم (2.8٪) رجفان أذيني (العمر الوسطي لديهم 74 عاماً)، وكان معظمهم من النساء (10 من بين 230) مقابل الرجال (3 من بين 233). وبخلاف الدراسات السابقة التي أجريت في البلدان النامية فإن معدل الانتشار يزداد بمقدار ثلاثة أضعاف بمرور عَقْد من العمر، فقد ازداد زيادة ملحوظة من 0.6٪ في سنوات العمر 50-59 ليصبح 6.4٪ في سنوات العمر 70-79. ومع ازدياد التشيخ في بعض البلدان النامية فإن الرجفان الأذيني وما يسببه من سكتات دماغية قد يصبح من المشكلات الصحية البالغة الأهمية.

ABSTRACT This study determined the prevalence of atrial fibrillation at a primary health care centre in Fars province of the Islamic Republic of Iran. All ambulatory people aged ≥ 50 years visiting the centre between April and October 2001 were screened for atrial fibrillation using a standard 12-lead ECG. The mean ± SD age of participants was 64.0 ± 8.9 years. Of 463 participants aged 50–79 years, 13 (2.8%) had atrial fibrillation (median age 74 years), significantly more women (10/230) than men (3/233). Unlike previous studies in industrialized countries, the prevalence tripled with each decade of life and increased significantly from 0.6% in the 50–59 years to 6.4% in the 70–79 years age group. With increasing longevity in some developing countries, atrial fibrillation and consequently stroke may become major health problems.

Prévalence de la fibrillation auriculaire dans un centre de soins de santé primaires de la province de Fars (République islamique d'Iran)

RESUME Cette étude a permis de déterminer la prévalence de la fibrillation auriculaire dans un centre de soins de santé primaires de la province de Fars (République islamique d'Iran). Tous les patients ambulatoires âgés de 50 ans ou plus qui ont consulté au centre entre avril et octobre 2001 ont subi un examen à la recherche d'une fibrillation en réalisant un ECG standard à 12 dérivations. L'âge moyen des participants était de 64,0 ± 8,9 ans. Sur les 463 participants âgés de 50-79 ans, 13 (2,8 %) avaient une fibrillation auriculaire (âge médian 74 ans) ; il y avait un nombre significativement plus important de femmes (10/230) que d'hommes (3/233). Contrairement aux études précédentes réalisées dans les pays industrialisés, la prévalence triplait à chaque décennie de vie et augmentait de manière significative, de 0,6 % dans le groupe d'âge des 50-59 ans à 6,4 % dans celui des 70-79 ans. Compte tenu de l'accroissement de la longévité dans certains pays en développement, la fibrillation auriculaire et donc les accidents vasculaires cérébraux peuvent devenir des problèmes de santé majeurs.

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Introduction

First described almost 100 years ago, atrial fibrillation is now the most common clinically important sustained cardiac arrhythmia [*1,2*]. Atrial fibrillation is associated with a substantial morbidity [*2,3*] and is a major health care burden for many countries. The arrhythmia is the complete absence of coordinated atrial systole, characterized on the electrocardiogram (ECG) by the absence of 'P' waves before each QRS complex and the presence of rapid, irregular 'F' waves that vary in size, shape and timing. Atrial fibrillation is a potent predisposing factor for ischaemic stroke, and is associated with a 5-fold increase in risk [*4,5*]. Those with rheumatic heart disease and atrial fibrillation have an even higher (17-fold) increased risk of stroke [*1*]. It may also be an independent risk factor for death, with a relative risk of around 1.5 for men and 1.9 for women after adjustment for known risk factors [*6*].

The prevalence of atrial fibrillation reported in various studies, mostly conducted in industrialized countries, doubles with each decade of life and ranges from 0.05% among persons aged 25–35 years to 13.7% in those aged 80 years or older [*7,8*]. Nevertheless, little information is available about the prevalence of the disorder in developing countries. This study was conducted to determine the prevalence of atrial fibrillation in people aged 50 years and older attending a primary health care centre in Fars province in the south of the Islamic Republic of Iran.

Methods

Two general practitioners who practise in a primary health care centre affiliated to the National Iranian Oil Company (NIOC) health care system were assigned to screen patients for atrial fibrillation between April and October 2001. This centre sees about 400 patients a day in its 8 general practice clinics. The patients are employees of NIOC and their family members. They therefore range in age from children to old people. Around 70%–80% of the attendees are from urban areas and the remaining patients come from suburban/rural regions. Participants were selected from those who attended the centre for either a medical problem or routine check-up through simple random sampling; each patient attending the health care centre was assigned to 1 of 8 general practice clinics at random. The assignment was done by computer program, so that each doctor saw almost equal number of patients in each working day. Regardless of their presenting signs and symptoms, all ambulatory persons aged ≥ 50 years who were seen by each of the 2 general practitioners were included in the study. The patients were informed of the study objectives, the time needed to complete it and the procedure in detail. None of them refused to participate.

The diagnosis of atrial fibrillation (complete absence of coordinated atrial systole, characterized by the absence of 'P' waves before each QRS complex and the presence of rapid, irregular 'F' waves that vary in size, shape and timing) was made after recording a single standard resting 12-lead surface ECG (Fukuda Cardisuny 501B-III, Japan) with a 1-minute rhythm strip.

For analysis, the participants were categorized into 50–59, 60–69, 70–79 and ≥ 80 years age groups. Data were analysed using *SPSS*, version 10.

Results

Between April and October 2001, 480 Caucasian patients were entered into the study.

As only 17 participants were aged ≥ 80 years, further analysis of prevalence was not performed on this age group. The age distribution of the remaining 463 individuals (233 males, 230 females) is shown in Table 1. The mean ± SD age was 64.0 ± 8.9 years.

Overall, 13 patients were found to have atrial fibrillation, a prevalence of 2.8% (95% confidence interval 1.3%–4.3%). The prevalence increased significantly (χ^2 for trend = 9.608, $P < 0.002$) from 0.6% (SE = 0.6) in patients aged 50–59 years to 1.4% (SE = 1.0) and 6.4% (SE = 2.0) in the 70–79 year age group (Figure 1). The median age (25th percentile, 75th percentile) for those who had atrial fibrillation was 74.0 (68.5, 76.5) years. Of the 13 patients with atrial fibrillation, women (n = 10) were affected significantly more than men (n = 3) (Pearson's χ^2 =3.972, df = 1, $P <$ 0.05).

Discussion

The epidemiology of atrial fibrillation in this health centre in the south of the Islamic Republic of Iran is different from that of industrialized countries. In contrast to previous studies that found higher rates of atrial fibrillation among men [*1,2,5*], we found the prevalence was higher in women (10/13) than men (3/13) ($P < 0.05$).

Nowadays, atrial fibrillation is more often treated as a sign of an underlying disease than a disease entity in its own right. The most important underlying cardiac disorders consist of heart failure, coronary artery disease, hypertensive cardiovascular disease and valvular heart diseases [*9,10*]. Mitral stenosis is important in the etiology of atrial fibrillation [*11*]. Mitral stenosis mostly results from rheumatic fever, a disease that is currently not frequent in industrialized countries, owing to better treatment with antibiotics and increased health standards [*10*]. In developing countries, however, where rheumatic fever is still a health problem, mitral stenosis is much more frequent. Since mirtal stenosis affects females twice as often as males [*10*], in developing countries the sex ratio of patients with atrial fibrillation would be reversed, and women might be expected to develop atrial fibrillation much more often than men.

Like previous studies, mostly conducted in industrialized countries, we observed that the prevalence of atrial fibrillation increases with age [*5*]. The median age of 74 years in our patients is in keeping with that reported in other studies [*4,5*]. Go et al. [*4*] reported a prevalence of 1.8% in patients aged 60–69 years and 4.9% in 70–79 years, compared with 1.4% and 6.5% respectively in our study. Comparing our results with these, we found no statistically significant difference between the reported prevalences over the age range 60 to 80 years. However, unlike previous reports that show a 2-fold increase in the prevalence of atrial fibrillation with each decade of life [*2,5*], in our population this trend was much steeper and instead increased 3-fold with each decade over the age range studied. This accelerated trend may be due to ethnic

Table 1 **Age and sex distribution of participants and prevalence of atrial fibrillation**

Age group (years)	No. of participants			No. (%) with atrial fibrillation	
	Male	Female	Total		
50–59	89	74	163	1	(0.6)
60–69	60	83	143	2	(1.4)
70–79	84	73	157	10	(6.4)
Total	233	230	463	13	(2.8)

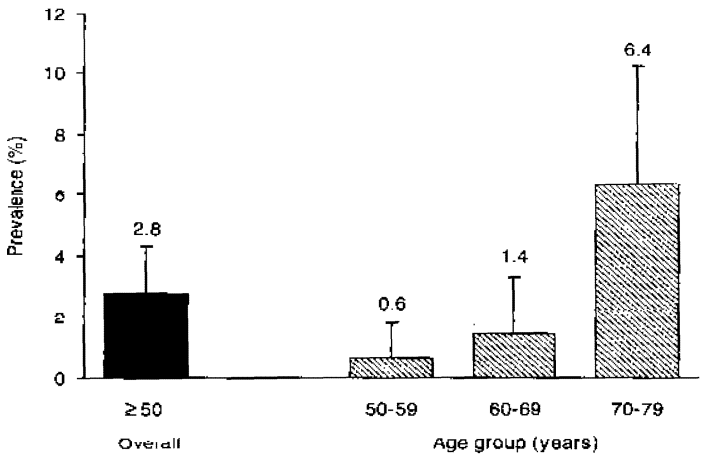

Figure 1 **Prevalence of atrial fibrillation by age group (error bars represent 95% confidence interval)**

differences and/or to the underlying causes in our population. In industrialized countries, ischaemic heart disease is the most important cause [*9*], whereas in developing countries, though it was not studied here, valvular heart disease, particularly mitral stenosis, seems to be the cause of atrial fibrillation in the majority of cases.

This study suffers from some limitations. Estimates of the prevalence of atrial fibrillation vary according to the characteristics of the population studied and the way atrial fibrillation is ascertained. In the current study, the diagnosis of atrial fibrillation was made on a single ECG. Hence, we might miss some people with non-sustained atrial fibrillation. Moreover, given the small sample size used in this study as compared with those conducted in industrialized countries, the statistical power of the current research is low. Likewise, the small number of cases with atrial fibrillation diagnosed in this study, particularly when they were categorized into age groups, means that we were only able to derive a rough estimate of the prevalence rates. Nevertheless, the results obtained from this work were statistically significant and the steep trend of increasing prevalence of atrial fibrillation at older ages is noteworthy. With improved health standards and increasing longevity in some developing countries [*12,13*], atrial fibrillation and consequently stroke may become increasingly important health problems.

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Frequency of *Yersinia* species infection in paediatric acute diarrhoea in Tehran

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تواتر العدوى بأنواع اليرْسَنيّات في الإسهال الحاد لدى الأطفال في طهران

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الخلاصة: توضح هذه الدراسة تواتر العدوى باليرْسَنيّات المُمْلِهة للمعى والقولون لدى 300 طفلاً أصيبوا بالإسهال الحاد وتقل أعمارهم عن 12 عاماً، ممن راجعوا مستشفى الأطفال في طهران. واستمرت الدراسة خمسة أشهر (من أيار/مايو إلى أيلول/سبتمبر 2002)، وتم خلالها إجراء الزرع لأنواع اليرْسَنيّات ولغيرها من العوامل المرضة، مع تعيين النمط السيرولوجي من العينات أو المسحات البرازية. وقد أمكن كشف أنواع اليرْسَنيّات في 8 حالات (2.7٪) والإيشريكية القولونية المرضة للأمعاء في 5.7٪ من الحالات وأنواع الشيغيلات في 3٪ من الحالات وأنواع السالمونيلات في 2٪ من الحالات. ولم تكشف أيٌّ حالة من اليرْسَنيّات المُمْلِهة للمعى والقولون التي تنضوي تحت النمطين السيرولوجيين الشائعين O:9 وO:3. في حين تم استفراد بعض الأنواع اللاّنموذجية من اليرْسَنيّات (اليرْسَنيّات المتوسطة واليرْسَنيّات الفريدريكسونية). وقد أبدَتْ جميع مستفردات اليرْسَنيّات غراراً متشابهاً من حيث المقاومة لمضادات المكروبات. على أن العدوى بأنواع اليرْسَنيّات غير شائع في أشهر الصيف في طهران.

ABSTRACT This study determined the frequency of *Yersinia enterocolitica* infection in 300 children with acute diarrhoea aged 0–12 years who were attending a paediatric hospital in Tehran. Over the 5-month study (May–September 2002), *Yersinia* species and other organisms were cultured and serotyped from stool samples or swabs. *Yersinia* spp. were found in 8 cases (2.7%). Enteropathogenic *Escherichia coli* was isolated in 5.7% of cases, *Shigella* spp. in 3.0% and *Salmonella* spp. in 2.0%. None of the *Y. enterocolitica* belonged to the common serotypes of O:3 and O:9. Atypical *Yersinia* spp. (*Y. intermedia* and *Y. frederiksenii*) were isolated. All *Y. enterocolitica* isolates had a similar pattern of antimicrobial resistance. *Yersinia* spp. infections are not common in the summer months in Tehran.

Fréquence de l'infection par les espèces de *Yersinia* dans la diarrhée aiguë de l'enfant à Téhéran
RESUME Cette étude a déterminé la fréquence de l'infection à *Y. enterocolitica* chez 300 enfants âgés de 0 à12 ans souffrant de diarrhée aiguë qui consultaient dans un hôpital pédiatrique de Téhéran. Pendant l'étude qui a duré cinq mois (mai-septembre 2002), les espèces de *Yersinia* et d'autres organismes ont été mis en culture et sérotypés à partir d'échantillons fécaux ou de prélèvements effectués à l'aide d'un écouvillon. On a trouvé *Yersinia* spp dans 8 cas (2,7 %). *Escherichia coli* entéropathogène a été isolé dans 5,7 % des cas, *Shigella* spp dans 3,0 %, et *Salmonella* spp dans 2,0 %. Aucune des *Y. enterocolitica* spp n'appartenait aux sérotypes courants O:3 et O:9. Des espèces atypiques du genre *Yersinia* (*Y. intermedia* et *Y. frederiksenii*) ont été isolées. Tous les isolats de *Y. enterocolitica* avaient un profil de résistance antimicrobienne similaire. Les infections à *Yersinia* spp ne sont pas fréquentes durant les mois d'été à Téhéran.

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Introduction

Diarrhoeal diseases are a major cause of childhood morbidity and mortality worldwide, especially in developing countries [1]. They account for an estimated annual 5 million deaths among infants aged under 5 years around the world [2].

Yersinia enterocolitica is a pathogen that causes self-limiting gastroenteritis or enterocolitis [3,4]. The organism is particularly common among children, causing outbreaks in day-care centres and schools. Symptoms range from mild (diarrhoea, abdominal pain) to severe (fever, severe abdominal pain often mistaken for appendicitis). Occasionally *Y. enterocolitica* gastrointestinal infection is followed by arthritis of the peripheral joints [5,6]. In different parts of the world, such as southern and western Europe (Scandinavia, Belgium, Holland, France, Germany, Denmark, etc), the United States of America, Canada, Australia, Japan and many other countries, *Y. enterocolitica* has been shown to be a primary human pathogen [4,7–9].

Although *Yersinia* species (spp.) have been reported in tropical areas [10], infections are more prevalent in cold European countries and North America [11,12]. Since the first isolation of *Y. enterocolitica* in the Islamic Republic of Iran in 1977 [13], there have been only a few studies on the epidemiology of this pathogen in our country and only one study has reported its isolation from drinking-water in Tehran [14,15]. *Y. enterocolitica* has been isolated from humans in many countries with varying rates [6,16]. Infrequent isolation in some areas has led some investigators to conclude that routine culturing of stool specimens for *Yersinia* spp. is not cost-effective [17]. The current study aimed to record the frequency and serotypes of *Yersinia* spp. and other enteropathogens in children with acute diarrhoea attending a paediatric hospital in Tehran. We focused our study on children as they are two to three times more susceptible to infection with *Yersinia* spp. than adults.

Methods

Subjects

The subjects were 300 children between the ages of 0 and 12 years who were suffering from acute diarrhoea (3 or more loose or watery stools per day for a period of less than 2 weeks). We included all patients with a diagnosis of acute diarrhoea visiting the Children's Treatment Centre at Markaz Tebbi Koodakan during a 5-month period from May to September 2002. Markaz Tebbi Koodakan is the largest paediatric hospital in Tehran, the capital city of the Islamic Republic of Iran

Data collection

All the patients' demographic and clinical data were collected by the primary care physicians at the centre using a questionnaire. Stool samples were collected from the children or, when this was not possible, rectal swabs were taken. For the detection of *Yersinia* spp., the samples were transported to the laboratory in phosphate-buffered saline transport medium (pH 7.0), according to World Health Organization recommendations [18] (0.5–1 g stool in 5 mL of buffer). To isolate *Escherichia coli*, *Salmonella* spp. and *Shigella* spp., an additional swab was obtained from the stool sample or directly from the patient and was placed in Cary -Blair transport medium.

Laboratory analysis

For the detection of *Yersinia* spp. the cold enrichment method was used. Samples were incubated at 4 °C for 4 weeks. At the end of the first, second, third and fourth week of incubation, samples were then cul-

tured on *Yersinia*-selective agar (CIN, 1.16434.0500, Merck, Darmstadt, Germany) with *Yersinia*-selective supplement (CIN, 1.16466.0001, Merck) and on MacConkey agar (CIN, 1.05465.0500/5000, Merck, Darmstadt, Germany) and incubated at 22 °C for 24 and 48 hours. After 24-hour incubation on *Yersinia*-selective agar, the samples were inspected carefully for suspicious colonies: pinpoint, round pink to red colonies, with a clear colourless surrounding halo which was clearly visible after 48 hours. After 24 hours on Mac Conkey agar, tiny, round and colourless (lactose-negative) colonies were considered suspicious for *Y. enterocolitica*. Because of the lack of necessary anti-sera at the laboratory, the isolates were sent to the Pasteur Institute of Paris for serotyping.

To identify other organisms, the samples were transferred immediately to culture media. *E. coli* were cultured on Endo agar medium (CIN, 1.04044.0500/5000, Merck, Darmstadt, Germany) and suspicious colonies (red, sometimes metallic colonies) were transferred to differential media (Kligler iron agar, SIM medium, urea agar and Simmons citrate agar) and incubated for 18–24 hours at 37 °C. Identification of enteropathogenic *E. coli* was done by slide agglutination with commercial polyvalent antisera (BioMericux, Lyon, France). For isolation of *Salmonella* spp. and *Shigella* spp. samples were transferred to Salmonella Shigella (SS) agar (CIN, 1.07667.0500, Merck, Darmstadt, Germany). Selenite-F broth (CIN, BO0354, Oxoid, UK) was used to augment the isolation of *Salmonella* spp. Suspicious colonies for *Shigella* sp. (colourless colonies) and for *Salmonella* spp. (colourless sometimes with a black precipitate) were transferred to differential media and incubated as before. *Salmonella* and *Shigella* spp. were identified through standard techniques [*19*] and were seroptyped using commercially available antisera (Difco, Detroit, Michigan, USA). Stool were examined for ova and parasites using the formalin ethyl acetate concentration method.

The *in vitro* susceptibilities to a range of antibiotics were determined for all isolates by the disk diffusion method [*20*]. The antibiotics tested were: tetracycline, chloramphenicol, gentamicin, kanamycin, streptomycin, amikacin, colistin, polymyxin B, co-trimoxazole, nitrofurantoin, nalidixic acid, lincomycin, penicillin G, ampi- cillin, cephalothin and rifampicin.

Results

A total of 300 children were studied: 122 females (40.7%), 178 males (59.3%). The mean age ± standard deviation was 3.34 ± 3.21 years. Table 1 shows the age and sex distribution of the children. Most of the patients were younger than 1 year old, followed by those aged 1–3 years.

Enteropathogenic *E. coli* was the most common pathogen isolated (17 out of 300 cases, 5.7%). *Shigella* spp. were isolated from 9 cases (3.0%), *Yersinia* spp. from 8 (2.7%) and *Salmonella* spp. from 6 (2.0%) (Table 2). More than one pathogen was isolated from 2 children, who were positive

Table 1 Age and sex distribution of 300 children with acute diarrhoea

Age (years)	Male No.	Female No.	Total No.	Total %
< 1	63	47	110	36.7
1–3	46	33	79	26.3
4–6	31	22	53	17.7
7–9	28	14	42	14.0
10–12	10	6	16	5.3
Total	178	122	300	100.0

for both enteropathogenic *E. coli* and *Shigella* spp.

The most common serotypes of the isolated *E. coli* were O26:B6 (8 cases, 47.1%) and O119:B14 (3 cases, 17.7%). Among the *Shigella* spp., *Sh. flexneri* type 2 (4 cases, 44.4%) and *Sh. sonnei* (2 cases, 22.2%) were most common. In cases of *Salmonella* spp., the two serotypes isolated were *S. typhimurium* (5 cases, 83.3%) and *S. havana* (1 case, 16.7%).

Among the 8 *Yersinia* spp. isolates, 5 cases (62.5%) were *Y. enterocolitica*. The results of the serotyping for isolated *Y. enterocolitica* showed that none of them belonged to the common serotypes of O:3 and O:9, and they were all environmental serotypes. Furthermore, the atypical *Yersinia* species *Y. intermedia* or *Y. frederiksenii* were isolated from the other 3 cases (Table 3).

All of our *Y. enterocolitica* isolates had a similar pattern of antimicrobial resistance (Table 4). The *E. coli* were all were sensitive to nalidixic acid and colistin, while the pattern of resistance for the rest of the antibiotics differed for each strain. Among the *Salmonella* and *Shigella* spp., all were sensitive to nalidixic acid, colistin and amikacin and were resistant to the other antibiotics.

Among the cases with *Y. enterocolitica*, 60% (3 out of 5) lived in rural areas in Tehran province where the drinking-water supply is from wells; the rest were living in urban areas. Diarrhoea and abdominal pain and fever (38–39 °C) were the most common clinical manifestations of infection with *Yersinia* sp. and were observed in all cases. Vomiting, headache and anorexia were seen less frequently. Diarrhoea was sometimes accompanied by mucous but no blood was found in stools.

All of the 17 patients with enteropathogenic *E. coli* had a negative direct smear for parasites, except 1 patient who was positive for *Hymenolepis nana* and had a past medical history of long-term weakness, malaise and chronic diarrhoea. Eight (47.1%) of the enteropathogenic *E. coli* cases were less than 1 year old.

Table 2 **Distribution of isolated pathogens by sex from 300 children with acute diarrhoea**

Species	Male No.	Female No.	Total No.	%
Enteropathogenic *E.coli*	10	7	17	5.7
Shigella spp.	5	4	9	3.0
Yersinia spp.	5	3	8	2.7
Salmonella spp.	3	3	6	2.0

Table 3 **Biotypes and serotypes of *Yersinia* species isolated from 8 cases of acute diarrhoea**

Case no.	Species	Biotype	Serotypes
1	*Y. enterocolitica*	1	O:7, O:8, O:19
2	*Y. enterocolitica*	1	O:7, O:8, O:19
3	*Y. enterocolitica*	1B	Autoagglutinable
4	*Y. enterocolitica*	1	Non autoagglutinable
5	*Y. enterocolitica*	1	Non-autoagglutinable
6	*Y. frederiksenii*	–	O:39
7	*Y. intermedia*	2	O:17
8	*Y. intermedia*	1	O:17

Discussion

The main objective of our study was to isolate *Yersinia* spp. to find out whether this organism and its common serotypes exist in the Islamic Republic of Iran or not. We were able to isolate *Yersinia* spp. from 8 of our patients in whom the other enteric bac-

Table 4 **Antimicrobial susceptibility of *Yersinia* species isolated from 8 cases of acute diarrhoea**

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Tetracycline	8	100	0	0
Chloramphenicol	8	100	0	0
Gentamicin	8	100	0	0
Kanamycin	8	100	0	0
Streptomycin	8	100	0	0
Amikacin	8	100	0	0
Colistin	8	100	0	0
Polymyxin B	8	100	0	0
Co-trimoxazole[a]	8	100	0	0
Nitrofurantoin	8	100	0	0
Nalidixic acid	8	100	0	0
Lincomycin	0	0	8	100
Penicillin G	0	0	8	100
Ampicillin	0	0	8	100
Cephalothin	0	0	8	100
Rifampicin	0	0	8	100

[a] *Sulfamethoxazole/trimethoprim.*

terial pathogens mentioned were excluded. Our findings clearly show that *Y. enterocolitica* is present as a pathogen of diarrhoea in this country and can be isolated from the stool samples of children suffering from acute diarrhoea. We found a frequency of 2.7% for this organism, which is near the frequency of about 1% reported by other studies in this country [*14*] but is lower than some parts of the world especially northern European countries with a frequency up to 13% [*7,11*]. This might be partly due to the warmer climate in our country, especially as the study was carried out during summer, and partly due to different dietary patterns in the Islamic Republic of Iran where pork is not consumed. We would expect a higher frequency during autumn and winter, based on the fact that this organism increases greatly in comparison with other species in cold seasons [*4,16*].

We used CIN medium and the cold enrichment method, which has been shown by many researchers to be an effective method for the isolation of *Yersinia* spp. from stool samples [*21–23*]. The *Y. enterocolitica* isolated were considered to be the cause of the presentation of acute diarrhoea, despite the fact that none of them belonged to the group of serotypes that are dominant in Europe, Asia, and Canada (O:3 and O:9) [*5,6*].

According to previous studies, the highest frequency of *Y. enterocolitica* is in cool-weather rural areas, based on the presence of the most important sources of contamination: pigs, cows, rabbits, and dogs and the surfaces and drinking-water sources contaminated with their faeces [*24–27*]. Our findings are in harmony with the previous studies since 3 out of 5 of our *Y. enterocolitica* cases lived in rural areas with a cooler climate and probably used contaminated drinking-water. This might also be the reason why all the isolated species belonged to the groups whose pathogenicity has been reported [*25,28–30*]. The main risk factors for the morbidity and mortality of diarrhoea are well known and relate to a poor quality of life, lack of sanitation and clean water supply for most of the population living in poor areas of developing countries [*29,30*].

The clinical manifestations of *Y. enterocolitica* infection in our children were mild, in accordance with studies from northern Europe [*5*] and in contrast with the study of Naqvi et al. [*4*]. Six of them had fever but we did not find any patients with bloody stools. The results of the antimicrobial sensitivity tests for *Yersinia* spp. iso-

lates showed a similar pattern to other studies [*4,5*].

Overall, enteropathogenic *E. coli* was the most common isolated pathogen in our study (5.7%), and this is consistent with other studies in the Islamic Republic of Iran [*14*] and in other developing countries [*31*]. The findings for *Salmonella* and *Shigella* spp. isolates were also consistent with these studies [*14,31*].

In spite of the fact that *Y. enterocolitica* is an important cause of diarrhoea in some European and Scandinavian countries with a colder climate, this study has shown that this organism is rarely isolated from stool cultures of children with diarrhoea seen in a hospital setting in the summer months in Tehran.

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Quinine therapy in severe *Plasmodium falciparum* malaria during pregnancy in Sudan

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معالجة الملاريا الناجمة عن المتصورات المنجلية بالكينين أثناء الحمل في السودان

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الخلاصة: أجريت دراسة استباقية في منطقة تعاني من سراية غير مستقرة للملاريا في وسط السودان للتعرف على نجاعة وسمية الكينين أثناء الحمل. فقد عولجت بالكينين 33 حاملاً مصابة بملاريا وخيمة ناجمة عن المتصورات المنجلية وكان وسطي عمر الحمل لديهن 288 أسبوعاً، واستمرت المعالجة سبعة أيام، وكان وسطي درجة حرارة الجسم أثناء القدوم لدى 3 حوامل ممن ولدن قبل الأوان أعلى بشكل ملحوظ (0.7 ± 392) من درجة حرارة الجسم لدى الحوامل اللواتي ولدن في تمام الحمل (1.3 ± 38.7). ولم يكن هناك فرق بين المجموعتين من حيث المتثابتات السريرية أو البيولوجية والطبية الأخرى. ولم يكن هناك تشوهات خلقية يمكن كشفها ولا عيوب بصرية أو سمعية أو عصبية في المولدان وقت ولادتهم ولا بعد مرور 6 شهور على ولادتهم. وعلى هذا، فقد يكون الكينين دواءً مأموناً لمعالجة الملاريا الوخيمة الناجمة عن المتصورات المنجلية أثناء الحمل.

ABSTRACT A prospective study was carried out in an area of unstable malaria transmission in central Sudan to determine the efficacy and toxicity of quinine in pregnancy. Thirty-three pregnant women with severe *Plasmodium falciparum* malaria at mean 28.8 weeks gestational age were treated with quinine for 7 days. The mean body temperature on presentation for 3 patients who delivered prematurely was significantly higher than for those who delivered at term (39.2 ± 0.7 °C versus 38.7 ± 1.3 °C). There were no significant differences between the 2 groups in other clinical or biochemical parameters. There were no clinically detectable congenital malformations and no auditory, visual or other neurological deficits in the babies at birth or 6 months later. Quinine may be safe in the treatment of severe falciparum malaria during pregnancy.

Traitement par quinine du paludisme sévère à *Plasmodium falciparum* pendant la grossesse au Soudan

RESUME Une étude prospective a été réalisée dans une zone de transmission instable du paludisme au centre du Soudan afin de déterminer l'efficacité et la toxicité de la quinine pendant la grossesse. Trente-trois femmes enceintes atteintes de paludisme sévère à *Plasmodium falciparum* à un âge gestationnel moyen de 28,8 semaines ont été traitées par quinine pendant 7 jours. La température corporelle moyenne au moment de la présentation pour trois patientes ayant eu un accouchement prématuré était significativement plus élevée que pour celles ayant accouché à terme (39,2 ± 0,7 °C versus 38,7 ± 1,3 °C). Il n'y avait aucune différence significative entre les deux groupes pour les autres paramètres cliniques ou biochimiques. Il n'y avait pas de malformations congénitales cliniquement détectables ni de déficits auditifs, visuels ou autres déficiences neurologiques chez les bébés à la naissance ou six mois après. La quinine peut être utilisée en toute sécurité pour le traitement du paludisme sévère à *Plasmodium falciparum* pendant la grossesse.

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Introduction

In Africa, each year some 24 million women become pregnant in malaria endemic areas. Pregnancy increases susceptibility to malaria and pregnant women are more likely to develop clinical attacks of malaria and serious complications than non-pregnant women of the same age. The increased susceptibility of pregnant women to malaria is thought to be in part the result of a certain degree of immune suppression during pregnancy required for fetal allograft retention [*1*]. Malaria in pregnant women is associated with serious adverse effects on pregnancy causing abortion, preterm labour, low birth weight, intrauterine fetal death and maternal death [*2–4*].

The epidemiological profile and clinical pattern of malaria vary according to the endemicity of the disease. In areas where malaria transmission is seasonal and unstable, both the mother and her fetus suffer from severe complications of the disease [*5*].

High-grade resistance of the malaria parasite to chloroquine has been reported in eastern and central Sudan [*6,7*]. This has led to the use of alternative drugs, such as quinine. There are few detailed reports on the effect of quinine in pregnant women and its effect on the outcome of pregnancy, and doubts remain about its safety in pregnancy. Through its oxytocic effect, quinine is capable of inducing abortion and labour [*8*]. Moreover, it can cause maternal hypoglycaemia through the release of insulin [*9*]. However, quinine is still the drug of choice for treatment of severe falciparum malaria in Sudan. The objectives of this work were to study the clinical efficacy and toxicity of quinine on mothers with severe falciparum malaria and their babies and to demonstrate the manifestations of severe falciparum malaria during pregnancy in the study group

Methods

Study area and patients

This study was carried out at Wad Medani teaching hospital, Gezira state, central Sudan, an area of seasonal unstable malaria transmission [*6*]. It is the main hospital where all seriously ill patients are referred from health centres and other single-doctor hospitals in the area.

All patients included in the study were selected from around 850 pregnant women seen and admitted to the hospital during the period September 1997 to January 1998 (the main malaria transmission season). Pregnant women were included in the study if they had a positive blood film for *Plasmodium falciparum* and 1 or more of the following criteria for severe malaria [*10*]: cerebral malaria (unarousable coma for > 30 min), repeated generalized convulsions, hyperpyrexia (rectal temperature > 40 °C), severe anaemia (haematocrit < 15% or haemoglobin < 50g/L), hypotension or shock (systolic blood pressure < 70 mmHg), jaundice (high serum bilirubin), pulmonary oedema, hypoglycaemia (blood glucose < 2.2 mmol/L), renal failure (< 400 mL urine/24h despite rehydration, and serum creatinine > 265 µmol/L), spontaneous bleeding or evidence of disseminated intravascular coagulation and hyperparasitaemia (> 250 000 rings/µL). Patients in labour, and those with twin pregnancy, intrauterine fetal death or vaginal bleeding were excluded from the study.

The patients or their relatives gave oral consent for admission to the study after full explanation of the purpose of the study and its expected risks.

Data collection

A full medical and obstetric history and physical examination were performed on

the participants and recorded using a case report form.

Parasitological diagnosis of malaria was confirmed by thick and thin blood films using Giemsa stain. The parasite was counted against 200 white blood cells and the extent of parasitaemia was calculated using the patients' white blood cells.

The following investigations were performed for all patients included in the study. Urine analysis was carried out (including presence of haemoglobin). Blood was analysed for haemoglobin level, white blood cell count and reticulocyte count. Serum levels of bilirubin, albumin, urea, creatinine, calcium alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also determined. Blood glucose level was determined on presentation and repeated if hypoglycaemia was suspected clinically.

Ultrasound examination to confirm the gestational age and viability of the fetus was performed on admission and every 4–6 weeks. Chest X-ray was performed using necessary protective precautions if pulmonary oedema was suspected and the pregnancy was more than 28 weeks.

Treatment and follow-up

The women were treated with quinine (Laboratoires Renaudin, France) at a dose of 30 mg salt/kg per day for 7 days. It was given first by intravenous infusion in 5% dextrose solution over 2–4 hours 3 times a day, and when the patient could tolerate it, therapy was continued orally in the form of tablets. Paracetamol was used to lower the fever. When haemoglobin was less than 70 g/L, packed red cells were infused as necessary. The patients were discharged after completing the full dose of quinine on day 8. They were seen at the antenatal clinic every 2 weeks until delivery.

The obstetrician supervised all hospital deliveries and kept close links with those who decided to deliver at home. Delivery followed the standard management procedure adopted in Wad Medani teaching hospital. A paediatrician examined and followed up all the babies to exclude congenital malformations. Weight and head circumference were recorded for babies delivered in hospital or at home. All the babies, whether hospital or home delivered, were followed up until 6 months of age by the same paediatrician to make an initial assessment of hearing (ability to respond to a rattle in a calm quite room) and vision (ability to respond to a coloured object moving in front of the child). If defects were suspected, more specialized tests would be performed, e.g. brain stem test and fundoscopy.

Definitions

Abortion was defined as expulsion of a dead fetus before 28 weeks of gestation. Premature labour was delivery after 28 weeks of gestation and before the 37 weeks. Perinatal death was death of the baby from 28 weeks *in utero* until the age of 1 week. Anaemia was defined as haemoglobin < 100 g/L.

Statistics

Data was entered into the computer using *SPSS*, version 10.0 batching for data analysis. Simple frequency distributions, descriptive statistics, mean, standard deviation (SD), *t*-test and chi-squared tests were used, with a probability level of < 0.05 for statistical tests.

Ethics

Ethical clearance for the study was obtained from the ethical committee of the Faculty of Medicine University of Khar

toum and national ethical committee at the Sudanese Federal Ministry of Health.

Results

Clinical presentation on admission

Thirty-five pregnant women with severe *P. falciparum* infection were initially admitted to the study: 33 patients completed the study and 2 patients decided to discontinue after the first dose of quinine (initial data collected from these patients is reported here).

The mean ± SD parity was 1.8 ± 2.2. Of the 35 patients, 11 were primigravidae, 9 were in the second pregnancy and 15 were in the third pregnancy or more (1 patient was a grand multipara in her ninth pregnancy). On presentation, the mean gestational age was 28.8 + 8.7 weeks. One patient had received quinine during the first 8 weeks of pregnancy.

The main presenting symptoms among the initial sample were: fever (77.1%), vomiting (68.6%), headache (57.1%), cough (22.9%) and diarrhoea (14.3%). Table 1 summarizes the clinical and laboratory findings obtained on admission. The mean values for all biochemical parameters measured were within normal ranges, except for bilirubin which was slightly raised.

The major manifestations of severe malaria among the 35 patients who started quinine therapy were: cerebral malaria (unarousable coma) (2 patients), severe anaemia (4), jaundice (11), hyperpyrexia (17), haemoglobinuria (11), hypotension (2), hypoglycaemia (1), and pulmonary oedema (1). Some patients presented with more than 1 symptom and 1 patient developed hypoglycaemia on day 7 of quinine treatment. However, no patient presented with renal failure or spontaneous bleeding from the gums or nose, and none were defined with hyperparasitaemia (> 250 000 rings/μL). The spleen was palpable in 8 patients on admission.

The 2 comatose patients (with cerebral malaria) also had repeated generalized convulsions, haemoglobinuria, body temperatures of 38.6 °C and 38.9 °C, parasite counts of 7600 rings/μL and 6250 rings/μL, haemoglobin concentrations of 98 g/L

Table 1 **Major clinical and laboratory findings on admission in 35 pregnant women with severe malaria**

Parameter	Mean	SD
Age (years)	26.3	4.7
Gravidity (No.)	3.2	2.5
Parity (No.)	1.8	2.2
Weight (kg)	60.5	10.9
Gestational age (weeks)	28.8	8.7
Systolic blood pressure (mmHg)	102.3	11.5
Temperature (°C)	38.8	1.3
Parasite count (rings/μL)	18735	21819
Haemoglobin (g/L)	89	25
White blood cells (cells/mm³)	4400	900
Blood urea (mmol/L)	23.7	5.5
Blood glucose (mmol/L)	5.70	2.50
Creatinine (mg/dL)	0.83	0.11
Bilirubin (mg/dL)	1.90	1.40
Albumin (g/L)	31.4	5.2
Calcium (mg/dL)	10.2	0.4
ALT (IU/L)[a]	5.14	9.70
AST (IU/L)[b]	3.17	6.11
Alkaline phosphatase (IU/L)	23.6	12.1

[a]ALT = alanine aminotransferase; normal values 0–45 IU/L.
[b]AST = aspartate aminotransferase; normal values 0–41 IU/L.
SD = standard deviation.

and 91 g/L, and blood glucose levels of 7.8 mmol/L and 3.9 mmol/L, respectively.

The mean ± SD haemoglobin of all the patients was 89 ± 25 g/L. However, 4 patients presented with severe anaemia (haemoglobin < 50 g/L). In these patients the peripheral blood picture showed normocytic normochromic red cells, with anisopoikilocytosis, and the sickling test was negative.

Treatment response

Twenty-one of the patients included in the study had received chloroquine before admission and had not improved. All patients responded well to quinine with dramatic relief of symptoms. The comatose patients recovered consciousness within 24 hours, and the fever subsided within 72 hours. Vomiting was not noticed in any of the patients after 72 hours and treatment was continued with oral quinine. Jaundice disappeared within 5 days in all except 2 patients where it persisted for 7 days. Severe anaemia was corrected by transfusion of packed red cells and the response was satisfactory. Quinine was well tolerated by the patients with limited side-effects (tinnitus and dizziness in about 54% of patients), which were mild and transient.

All patients had negative blood films on day 7. However, 2/33 (6.1%) patients presented on days 17 and 20 with recurrence of malaria symptoms and their blood films were positive. They were admitted and given artemether intramuscularly, 80 mg initially then 80 mg after 12 hours and then daily for 4 days. They were discharged after completing the treatment with full recovery and were followed up closely until delivery.

Pregnancy outcome

Thirty patients delivered at term and 3 patients delivered prematurely (at 32, 33, 34 weeks). One of these 3 patients delivered after the third dose of quinine; the other 2 patients delivered prematurely, but 30 and 50 days after completing quinine therapy which makes it unlikely to be due to quinine.

When we compared the clinical and laboratory findings in the 2 groups of patients (term and pre-term deliveries), there was no significant difference between them regarding mother's age, parity, gestational age when they received quinine therapy, systolic blood pressure, parasitaemia or biochemical findings (blood glucose level, serum bilirubin, blood urea level and haemoglobin concentration). However, the mean ± SD temperature on presentation for the 3 patients who delivered prematurely was 39.2 ± 0.7 °C, whereas it was 38.7 ± 1.3 °C in those who delivered at term, a difference that was significant ($P = 0.05$). On the third day, the temperature was normal in both groups (Table 2).

Fifteen patients delivered in Wad Medani hospital and 18 at home. The mean birth weight of the babies delivered at hospital and at home was 3.0 kg (range 2.2–4.0 kg); the mean head circumference at birth was 35.5 cm. There were no auditory, visual or neurological defects recorded. There were no maternal deaths, abortions, stillbirths or perinatal deaths.

Discussion

The present study was carried out in an area of unstable malaria transmission. More than 50% of the women were parous and different forms of clinical presentation of severe malaria were observed among our study group, including cerebral malaria and hyperpyrexia. This supports the belief that falciparum malaria in areas of low and unstable transmission is usually symptomatic and affects all parities, whereas in areas of

Table 2. **Comparison of the major characteristics of severe malaria in women with term and pre-term deliveries**

Parameter	Women with term delivery (n = 30) Mean	SD	Women with pre-term delivery (n = 3) Mean	SD	P-value[a]
Age (years)	26.6	4.6	22.3	6.8	0.42
Gravidity (No.)	3.2	2.5	3.3	3.2	0.70
Parity (No.)	1.8	2.2	2.3	3.2	0.34
Gestational age at treatment (weeks)	29.3	9.0	29.3	6.0	0.50
Temperature on admission (°C)	38.7	1.3	39.2	0.7	0.05
Temperature on day 3 (°C)	37.2	0.8	37.2	0.9	0.90
Systolic blood pressure (mmHg)	102.3	12.1	103.3	16.7	0.12
Parasite count (rings/μL)	18855	23472	15200	8265	0.32
Haemoglobin (g/L)	87	26	104	12	0.24
Random blood glucose (mg/dL)	106.6	44.7	80.6	13.6	0.33
Blood urea (mg/dL)	24.1	5.3	23.3	5.8	0.90
Serum bilirubin (mg/dL)	1.90	1.50	2.50	0.63	0.38

[a] *Using* t-*test.*
SD = standard deviation

high endemicity, falciparum malaria is more common among primigravidae and patients are usually asymptomatic or present with severe anaemia [*11–13*].

It is of interest to note that, although the patients presented with severe illness, the mean parasite count was relatively low at 18 735 rings/μL, and the highest count was 100 380 rings/μL. The explanation of this phenomenon is not clear. It is possible that the threshold for complications is low in this epidemiological setting. Hypoglycaemia was found in 1 patient on presentation, and only 1 patient developed hypoglycaemia after quinine therapy. However, hypoglycaemia has been reported in around 50% of pregnant women at one stage or another of severe falciparum malaria [*9*].

In this study, the patients responded readily to quinine and all symptoms cleared rapidly as described in the results. Only 2 patients (6%) developed symptoms of malaria with detectable parasitaemia in the third week. We are not sure if this was due to some degree of declining efficacy or even resistance to quinine or due to re-infection. Quinine resistance or failure has been reported in the eastern parts of Sudan among non-pregnant patients [*7,14*], and quinine resistance during pregnancy of around 30% has been documented in Thailand [*15,16*].

Quinine was well tolerated by the mothers and had no adverse effect on the children up to the age of 6 months. There were no maternal deaths, abortions, stillbirths or perinatal deaths. The mean gestational age of the patients at the time of enrolment was 28.8 weeks, which might explain why there was no abortion. This was also reported in 2 recent studies [*15,16*].

In the present report, we showed that 3 patients (9%) had pre-term labour; 1 of them delivered during quinine therapy and the other 2 patients delivered prematurely, 30 and 50 days after completion of quinine therapy. This makes it difficult to relate premature labour to quinine. However, no pre-term labour was reported by Mc Gready et al. in 1998 and 2000 [*15,16*]. This might be explained by the difference in morbidity of the patients included in those studies. In our study, all patients presented to hospital with manifestations of severe falciparum malaria, whereas in the above-mentioned studies, which were community based, the patients presented with uncomplicated malaria. Only 16% and 18% of the patients presented with fever, whereas the mean temperature of our patients on presentation was 38.8 °C. Furthermore, in our study 17 patients presented with hyperpyrexia and the difference in temperature on presentation between patients who delivered prematurely and those who delivered at term was significantly higher. High temperature was the main reason for uterine contraction [*9*]. Malaria can cause abortion and pre-term labour as well [*2*] and in central Sudan it was found to be the leading cause of low birth weight by causing pre-term labour [*17*]. However, the oxytocic effect of quinine on the pregnant uterus cannot be excluded totally, at least in the patient who delivered during quinine treatment. Quinine has been used as an abortifacient and labour inducing agent [*8*].

In this study, the paediatrician did not note any hearing or visual defects, and no congenital or developmental abnormalities in the babies. This agrees with other recent studies [*15,16*]. However, deafness and hypoplasia of the optic nerve have been described in children born after unsuccessful attempts to induce abortion in women taking quinine overdoses [*18*]. In humans, organogenesis is in the first 12 weeks of intrauterine life, and during this time only 1 patient in this study received quinine. Very few studies have been made on the safety of quinine during early pregnancy. We think it is necessary to carry out such studies in view of the emerging multidrug-resistant strains of *P. falciparum* and the limited choice of alternative safe and effective drugs for malaria treatment.

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Anopheles arabiensis: abundance and insecticide resistance in an irrigated area of eastern Sudan

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توافر الأنوفيلة العربية ومقاومتها للمبيدات في منطقة مرويّة في شرق السودان

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الخلاصة: درس توافر الأنوفيلة العربية ومدى تأثرها بالمبيدات في منطقة حلفا الجديدة، شرق السودان، خلال الفترة من آذار/مارس 1999 وحزيران/يونيو 2000. ومن بين الإناث من الأنوفيلات التي تم جمعها والتي بلغ عددها 4854 كان 4847 منها (99.9٪) من الأنوفيلات العربية، فيما كان 7 منها (0.1٪) من الأنوفيلات الفرعونية. إن الإناث من الأنوفيلات العربية تواصل سرأها (تكاثرها) طيلة العام مع فصلين للذروة الكثيفة، أحدهما خلال الفصل المطير (حيث وجدت 158.4 أنثى/اليوم/الغرفة مع 84.7 من اليرقات في كل عشر غمسات) والثاني خلال فصل الري (حيث وجد 136.8 من الإناث/اليوم/ الغرفة مع 44.8 من اليرقات في كل عشر غمسات). وكان متوسط نشاط العض 28.8 عضة/شخص/ليلة، وتتوزع العضات على مدار الليل، وبشكل خاص خارج المنزل. وقد كان معدل تأثر الأنوفيلات العربية بالد.د.ت 97.8٪ وبالملاثيون 96.3٪ وبالفينيتروثيون 100٪. إن الأنوفيلة العربية هي الناقل الوحيد للملاريا في المنطقة، وهي ذات نشاط يستمر كامل العام ولا يقتصر على فصل دون آخر.

ABSTRACT The abundance of *Anopheles arabiensis* and its susceptibility to insecticides was studied in New Halfa, eastern Sudan, from March 1999 to June 2000. Of 4854 females anophelines collected, 4847 (99.9%) were *An. arabiensis* and 7 (0.1%) *An. pharoensis*. Female *An. arabiensis* were breeding throughout the year, with 2 peak densities, during the rainy (158.4 females/room/day and 84.7 larvae/10 dips) and irrigated seasons (136.8 females/room/day and 44.8 larvae/10 dips). The mean biting activity was 28.8 bites/person/night, found throughout the night, mainly outdoors. Susceptibility of *An. arabiensis* to insecticides dichloro-diphenyl-trichloroethane (DDT), malathion and fenitrothion was 97.8%, 96.3% and 100% respectively. *An. arabiensis* is the sole malaria vector in the area and is perennial rather than seasonal.

***Anopheles arabiensis* : abondance et résistance aux insecticides dans une zone irriguée du Soudan oriental**

RESUME L'abondance d'*Anopheles arabiensis* et sa sensibilité aux insecticides ont fait l'objet d'une étude à New Halfa (Soudan oriental) de mars 1999 à juin 2000. Sur 4854 anophèles femelles récoltées, 4847 (99,9 %) appartenaient à l'espèce *An. arabiensis* et 7 (0,1 %) à *An. pharoensis*. Les femelles *An. arabiensis* se reproduisaient toute l'année, avec 2 pics de densité, pendant la saison des pluies (158,4 femelles/pièce/jour et 84,7 larves/10 prélèvements) et la saison d'irrigation (136,8 femelles/pièce/jour et 44,8 larves/10 prélèvements). La densité agressive moyenne était de 28,8 piqûres/personne/nuit, constatée pendant toute la nuit, principalement à l'extérieur. La sensibilité d'*An. arabiensis* aux insecticides dichloro-diphényl-trichloroéthane (DDT), malathion et fénitrothion était de 97,8 %, 96,3 % et 100 % respectivement. *An. arabiensis* est le seul vecteur du paludisme dans la région et la transmission y est pérenne plutôt que saisonnière.

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Introduction

Malaria is a major health problem in the tropical countries, especially sub-Saharan Africa, where about 90% of the clinical cases occur. There are nearly 500 million clinical cases of malaria worldwide each year and 1.1 to 2.7 million people die annually [*1*]. The *Anopheles gambiae* complex is the main malaria vector [*2*]. In Sudan, malaria constitutes around 40% of all infectious diseases and *Plasmodium falciparum* is the predominant species, which is responsible for over 90% of the infections [*3*]. The spread of drug-resistant *P. falciparum* strains worldwide has hampered the control of malaria, and vector control remains the best approach for protecting the community against malaria [*4*].

An. arabiensis is the major malaria vector reported from all parts of the country, co-existing with *An. gambiae sensu stricto (s.s.)* and *An funestus* in southern Sudan [*5–8*]. Vector control by chlorinated hydrocarbon insecticides was started in Sudan in 1948, when dichloro-diphenyl-trichloroethane (DDT) sprayed with oil was used as a residual indoor insecticide. Thereafter, organized control by these chemicals was initiated in 1950/51 in the Gezira scheme (central Sudan) but by the early 1970s, resistance to DDT was reported. Thereafter, the organophosphate insecticides malathion and fenitrothion replaced DDT [*9,10*].

Understanding the behaviour of the malaria vectors and their abundance is essential for malaria control operations. Furthermore, understanding the resting, biting and breeding habits of the vectors and their susceptibility to insecticides is extremely important for planning, implementing and monitoring vector control measures. The objective of this study was to establish this data for the New Halfa area of Sudan.

Methods

Study area

The study was carried out at 2 localities (Dibaira camp and Heielmasakine) in the north and south of New Halfa town, an area which is surrounded by freehold farms. The New Halfa area is located in the semi-arid belt of the Sudan approximately 500 km east of Khartoum in the middle of an agricultural scheme. During the study period, the total rainfall was 431.6 mm and the average temperature was 30 °C. *P. falciparum* is the predominant malaria parasite species, and has been shown to be 75% and 9.6% resistant to chloroquine and quinine respectively [*11*].

Mosquito collection

Hand capture and pyrethrum spray methods were used to make monthly collections of indoor resting mosquitoes during the period March 1999 to June 2000. Collection by hand capture was carried out from 06:00 to 08:00 hours. Two collectors spent about 10–15 minutes in each hut (total 10 huts) using a torch, aspirators and paper cups to search for resting anopheline mosquitoes. Then 0.3% pyrethrum in kerosene was sprayed and knocked-down mosquitoes were collected from 08:00 to 10:00 hours. Females collected were classified as according to their blood meal stages unfed, fed, half-gravid/gravid.

The monthly night biting collection was carried out in fixed collection sites during the main transmission season by 2 people (1 indoors and 1 outdoor) sitting from 18:00 to 06:00 hours. Females collected were kept in separate paper cups until identification and dissection for parity determination. The ovaries were dissected to determine the parity rate using the method of Detinova [*12*]. Blood spots from freshly fed females of *An. arabiensis* caught by

pyrethrum spray catches were harvested on filter paper, the source of each blood determined (human biting index) using the Ouchterlony radial diffusion technique [*8*].

The surveys of immature stages were carried out from 13:00 to 15:30 hours using a standard dipper. Monthly samples of 100 dips were taken at 10 positive breeding sites selected randomly to cover the whole town. Adult and larvae mosquitoes were identified on a morphological basis using the standard keys [*13,14*].

Susceptibility testing

The susceptibility of *An. arabiensis* to DDT, malathion and fenitrothion was examined in 3 locations (Wad el Naeem, Heielmasakine and Umgargoor). According to World Health Organization (WHO) recommendations [*15*], fed females were exposed for 1 hour to 4% DDT, 5% malathion and 1% fenitrothion to determine their susceptibility to these insecticides.

Statistical analysis

Data was entered into a computer database and *SPSS* software was used for statistical analysis. The difference between variables was evaluated using the chi-squared test. Student *t*-test was used to evaluate the difference in the density of the vector in the 2 localities and the human biting rate between the outdoor and the indoor sites. One-way analysis of variance was used to compare the human biting rates at different times of the night. A P value of less than 0.05 was considered significant.

Results

Out of 4854 females collected, 4847 (99.9%) were *An. arabiensis* and 7 (0.1%) were *An. pharoensis*. Most of the *An. arabiensis* specimens were collected by pyrethrum spray (4164, 85.9%) rather than hand capture (683, 14.1%).

The mean vector density in the Heielmasakine area (pyrethrum collection) was 29.3 females/room/day, while it was 23.1 females/room/day in Dibaira camp (Table 1). The highest density was recorded in September 1999 by the end of the rainy season (158.4 females/room/day) in Heielmasakine while the lowest density in this area was recorded in April 1999 (0.2 females/room/day). The peak density of adults was reflected by the peak of immature stages in the same month (84.7 larvae/10 dips). The lowest density of adult females was recorded in March 2000 (0.4 females/room/day) in Dibaira camp. The

Table 1 Total number of adult *Anopheles arabiensis* specimens collected during the study period by hand capture and pyrethrum spray and mean density per room per day in the 2 localities

Locality	Pyrethrum spray		Hand capture		Total	
	Total collected No.	Mean ± SE density No./room/day	Total collected No.	Mean ± SE density No./room/day	Total collected No.	Mean ± SE density No./room/day
Dibaira camp	1821	23.1 ± 8.8	311	3.7 ± 1.4	2132	26.9 ± 10.1
Heielmasakine	2343	29.3 ± 13.0	372	4.6 ± 1.5	2715	33.9 ± 14.5
Total	4164	26.0 ± 7.9	683	4.2 ± 1.0	4847	30.3 ± 8.9

SE = standard error.

lowest density of immature stages was recorded in that month (0.54 larvae/10 dips). A minor peak of female density was recorded during the period of irrigation (March 1999), 136.8 females/room/day in Dibaira camp, coupled with a minor peak of immature stages (44.8 larvae/10 dips) in the same month (Figures 1 and 2).

Of 2132 females collected from indoors resting sites in Dibaira camp, 14.1%, 42.5% and 43.3 % were unfed, fed and half-gravid/gravid respectively. While from Heiclmasakine, 14.4%, 36.5% and 49.1% were unfed, fed and half-gravid/gravid. The ratio of fed to gravid/half gravid females in the 2 localities considered together was 1:1.2 in favour of gravid/half-gravid. This indicates that this species is more endophilic than exophilic. A total of 4.1 females/room/day were captured on thatched walls compared with 1.6 females/room/day on mud walls.

Of the *An. arabiensis* females captured while trying to bite human baits, 76.2% were caught outdoors and 23.8% indoors. The average human biting rate during the rainy season was significantly higher for outdoor biting activity (28.8 bites/person/night) than indoors (9.0 bites/person/night) ($P < 0.001$). The indoor biting activity started initially with a moderate value at 18:00–02:00 hours (0.88 bites/person/hour), followed by a peak of biting activity at 20:00–22:00 (1.13 bites/person/hour) and then dropped to lower activity at 24:00–02:00 (0.25 bites/person/hour). A minor peak was observed at 02:00–06:00 (0.75 bites/person/hour). In contrast, the outdoor biting activity started with a peak value at 18.00–20.00 hours (5.0 bites/per-

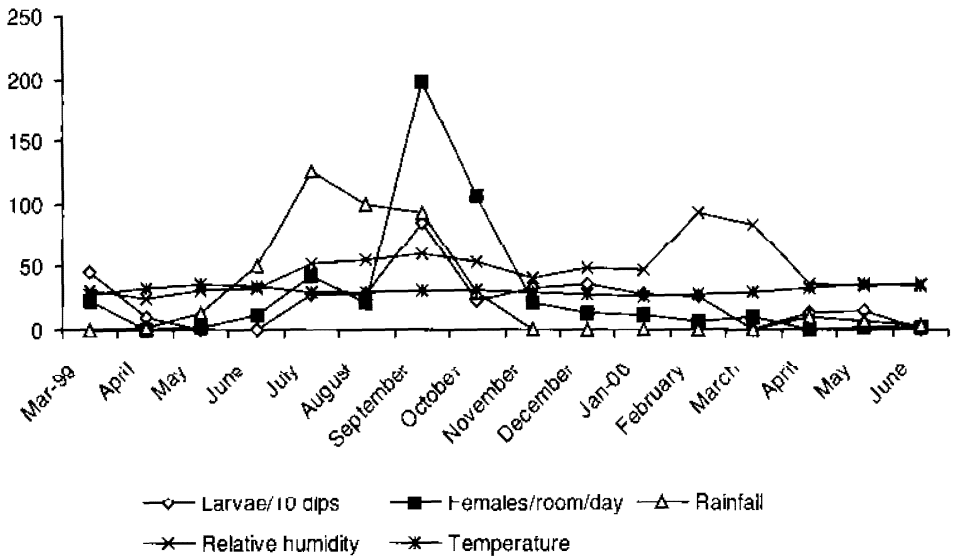

Figure 1 **Abundance of both adult females and larvae of *Anopheles arabiensis* in relation to rainfall (mm), relative humidity (%) and temperature (°C) in Dibaira camp**

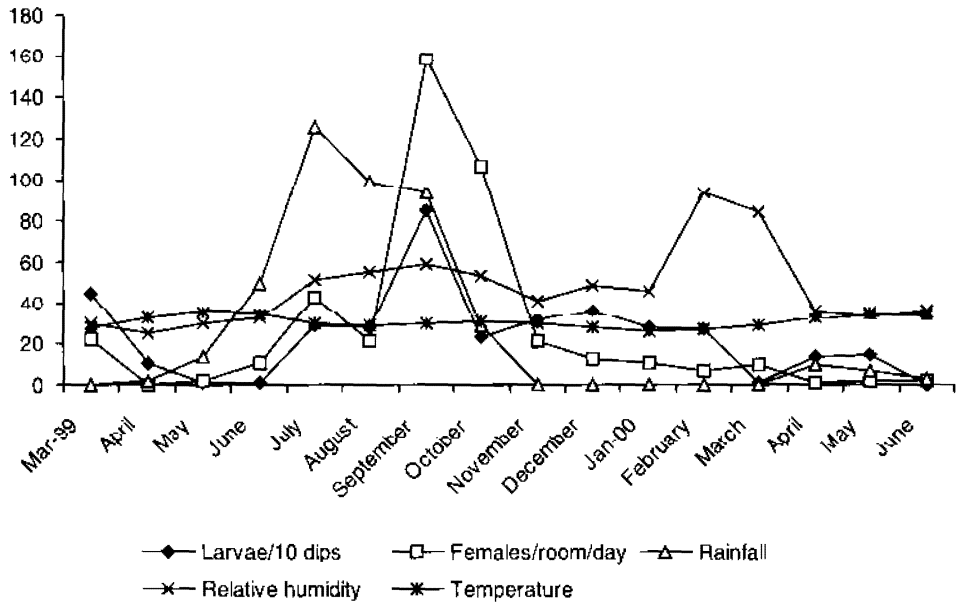

Figure 2 **Abundance of both adult females and larvae of *Anopheles arabiensis* in relation to rainfall (mm), relative humidity (%) and temperature (°C) in Heielmasakine**

son/hour) decreased at 20:00–22:00 to 3.25 bites/person/hour and dropped to lower values (0.25 bites/person/hour) between 02:00–06:00. The difference was not statistically significant ($P > 0.05$).

The monthly parity rates of *An. arabiensis* during the rainy season were 21.4%, 6.7%, 42.5% and 36.4% in July, August, September and October respectively, with a mean of 32.2% during this period. The human biting index of the 2 localities were 76.4% and 81.1% in Dibaira camp and Heielmasakine respectively, with a mean of 78.7% of the total mosquitoes examined.

Out of a total of 3849 larvae collected, 21.1%, 29.2%, 24.6% and 15.7% were first, second, third and fourth instar larvae respectively. Most (82.5%) of these larvae were collected from the shallow sunlit pools resulting from broken water pipes. Other breeding sites were rain pools (11.6%), leakage from irrigation canals (3.1%) and water excavations for human and animal use (2.9%). The mean total number of larvae collected during the study period was 24.3 larvae/10 dips.

The susceptibility of *An. arabiensis* to the 3 insecticides was 97.8%, 96.3% and 100.0% for 4% DDT, 5% malathion and 1% fenitrothion respectively (Table 2).

Discussion

This entomological study was conducted in an agricultural area in eastern Sudan, characterized by a high level of chloroquine-resistant falciparum malaria [*11*]. *An.*

Table 2 **Susceptibility to insecticides of adult *A. arabiensis* collected from 3 localities**

Locality	DDT 4%			Malathion 5%			Fenitrothion 1%		
	Total tested No.	Suscep %	Resist %	Total tested No.	Suscep %	Resist %	Total tested No.	Suscep %	Resist %
Wad el Naeem	75	97.3	2.7	75	98.7	1.3	75	100.0	0
Heleimasakine	60	98.3	1.7	40	100.0	0	40	100.0	0
Umgargoor	100	100.0	0	100	93.0	7.0	100	100.0	0
Total	235	97.8	2.2	215	96.3	3.7	215	100.0	0

Suscep = susceptible.
Resist = resistant.

arabiensis was the main vector (99.9%) found in the area; only 0.1% were *An. pharoensis* and no other species were detected. This agrees with a previous study from the nearby area (Gedaref) in the eastern Sudan where *An. arabiensis* was the main vector, besides 2 other species, *An. pharoensis* and *An. funestus* [*8*].

An. arabiensis existed throughout the year with 2 peaks, a major one at the end of the rainy season (September) and the other during the irrigated season of the scheme. The second peak is most likely due to presence of breeding sites that were formed from the puddles of irrigation canals around the area. Thus *An. arabiensis* has become perennial instead of seasonal because of irrigation and agricultural practices. Similar findings were reported from an irrigated area in the Gezira scheme in central Sudan [*16*], but unlike our study, a single peak of vector density was observed at the end of the short rainy season that dropped gradually to disappear in the long, hot dry season [*8*]. The most important factor leading to high breeding density during the rainy season was the optimal temperature (28.9–30.1 °C) and high relative humidity, as it is known that high temperature and low humidity adversely affect adult and immature stages [*17*]. This trend of mosquito abundance differs from other tropical areas that are characterized by longer rainy seasons and more humid conditions, e.g. southern Sudan and many other African countries. In these regions, anopheline mosquitoes are known to be prevalent throughout the year [*18*].

Our study showed that *An. arabiensis* clearly preferred thatched to mud walls as a resting surface. A similar finding was observed from central Ethiopia [*19*].

In this area *An. arabiensis* is strongly exophagic. This finding is supported by the previous study in Gedaref state and central Ethiopia [*8,19*]. Three factors seem to determine the biting cycle of the *An. arabiensis*: rhythmic activity of the mosquitoes, the microclimate and human habits [*20*]. Winds and temperature are the climatic factors affecting the peaks of the biting cycle [*18*]. In the Zwai area of central Ethiopia the peak of outdoor biting occurred from 22:00 to 24:00 hours, whereas the peak of indoor biting took place early in the evening from 18:00 to 20:00 [*19*]. However, the vector was aggressive throughout the night and exhibited 2 peaks in the indoor biting activity at 20:00 to 22:00 and later at 02:00 to 06:00, with the peak outdoor biting activity at 18:00 to 22:00.

The low parity rate of female mosquitoes in this area is likely to be related to the application of the insecticide K-Othrine (a water-based formulation of deltamethrin) during the period of the study in August 1999, as shown by the low parity rate that month (6.7%). Mixed feeding by mosquitoes is common, but with *An. arabiensis* this may be determined by the host available [*21*]. In this area, the presence of domestic animals is most likely to be very rare in human dwellings. Therefore, the human biting index recorded (78.7%) was relatively high, similar to that in Gedaref state [*8*].

Although there is seldom a large difference in susceptibility between the sexes, female mosquitoes (preferably blood-fed) should be used exclusively in field tests. This is because they survive better and show lower control mortality [*15*]. The susceptibility to DDT, malathion and fenitrothion was found to be 97.8%, 96.3% and 100.0% respectively, indicating that the mosquito population in this area had a low level of resistance to insecticides. Similar findings were reported in central Ethiopia [*19*]. The comeback susceptibility of *An. arabiensis* to DDT may be due to stoppage of chlorinated hydrocarbon insecticides for more than 20 years. Nevertheless, in the Gezira irrigated scheme the vector developed resistance to several insecticides in the 1970s, which was influenced by the overuse of crop-spraying with insecticides [*10*].

It is important to note that the establishment of the New Halfa agricultural scheme in the area has resulted in a serious abundance of *An. arabiensis* throughout the year. More studies are needed in order to assess the role of this species in malaria transmission in different seasons.

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Characteristics of tuberculosis patients in Yazd province, Islamic Republic of Iran, 1997–99

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خصائص مرضى السل في مقاطعة يزد في إيران 1997-1999

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الخلاصة: حددت الدراسة معدل وقوع السل وبعض الخصائص الديمغرافية لدى 604 من المرضى خلال 3 سنوات (1997-1999) في مقاطعة يزد في إيران. وقد اتضح أن المعدل السنوي الوسطي لوقوع السل 26.8 لكل مئة ألف (22.9 لدى الذكور و31.0 لدى الإناث لكل مئة ألف)، وقد كان أعلى معدل للسل بين من يزيد عمرهم على 50 عاماً (111.1 لكل مئة ألف). و كان العدد السنوي الوسطي لحالات السل الرئوي 75.8٪ وخارج الرئوي 24.2٪، وقد شفي من مرضى السل 66.7٪، فيما نقل 8.4٪ إلى مناطق احتواء مختلفة، وعانى 17.7٪ منهم من فشل المعالجة، ومات 7.1٪ منهم. ومن بين المصابين كان 63.2٪ منهم إيرانيين و36.1٪ من اللاجئين الأفغان. ورغم جهود الوقاية والتشخيص والمعالجة للمرضى وللاجئين فإن السل لايزال يمثّل مشكلة هامة في هذه المقاطعة.

ABSTRACT We determined the incidence of tuberculosis and some demographic characteristics among 604 patients in a 3-year period (1997–99) in Yazd province, Islamic Republic of Iran. The average annual rate of tuberculosis was 26.8 per 100 000 (22.9 in males and 31.0 per 100 000 in females). The highest rate of tuberculosis was in the > 50 years age group (111.1 per 100 000). The average annual proportion of pulmonary and extra-pulmonary tuberculosis cases was 75.8% and 24.2% respectively. On average 66.7% of TB patients were cured, 8.4% transferred to a different catchment area, 17.7% were treatment failures and 7.1% died. Of the total, 63.2% were Iranian, 36.1% Afghan refugees. Despite efforts in prevention, diagnosis and treatment of patients and refugees, tuberculosis is still an important problem in this province.

Caractéristiques des patients tuberculeux dans la province de Yazd (République islamique d'Iran), 1997-1999

RESUME Cette étude a permis de déterminer l'incidence de la tuberculose ainsi que certaines caractéristiques démographiques chez 604 patients sur une période de trois ans (1997-1999) dans la province de Yazd (République islamique d'Iran). L'incidence annuelle moyenne de la tuberculose était de 26,8 pour 100 000 (22,9 chez les hommes et 31,0 chez les femmes). L'incidence la plus élevée se trouvait dans le groupe d'âge des plus de 50 ans (111,1 pour 100 000). La proportion annuelle moyenne de cas de tuberculose pulmonaire et extrapulmonaire était de 75,8 % et 24,2 % respectivement. En moyenne, 66,7 % des patients tuberculeux ont guéri, 8,4 % ont été transférés à un autre secteur, 17,7 % ont connu un échec thérapeutique et 7,1 % sont décédés. Au total, 63,2 % étaient des Iraniens, 36,1 % des réfugiés afghans. Malgré les efforts déployés pour la prévention, le diagnostic et le traitement des patients, la tuberculose demeure un problème important dans cette province.

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Introduction

Tuberculosis (TB) remains a major global public health problem. The World Health Organization (WHO) estimated that in 1997 there were about 8 million new cases of TB and 2 million deaths worldwide; the great majority (95%) of these cases and deaths (98%) were in developing countries [*1,2*]. It was estimated that by 2000, the annual global number of new cases would have increased to over 10 million (163 per 100 000 population), and the annual expected number of deaths to 3.5 million per year (nearly 46 per 100 000 population) [*3,4*]. Today, TB remains common throughout most of the world with one-third of the world's population estimated to be infected with *Mycobacterium tuberculosis*. The incidence of TB has recently started to rise again due to increased migration, a higher rate of direct transmission of *M. tuberculosis* and co infection with HIV [*5*].

In 1999, the highest and lowest rates of TB in provinces of the Islamic Republic of Iran were reported to be 137 per 100 000 and 10 per 100 000 respectively [*6*]. The main goal of this study was to describe the incidence of TB and some demographic characteristics of TB patients over a 3-year period (1997–99) in Yazd province, in the central part of the Islamic Republic of Iran.

Methods

Yazd province covers an area of about 74 214 km^2 and has an estimated population of 750 769 (385 936 males and 364 833 females). The study participants were patients with TB aged 8–65 years who were referred to the Yazd referral hospital during the study period (1997–99).

Samples of sputum, tissue or body fluids were obtained and investigated by Ziehl–Neelsen staining and culture methods. Specimens were transported in broth media (Becton Dickinson, Maryland, USA) and processed on the same day if possible, or if delay was unavoidable, the specimens were stored at 4 °C for not more than 1 night before processing. On arrival at the laboratory, the presence of visible fibrinous clots was noted and Ziehl–Neelsen staining was applied. Cultures were obtained by inoculation into conventional solid Lowenstein–Jensen media (Difco). All inoculated cultures were incubated at 37 °C under 5% CO_2. The culture media were investigated twice a week for the first 3 weeks and thereafter weekly for a total of 6 weeks incubation. All isolates were initially confirmed by Ziehl–Neelsen staining and subsequently identified by standard biochemical tests [*7,8*].

The collected data and results of laboratory tests were analysed by *SPSS*, version 6.

Results

A total of 604 cases of TB were identified during the study period. The overall mean annual rate of TB over the 3-year period was 26.8 per 100 000 population (Table 1).

There were 256 males and 339 females. There was no significant difference in the rate of TB between the sexes ($P > 0.05$); the mean annual rate was 22.9 per 100 000 for males and 31.0 per 100 000 for females. Breakdown of the data by age showed the lowest rate of TB was in the ≤ 10 years age group (7.0 per 100 000) and the highest rate among the > 50 years age group (111.1 per 100 000). Data analysis revealed that those aged > 50 years had a significantly higher rate than those in the younger age groups ($P < 0.05$).

The nationality of the TB patients over the 3-year period showed a high proportion of the group were Afghan refugees

Table 1 **Number of tuberculosis patients and rate per 100 000 population by sex and age**

Variable	Population	1997		1998		1999		Total	Mean
		No.	Rate	No.	Rate	No.	Rate	No.	annual rate
Sex									
Male	385 936	87	22.5	107	27.7	71	18.4	265	22.9
Female	364 833	112	30.7	122	33.4	105	28.8	339	31.0
Age (years)									
≤ 10	171 738	22	12.8	10	5.8	4	2.3	36	7.0
11–19	210 309	18	8.6	25	11.9	20	9.5	63	10.0
20–29	120 355	19	15.8	35	29.1	23	19.1	77	21.3
30–39	94 622	14	14.8	23	24.3	13	13.7	50	17.6
40–49	58 922	25	42.4	21	35.6	16	27.2	62	35.1
> 50	94 823	101	106.5	115	121.3	100	105.5	316	111.1
Total	750 769	199	26.5	229	30.5	176	23.4	604	26.8

(36.1%); 63.2% were Iranian and 0.7% other nationalities (Table 2).

Of the 604 patients, 458 patients had pulmonary TB and 146 extra-pulmonary TB. The mean annual percentage of extra-pulmonary TB cases (24.2%) was much lower than for pulmonary TB cases (75.8%) (Table 2).

Table 2 **Distribution of patients by nationality, type of tuberculosis and treatment outcomes**

Variable	1997 (*n* = 199)		1998 (*n* = 229)		1999 (*n* = 176)		Total (*n* = 604)	Mean annual
	No.	%	No.	%	No.	%	No.	%
Nationality								
Iranian	121	60.8	155	67.7	106	60.2	382	63.2
Afghan (refugee)	75	37.7	73	31.9	70	39.8	218	36.1
Other	3	1.5	1	0.4	–	–	4	0.7
Type of tuberculosis								
Pulmonary	147	73.9	186	81.2	125	71.0	458	75.8
Extra-pulmonary	52	26.1	43	18.8	51	29.0	146	24.2
Outcome								
Cured	132	66.3	149	65.1	122	69.3	403	66.7
Treatment failure	49	24.6	34	14.8	24	13.6	107	17.7
Transferred to different area	3	1.5	40	17.5	8	4.5	51	8.4
Death	15	7.5	6	2.6	22	12.5	43	7.1

n = *total number of TB patients.*

The outcome of TB for this group of patients showed that on average 66.7% of patients were cured, 8.4% transferred to a different catchment area, 17.7% were treatment failures and 7.1% died (Table 2).

Discussion

The technologically under-developed and developing countries carry an enormous share of the world's TB burden. The disease is not uniformly distributed and some countries show a continuing increase in TB infection rates, whereas others show declining infection rates [*9–11*]. In industrialized centres, a rise of TB incidence has been observed, due to increased migration, a higher rate of direct *M. tuberculosis* transmission and the HIV epidemic. TB is a continuing threat to health in all parts the world [*5*].

The present study was carried out to monitor the incidence of TB and some demographic characteristics of TB patients in Yazd province in a 3-year period (1997–99). The average annual rate of TB during the study period was 26.8 per 100 000 population (23 per 100 000 among males and 31 per 100 000 among females). The rate of TB in our study is higher than the rate in some other areas in the world [*6,12–15*]. But, it is nearly equal to the lowest rate for developing countries (25 per 100 000), and one-ninth the highest rate for developing countries (240 per 100 000) [*16*].

Real increases in the numbers of TB patients cured will only occur when the Iranian Ministry of Health and Medical Education is able to either directly treat all cases of TB in Yazd province or at least influence the method of treatment applied in private practice and other health care services, such as university hospitals and military services. If the National TB Control Programme can achieve its target of DOTS [directly observed treatment, short-course] for all, it will have had a real impact on the problem of TB, not only immediately on incidence and mortality rates, but also in the longer term on TB prevalence. In addition, clinical mycobacteriology laboratories play an important role in the control of the spread of TB through the timely detection, isolation, identification and drug susceptibility testing of *M. tuberculosis* [*17*].

Today's worldwide TB epidemic and the movement of a growing number of refugees have made TB control in refugee populations an issue of increasing importance. However, in developing countries TB control in refugee populations remains a largely unmet need. Experience shows that despite difficult field conditions, TB control programmes can be managed successfully in this setting [*18*]. It seems that despite the efforts which have been made in prevention, diagnosis and treatment of patients and refugees, TB is still a problem in this province.

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La fièvre typhoïde au nord du Liban : étude sur 8 ans (1992-1999) utilisant le test de Widal

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الحمى التيفية في شمال لبنان: دراسة لمدة 8 سنوات (1992-1999) باستخدام تفاعل فيدال

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الخلاصة: تقيم الدراسة تواتر وقوع الحمى التيفية في شمال لبنان على مدى ثمانية أعوام (1992-1999) لدى المرضى الذين أدخلوا إلى المستشفى الخيري الإسلامي في طرابلس بسبب إصابتهم بالحمى. وقد شمل التحليل 739 عينة مصلية لتحري العدوى بالسالمونيلات التيفية باستخدام تفاعلَيْ فيدال وفيلكس. وكانت القيم الفيصلية للعدوى هي حدوث التراص بالمستضد O بعيار يزيد على 160/1 (وهي قيمة اعتمدت في دراسات سابقة أجريت في نفس المنطقة). ومن بين العينات التي بلغ عددها 7 391 كان عيار التراص في 1131 منها (15.3٪) أكثر من 160/1. وقد أوضح الترصد على مدى السنوات الثماني أنه كان هناك تناقص مطرد في تواتر وقوع المرض. ومع حدوث ما يزيد على مئة حالة سنوياً تعتبر الحمى التيفية متوطنة في المنطقة وقد تسبب فاشيات كبيرة. وقد أوضح التحليل الشهري أن هناك ازدياداً في فصل الصيف مع تناقص ملحوظ في فصل الشتاء.

RESUME Nous avons évalué la fréquence de la fièvre typhoïde au nord du Liban sur une période de 8 ans (1992-1999) : 7391 sérums ont été analysés pour rechercher une infection par *Salmonella typhi* par le test de Widal et Félix. La valeur seuil retenue pour l'infection était celle d'un titre en agglutinine O supérieur ou égal à 1/160 (valeur validée par un travail antérieur mené dans cette même région). Sur les 7391 sérums, 1131 (15,3 %) présentaient un titre en agglutinine O ≥ 1/160. La surveillance sur les 8 années a permis de mettre en évidence une baisse progressive de la fréquence de la maladie. Mais avec plus de 100 cas chaque année, la typhoïde reste dans cette région une pathologie endémique qui peut s'aggraver d'épisodes épidémiques majeurs. L'analyse mensuelle montre qu'à la classique poussée estivale s'ajoute une recrudescence hivernale.

Typhoid fever in north Lebanon: a 8-year study (1992–1999) using the Widal test

ABSTRACT We evaluated the frequency of typhoid fever in north Lebanon over an 8-year period (1992–1999) in patients admitted with fever to the Islami de Bienfaisance Hospital in Tripoli. We analysed 7391 serum samples for *Salmonella typhi* infection using the Widal and Felix tests. The cut-off value for infection was an agglutinin O titre ≥ 1/160 (a value validated in an earlier study in the same region). Of the 7391 samples, 1131 (15.3%) had an agglutinin O titre ≥ l/160. The 8-year surveillance showed there was a progressive decrease in the frequency of the disease. However with over 100 cases annually, typhoid is endemic in the area and could cause major outbreaks. The monthly analysis shows that we have an increase in summer, whilst a decrease is observed in winter.

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Introduction

Alors qu'elle est devenue rare dans les pays industrialisés, la fièvre typhoïde reste un problème de santé publique dans les pays où l'hygiène collective et individuelle est déficiente. La maladie reste en effet endémique en Afrique, en Asie du Sud-Est, en Amérique centrale et en Amérique du Sud [*1-3*].

Dans les pays développés, le diagnostic repose sur l'isolement du germe. Mais en pays d'endémie, où les moyens de culture peuvent faire défaut [*2,4-7*], le sérodiagnostic de Widal-Félix reste le plus économique des moyens diagnostiques. Par ailleurs, dans ces régions, la prévalence plus forte de la maladie renforce la valeur prédictive positive de ce test. Il reste par conséquent couramment employé et il constitue la base du plus grand nombre de diagnostics [*8-10*].

Au Liban, la fièvre typhoïde sévit de manière endémique. Déjà par le passé, un rapport datant de 1895 avait signalé une épidémie survenue à Beyrouth, touchant 2 % de la population (2000 cas), avec un taux de mortalité de plus de 15 % [*11*].

Au cours des dernières décennies, et du fait de la guerre civile (1975-1990), la situation s'est aggravée dans le pays, en relation avec la dégradation ou la destruction des infrastructures : traitement et distribution des eaux potables, évacuation des eaux usées et évacuation des déchets. Au nord du pays, de graves épisodes de la maladie sont survenus. En effet, la plupart de la population de cette région consomme de l'eau non traitée. A cela s'ajoute l'absence totale de traitement pour les eaux usées et les déchets (ménagers mais également hospitaliers). Au total, la fièvre typhoïde fait partie des infections endémiques qui persistent dans cette région.

Le but de ce travail est de faire le point actuel sur la fréquence de la fièvre typhoïde au nord du Liban et d'étudier l'évolution survenue au cours des dernières années (période 1992-1999).

Méthodes

Période et lieu de l'étude

L'étude s'est déroulée de janvier 1992 à décembre 1999 dans le laboratoire de microbiologie de l'hôpital Islami de Bienfaisance à Tripoli. Cette ville (située à 80 km au nord de Beyrouth) est la capitale du nord du Liban. C'est la deuxième ville du pays. Le centre hospitalier comprend 189 lits, où la plupart des spécialités sont exercées (à l'exception des opérations à cœur ouvert et des transplantations). C'est le plus important au nord du Liban. Il est doté d'un secteur public et d'un secteur privé, et dessert toute la population de la région. Dans la mesure où les autres hôpitaux de la région sont principalement privés (9/10), on peut considérer que la pathologie vue dans ce centre reflète la situation de la population générale de la région.

Patients

Pendant les 96 mois de l'étude, 7391 patients ont fait l'objet d'une recherche sérologique de typhoïde par le test de Widal. Ce sérodiagnostic est demandé pour tout patient présentant des signes compatibles avec le diagnostic de typhoïde, une diarrhée fébrile ou une fièvre isolée qui persiste pendant plus de trois jours avec une température jusqu'à 40 à 41 °C associée à des céphalées, à une altération de l'état général et à des frissons. Avec celui de Wright, il constitue un examen de routine devant toute fièvre au Liban.

Le test de Widal

Pour chaque sujet, 5 ml de sang étaient prélevés dans un tube sec sans anticoagulant. Après coagulation, le tube était cen-

trifugé à une vitesse de 3000 t/mn pendant 3 à 5 minutes. Le sérum obtenu était analysé en utilisant des antigènes O et H commercialisés par la société Sclavo (Italie), suivant le protocole proposé par le fabriquant. Les sérums étaient dilués en série de 1/20 à 1/2560. Selon la définition habituelle, le titre retenu correspondait à l'inverse de la plus forte dilution pour laquelle on observait encore une agglutination. Chaque sérum avec un titre en agglutinine O supérieur ou égal à 1/160 était considéré positif. Ce seuil provient d'une étude antérieure où nous avons montré que la valeur critique qui permet la meilleure discrimination entre les sujets infectés et les autres dans cette population est un titre en agglutinine O supérieur ou égal à 1/160 [*12*] : dans cette étude, ce seuil donnerait une spécificité de 100 % par rapport aux donneurs, et de 94 % par rapport aux sujets fébriles sans typhoïde. L'élévation du seuil à 160 a permis de réduire le risque de porter à tort le diagnostic. L'association des agglutinines H ne permet malheureusement pas d'améliorer la fiabilité du test. En effet, s'ils sont plus faciles à détecter (taux plus élevés), les anticorps anti-H sont aussi plus fréquents dans la population générale en zone d'endémie.

Observation à partir de janvier 1996

Les résultats ont été enregistrés mois par mois et les 4 dernières années de l'étude (1996-1999) ont donc fait l'objet d'une analyse saisonnière.

Résultats

Les fièvres non typhoïdiennes (TO < 1/160)

Entre 1992 et 1999, les fièvres (avec ou sans diarrhée) nécessitant une hospitalisation ont augmenté progressivement (Tableau 1) passant de 641-645 cas annuels en 1992-1993 à plus de 800 à partir de 1996 (avec une poussée supplémentaire en 1999 : 1014 cas).

La période 1996-1999 permet d'analyser les variations saisonnières (Figure 1) : d'une année à l'autre, on a retrouvé une poussée estivale et un creux hivernal. Les trois premières années (1996, 1997 et

Tableau 1 **Distribution annuelle des cas de 1992 à 1999**

Année	Patients hospitalisés pour fièvre	Fièvres non typhoïdiennes	Typhoïde (TO ≥ 1/160)	
	Nbre	Nbre de cas	Nbre de cas	Proportion (%)
1992	812	641	171	21,0
1993	841	645	196	23,3
1994	877	716	161	18,4
1995	926	784	142	15,3
1996	924	807	117	12,7
1997	1000	823	177	17,7
1998	904	830	74	8,2
1999	1107	1014	93	7,8
Ensemble	7391	6260	1131	15,3

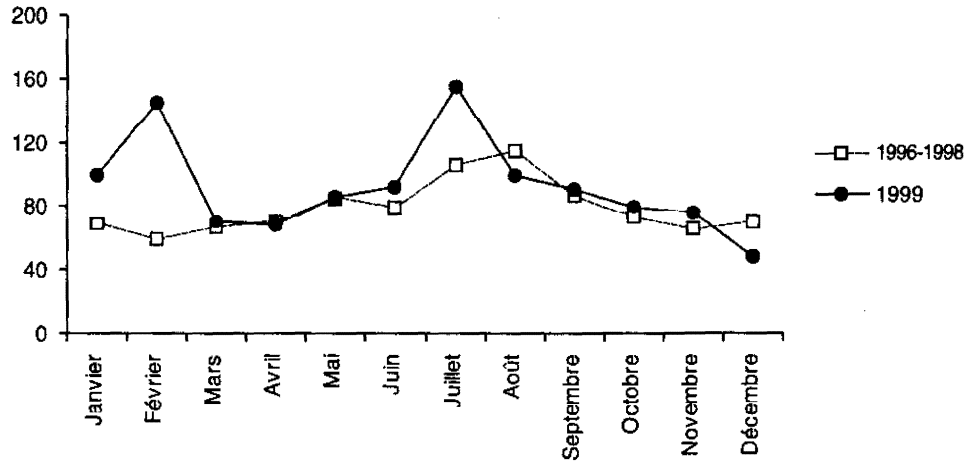

Figure 1 **Aspect saisonnier des diarrhées fébriles non typhoïdiennes (TO < 1/160) : moyenne pour les années 1996-1998, et année 1999**

1998) étaient très homogènes, avec 80 à 100 cas mensuels en été contre 40 à 60 en hiver. En 1999, 2 poussées se sont ajoutées à ce profil (janvier/février d'une part, et juillet d'autre part) expliquant à elles seules l'excès de cette année par rapport aux précédentes.

Typhoïde (TO ≥ 1/160)

Pendant la période de 1992 à 1999, le nombre de cas de fièvre typhoïde diagnostiqués par titrage des agglutinines TO a diminué, passant progressivement de 171 cas en 1992 à 93 cas en 1999 (Tableau 1), à l'exception d'une brève recrudescence en 1997 (177 cas).

Les variations saisonnières ont été étudiées sur la période 1996-1999 (Figure 2). Les années 1997 à 1999 ont montré un même profil, avec un minimum au printemps (le mois de mai étant la période la moins touchée) suivi d'une poussée estivale avec un creux vers le mois d'octobre puis une recrudescence hivernale. Contrastant avec ce profil, l'année 1996 a été marquée par une incidence élevée tout au long de l'année, et particulièrement pendant l'hiver 1996-1997 où il y a eu 15 à 40 cas mensuels de novembre jusqu'à avril (masquant ainsi l'habituelle récession post-estivale).

Discussion

Le sérodiagnostic de Widal, pratiqué au cours des fièvres hospitalisées à l'hôpital Islami de Tripoli (Liban) entre 1992 et 1999, révèle donc la situation épidémiologique dans le nord du pays. Alors que les hospitalisations pour fièvres justifiant une recherche par sérodiagnostic de Widal ont augmenté régulièrement, la typhoïde (TO ≥ 1/160) qui représente moins de 20 % de ces cas a baissé progressivement. Cependant, elle persiste tout au long de l'année avec une poussée estivale et une légère recrudescence hivernale et elle peut donner des épidémies importantes comme celle survenue pendant l'hiver 1996-1997.

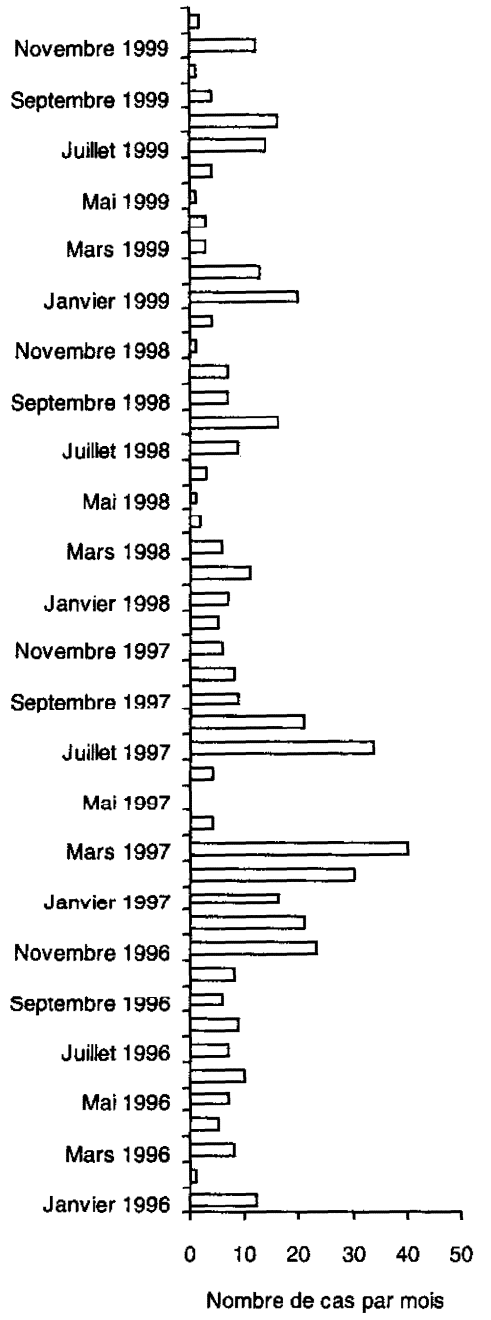

Figure 2 **Nombre de cas mensuels de typhoïde (TO ≥ 1/160) de janvier 1996 à décembre 1999**

Les limites du test de Widal sont connues. Ce test comporte en effet un risque de faux positifs par réactions croisées (autres salmonelles, *Yersinia pseudotuberculosis* type IV, autres entérobactéries) et un risque de faux négatifs (période initiale de l'infection, infection décapitée). Malgré ces limites, plusieurs travaux menés en zone d'endémie ont montré qu'en contrepartie d'un manque de sensibilité, l'augmentation du seuil de décision confère au test de Widal une haute spécificité [*8,13,14*], et plusieurs études ont conclu à l'intérêt de choisir pour le titre O une valeur de 160 ou plus pour affirmer la présence de l'infection [*6,15-17*] dans les pays en développement.

Il est donc possible que cette observation sous-estime le nombre de cas de typhoïde réellement survenus dans cette population pendant cette période, mais la valeur élevée du seuil retenue garantit par contre une bonne spécificité et nous assure que le phénomène n'est pas surévalué. De plus, l'emploi du même test avec la même valeur seuil tout au long de l'étude permet de comparer les années et les mois entre eux.

L'augmentation des fièvres non typhoïdiennes montre que la population recourant aux soins de notre hôpital n'a pas diminué pendant la période étudiée et permet donc de conclure, malgré le risque constant d'épisodes épidémiques, à une baisse réelle du niveau endémique de la typhoïde. Cette baisse est à rapprocher de l'amélioration relative des conditions sanitaires dans la région, tout particulièrement dans la ville de Tripoli, où depuis 1998 la station de traitement de l'eau potable a été remise en fonctionnement. Depuis plusieurs années, l'évolution saisonnière de la typhoïde dans notre région semble stabilisée, avec ses périodes creuses d'avril à juin (respectivement 8, 6 et 7 cas cumulés sur les 3 mois

pour 1997, 1998 et 1999), et novembre/décembre (respectivement 11, 5 et 13). Le profil de l'hiver 1996-1997 (22 cas pour avril, mai/juin, et 44 cas pour novembre/décembre) montre que l'absence de ces creux correspond à une poussée épidémique. Le suivi mensuel du test de Widal apparaît donc comme un bon outil de veille sanitaire.

Conclusion

Cette étude a montré que la fièvre typhoïde reste endémique dans le nord du Liban et présente toujours un risque de survenue d'épisodes épidémiques. Cependant l'amélioration des conditions sanitaires depuis la fin de la guerre civile s'accompagne d'une diminution relative du niveau d'endémie. Dans cette région où les difficultés économiques persistent, le test de Widal reste un bon moyen pour diagnostiquer l'infection. De plus, la surveillance de l'évolution au fil des mois permet d'utiliser ce test pour surveiller les épidémies.

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Note from the Editor

The EMHJ website (http://www.emro.who.int/EMHJ.htm) contains all issues of the Journal published to date from which the full text of all papers can be obtained free of charge. We would like to draw our readers' attention to the online evaluation form. We welcome comments from our readers and would appreciate it if readers could kindly take the time to complete this form.

Visual outcome of extracapsular cataract extraction and intraocular lens replacement in leprosy patients

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الحصيلة الإبصارية لاستئصال الساد خارج المحفظة والاستعاضة بعدسة داخل المقلة في مرضى الجذام

أكبر دراخشان

الخلاصة: أجريت الدراسة في مدينة مشهد، في إيران، في الفترة بين عامي 1998 و2000، لاستقصاء الحصيلة الإبصارية لإجراء جراحة عينية لاستئصال الساد خارج المحفظة مع الاستعاضة بعدسة داخل المقلة لدى 18 من مرضى الجذام أجريت لهم جراحة على عشرين عيناً. إن أكثر المضاعفات شيوعاً للجذام هو فقد الرموش والحواجب (90٪) ثم نقص الشفافية القرنية (55٪). وقد تراوحت حدة الإبصار قبل العملية الجراحية من إدراك الضوء إلى 10/1 ثم تحسنت بعد الجراحة لتصل إلى ما بين 10/5 و10/8 لدى 55٪ من المرضى. إن العدوى التالية للجراحة والتي أدت إلى التهاب باطن المقلة قد حدثت في مريض واحد فقط وعولجت دوائياً، وبقيت الحدة البصرية للمريض الذي أصيب بها على شكل رؤية وعد الأصابع على بعد 10 سنتيمترات. وقد تم تشخيص التصاق القزحية الناجم عن التهاب العنبية المزمن في الجذام لدى 70٪ من الحالات، وانسداد القزحية في 25٪ منها، والترسبات الكيراتينية في 25٪ منها، والضمور المعتدل في القزحية في 10٪ منها.

ABSTRACT The study was carried out in Meshed, Islamic Republic of Iran, from 1998 to 2000 to explore the visual outcome of eye surgery with extracapsular cataract extraction and intraocular lens replacement on 18 leprosy patients (20 eyes). The most common complications of leprosy were madarosis (90%) and partial or total corneal opacity (90%). Visual acuity before surgery ranged from 'light perception' to 1/10, and this improved after surgery to 5/10–8/10 for 55% of patients. Postoperative infection leading to endophthalmitis occurred in only 1 patient and was treated with drugs; this patient's visual acuity remained at 10 cm finger count. Posterior synechia due to chronic uveitis in leprosy was diagnosed in 70% of eyes, obstructed iris in 25%, keratic precipitates in 25% and moderate iris atrophia in 10%.

Résultat optique de l'extraction extracapsulaire du cristallin et du remplacement par une lentille intraoculaire chez des patients lépreux

RESUME Cette étude a été réalisée à Meshed (République islamique d'Iran) de 1998 à 2000 pour explorer le résultat optique de la chirurgie oculaire par extraction extracapsulaire du cristallin et remplacement par une lentille intraoculaire chez 18 patients lépreux (20 yeux). Les complications les plus courantes de la lèpre étaient la madarose (90 %) et une opacité cornéenne partielle ou totale (90 %). L'acuité visuelle avant l'intervention chirurgicale allait d'une « perception faible » à 1/10, et il y a eu une amélioration après l'intervention allant jusqu'à 5/10-8/10 pour 55 % des patients. Une infection postopératoire entraînant une endophtalmie est survenue chez un seul patient et a été traitée par médicaments ; l'acuité visuelle aux doigts de ce patient est restée à 10 cm. Une synéchie postérieure due à une uvéite chronique dans la lèpre a été diagnostiquée pour 70 % des yeux, une obstruction de l'iris pour 25 %, des précipités kératiques pour 25 % et une atrophie modérée de l'iris pour 10 %.

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Introduction

Leprosy is a stigmatizing disease affecting mainly the skin, peripheral nerves, and eyes and nose and is a notorious cause of blindness and nasal, hand and foot deformities. In 1986, 5.4 million leprosy patients were registered worldwide, a figure that had fallen to 3.1 million by 1992 [*1*].

The overall prevalence of leprosy in the Eastern Mediterranean Region in 1995 was 0.4 per 10 000. Although the prevalence in the Islamic Republic of Iran is less than 1 per 10 000, there are localized areas where it exceeds 1 per 10 000. Regional strategies are emphasized for the elimination and control of leprosy, early case detection, treatment with multidrug therapy and disability prevention. WHO supports national control programmes, providing technical assistance to the Islamic Republic of Iran in the promotion of surgery for leprosy impairment [*2*].

The rate of ocular involvement in leprosy ranges from 6%–90% in different studies. In a study in Nepal, the incidence of blindness (eyesight less than 2/200 or 6/60) was 3%–8% in patients with duration of illness up to 10 years, and 30% in patients with more than 20 years of illness [*3*]. In a study in Nigeria, the rate of blindness in leprosy patients was 8.7%, i.e. 10-fold more than the general population in the area, and one of the most common causes of blindness in that study was cataract [*4*]. The rate of eye problems and blindness vary with the type of leprosy; inflammatory eye is a common finding that can cause problems for eye surgery. Leprosy, especially the lepromatous type, causes impairment of the immune system, which increases the success of extracapsular cataract extraction and intraocular lens replacement surgery. In one study, blindness in patients who had aphakic surgery and pseudophakic surgery was 71% and 14% respectively [*5*].

Considering the high level of physical and psychological impairment associated with leprosy, all efforts should made to develop the full potential of leprosy patients. Rehabilitation of eyesight, even at finger count level is a valuable help to the patients. As there is little information about the nature or incidence of ocular pathology in leprosy patients and about the effectiveness of ocular surgery, the present study was conducted to explore the visual outcome of eye surgery with extracapsular cataract extraction and intraocular lens replacement. The study was carried out on leprosy patients in Imam Reza Hospital in Meshed, Islamic Republic of Iran, from 1998 to 2000.

Methods

In this study, 18 cured leprosy patients with cataracts (20 eyes) underwent extracapsular cataract extraction and ocular lens replacement surgery. For each patient, a primary ophthalmologic examination was conducted in the ophthalmology clinic of Imam Reza Hospital in Meshed.

After confirming leprosy and cataract, the patient was referred to the ophthalmology ward and a clinical assessment was performed by ophthalmologists or ophthalmology residents. Before the operation, intraocular pressure was measured. Surgery was performed on the eye with better vision, except for 1 patient who had no light perception in the other eye.

After surgery, all patients received betamethasone and chloramphenicol eye drops. All patients were followed on the 1st, 3rd and 7th days postoperation and at the end of the 3rd and 6th months and 1st year, and were examined by surgeons or ophthalmology residents. Postoperative vi-

sual acuity was defined by the eyesight at the end of the 1st year postoperation.

Results

The study sample was 12 males and 6 females, aged 54–70 years; operations were made on 20 eyes. The preoperative complications of the eyelid and cornea due to leprosy are presented in Table 1. Madarosis (loss of eyelashes) was the most common eyelid complication in 90% of eyes, followed by loss of eyebrows (65%), lagophthalmos (50%) and corneal hypoaesthesia (35%). Corneal problems were corneal infiltration (45%) and corneal neovascularization (45%), and partial or total corneal opacity (90%).

In all cases, intraocular pressure was normal before and after operation; no cases of increased intraocular pressure were detected. Mild postoperative ocular inflammation was seen in all cases, but the intraocular lens was well tolerated. In 14 cases (70%), in spite of iridotomy, slight iridoavulsion occurred at the time of the lens nucleus extraction, which is not a serious problem in intraocular lens replacement. Because of multiple ocular problems in 5 patients, tarsorrhaphy and corneal implantation was also performed in their operations.

In all cases, visual acuity before surgery ranged from 'light perception' to 1/10, and this improved in all cases after surgery, with 55% of eyes regaining a visual acuity from 5/10 to 8/10 (Table 2).

The postoperative complications were low. Posterior synechia was diagnosed in 14 eyes (70%), a very mild case in 1 eye. Obstructed iris was seen in 5 eyes (25%) and a mild case in 1. Keratic precipitates occurred in 5 eyes (25%) and very mildly in 1 eye. Iris atrophia was seen mildly in 14 eyes (70%) and moderately in 2 eyes (10%). Postoperative infection occurred in

Table 1 **Complications of the eyelid and cornea in 20 eyes from leprosy patients with cataract**

Complication	Mild		Moderate		Severe		Total	
	No.	%	No.	%	No.	%	No.	%
Eyelid								
Madarosis	16	80	1	5	1	5	18	90
Loss of eyebrows	10	50	2	10	1	5	13	65
Lagophthalmos	5	25	4	20	1	5	10	50
Corneal hypoaesthesia	4	20	3	15	0	–	7	35
Ectropion	3	15	2	10	0	–	5	25
Trichiasis	3	15	2	10	0	–	5	25
Cornea								
Inflammatory pannus	13	65	1	5	1	5	15	75
Partial corneal opacity	7	35	2	10	2	10	11	55
Total corneal opacity	4	20	3	15	0	–	7	35
Corneal infiltration	6	30	1	5	2	10	9	45
Corneal neovascularization	2	10	7	35	0	–	9	45

Table 2 **Visual acuity in 20 eyes from leprosy patients with cataract 1 year after extracapsular cataract extraction and ocular lens replacement surgery**

Visual acuity	No. of patients	%
5/10–8/10	11	55
1/10–4/10	5	25
CF = 5	2	15
Not determined	2	10

CF = counting fingers at 5 m.

1 patient, which led to endophthalmitis and was treated with drugs; this patient's visual acuity remained at 10 cm finger count.

Discussion

The prevalence of cataract in leprosy patients will increase as life expectancy continues to increase. Leprosy control programmes need to develop activities aimed at reducing the burden of cataract. Extracapsular cataract extraction and intraocular lens replacement is a suitable treatment for cataract in cured leprosy patients. The postoperative complications, followed up for 1 year, were very low in this study.

In our sample, the most common preoperative complication of leprosy was madarosis, which was similar to a study performed in the USA [6]. Loss of eyebrows, inflammatory pannus and partial corneal opacity were the next most common complications; conjunctival fibrosis was the second most common complication in leprosy patients in the American study [6].

Symptoms of neuromuscular involvement of leprosy, such as lagophthalmos and corneal hypoaesthesia, were more common in our study than other studies. In the USA, the rate of lagophthalmos was 11% and the rate of corneal hypoaesthesia was 16%; in our sample the rates of these complications were 50% and 35% respectively. This can be explained by the different rates of tuberculoid and lepromatous types of leprosy that occurred in these regions, which result in different complications. In a study in south India of 63 leprosy patients admitted for treatment of corneal ulcers, 34 had lagophthalmos, 28 had madarosis, 9 had ectropion, 6 had blocked nasolacrimal ducts, 3 had trichiasis and 39 had decreased corneal sensation [7]. Indigenous treatment and late presentation were notable in many cases and visual outcomes were not good.

In the present study, all patients had low visual acuity before surgery (between light perception and 1/10). One year after surgery 80% of them had highly improved visual acuity, which indicates the effectiveness of extracapsular cataract extraction and intraocular lens replacement in leprosy patients. In a study in Korea among patients with aphakic surgery, 71% were still blind in the operated eye, while among patients who had pseudophakic surgery, 14% remained blind [5]. In our study, postoperative posterior synechia was seen in 75% of patients due to chronic uveitis, which is one of the major causes of blindness in leprosy.

Considering the good visual outcomes in operated patients and the low rates of intraocular inflammation and reaction, we conclude that the results of intraocular lens replacement surgery for cured leprosy patients with cataract are satisfactory and can be recommended.

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Leprosy elimination

Leprosy continues to have public health importance in some countries of the Region at sub-national levels, although the number of newly registered leprosy cases has decreased significantly during the past several years. Drugs for treatment of leprosy are available free in all endemic countries, and diagnosis and treatment are integrated in the majority of countries, either within the primary health care system, or with other communicable diseases programmes. The main challenges for the endemic countries relate to reducing the backlog of untreated cases through passive and active case detection, achieving full compliance of cases with multidrug therapy (MDT), changing the negative image of leprosy and eliminating stigma through increased community awareness, preventing disabilities among cases, and achieving sustainability in diagnosis and treatment of cases in countries that have already eliminated leprosy.

Source: The work of WHO in the Eastern Mediterranean Region: annual report of the Regional Director, 1 January 31 December 2003

Distichiasis and dysplastic eyelashes in trachomatous trichiasis cases in Oman: a case series

R. Khandekar,[1] S. Kidiyur[2] and A. Al-Raisi[3]

ازدواج وخلل تنسج الأهداب في حالات الشعرة الناجمة عن التراخوما في عُمان: سلسلة من الحالات

راجيف خاند يكار، ساتيش كيديور، عبد اللطيف الرايسي

الخلاصة: تعد الدراسة تقييماً استباقياً لمعدل انتشار ازدواج وخلل تنسج الأهداب بين المصابين بالشعرة الناجمة عن التراخوما في وحدة رأب العين في أحد مستشفيات عُمان في فترة استغرقت ثلاثة شهور عام 2000. فقد فحص طبيب عيون متخصص برأب العيون هذه الحالات وأجرى لها تصويراً فوتوغرافياً باستخدام مجهر بيولوجي. ومن بين المرضى الثمانين المدروسين كان لدى 58 مريضاً (72.5٪) أهداب غير سوية بالإضافة إلى شعرة ناجمة عن التراخوما. وقد كان معدل ازدواج الأهداب 13.8٪ (بفاصلة ثقة 95٪ وتتراوح بين 6.2٪ و21.3٪) أما خلل تنسج الأهداب فقد كان 33.8٪ (وبتراوح بين 23.5٪ و44.1٪)، فيما عانى 25٪ منهم (15.5٪ إلى 34.5٪) من كلا الأمرين معاً. وقد كان انتشار ازدواج وخلل تنسج الأهداب أكثر من انتشار الشعرة الناجمة عن التراخوما لدى من يزيد أعمارهم على خمسين عاماً ولدى المصابين بالشعر الداخلي. إن وجود ازدواج وخلل تنسج الأهداب مع حالات الشعرة الناجمة عن التراخوما يتطلب المزيد من الدراسات لتوكيد هذه الملاحظة والتأكد من الترابط السببي بينهما.

ABSTRACT The study was a prospective evaluation of the prevalence of distichiasis and/or dysplastic eyelashes among trachomatous trichiasis cases at the oculoplasty unit of a hospital in Oman over 3 months in 2000. An oculoplasty surgeon examined and photographed cases using a bio-microscope. Out of 80 cases, 58 (72.5%) had abnormal eyelashes in addition to trachomatous trichiasis. The rate of distichiasis and dysplastic lashes were 13.8% (95% CI 6.2%–21.3%) and 33.8% (95% CI 23.5%–44.1%) respectively; 25.0% (95% CI 15.5%–34.5%) had both. Dysplastic and distichiasis eyelashes were significantly more prevalent in trachomatous trichiasis cases aged < 50 years and those with entropion. Presence of distichiasis and/or dysplastic eyelashes in trachomatous trichiasis cases warrants further analytical studies to confirm the observation and establish any causal association.

La distichiase et les cils dysplasiques dans les cas de trichiasis trachomateux à Oman : série de cas

RESUME Cette étude était une évaluation prospective de la prévalence de la distichiase et/ou des cils dysplasiques chez les cas de trichiasis trachomateux au Service d'oculoplastie d'un hôpital à Oman, portant sur 3 mois en 2000. Un spécialiste de chirurgie oculoplastique a examiné et photographié des cas à l'aide d'un biomicroscope. Sur les 80 cas, 58 (72,5 %) avaient des cils anormaux en plus d'un trichiasis trachomateux. Le taux de distichiase et de cils dysplasiques s'élevait à 13,8 % (IC 95 % : 6,2-21,3 %) et à 33,8 % (IC 95% : 23,5-44,1 %) respectivement ; 25,0 % (IC 95% : 15,5-34,5 %) avaient les deux. La distichiase et les cils dysplasiques etaient significativement plus courants chez les cas de trichiasis trachomateux âgés de plus de 50 ans et les cas d'entropion. La présence de distichiase et/ou de cils dysplasiques dans les cas de trichiasis trachomateux justifie la réalisation d'autres études analytiques pour confirmer cette observation et établir toute association causale.

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Introduction

Trachomatous trichiasis as graded by the World Health Organization (WHO) does not specify the presence of distichiasis and dysplastic eyelashes and limited information is available about these conditions among trachomatous trichiasis cases. In a long-term follow-up study of trachomatous trichiasis cases managed by the bilateral tarsal rotation procedure in Oman, the prevalence of distichiasis and dysplastic eyelashes were not noted [*1*]. The 61.8% recurrence rate of trichiasis observed in that study is very high and clinicians have proposed that this is due to dysplastic and distichiasis eyelashes which were not managed separately in addition to the bilateral tarsal rotation procedure. Our study was therefore undertaken to determine the frequency and determinants of dysplastic and distichiasis eyelashes among trachomatous trichiasis cases in Oman.

Methods

This was a case series study based at the Oculoplasty Unit of Al Nahdhah Hospital in Muscat, Oman. The study included all trachomatous trichiasis cases reporting to the unit between May 2000 and August 2000.

The field investigator was an oculoplasty surgeon. The ocular status of each patient was evaluated using a bio microscope with an attached photography unit. Patients' eyes were photographed after taking their written consent. The following were noted for each patient: personal details (age, sex), date of examination, details of trichiasis, past history of lid surgery, type of trichiasis, eye involved, presence of distichiasis or dysplastic lashes.

The trachoma gradings recommended by WHO in the 10th revision of the *International classification of diseases* [*2*] were used to define the eye conditions. Distichiasis was defined as the presence of an additional row of eyelashes at the lid margin. Dysplastic eyelash (Figure 1) was defined as any abnormal eyelash originating from the area of the lid margin posterior to the grey line or at the conjunctival edge of the lid margin of an in-turned eyelid. Presence of abnormal eyelashes in either eye was counted as one person with abnormal eyelashes.

The patients were offered laser treatment free of cost at the time of examination and then given bilateral tarsal rotation surgery.

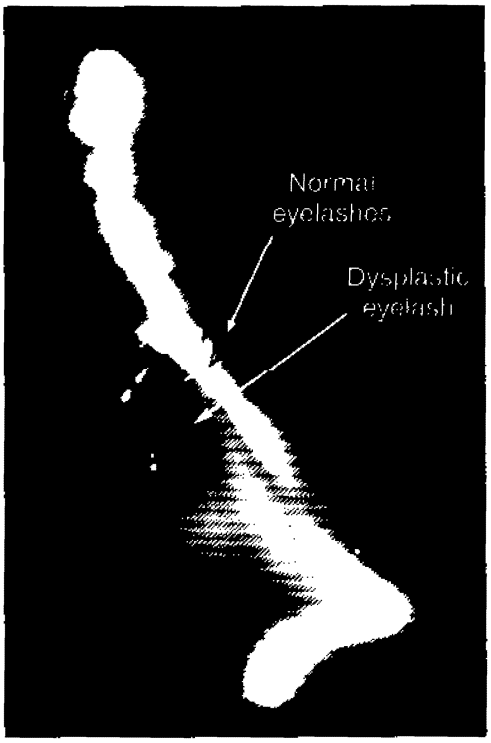

Figure1 **Dysplastic eyelashes in left upper lid with trachomatous trichiasis**

The data were collected on a standard form and entered into a *Microsoft Excel 2000* database. Two researchers computed the data separately using a pre-tested format. The frequency and percentage of patients and 95% confidence intervals were calculated. Relative risk (RR) and *P* values were calculated for comparison of subgroups.

Results

Eighty (80) trachomatous trichiasis cases were examined, 23 males and 57 females. They were divided into age groups < 50 years and 50+ years. Females in the age group 50+ years were the largest group in the study (Table 1).

Distichiasis and/or dysplastic eyelashes were observed in 58 (72.5%) of trachomatous trichiasis cases (Table 2). Analysing the data by sex showed that 17 of 23 male cases (73.9%) had abnormal eyelashes (distichiasis or dysplastic lashes) compared with 41 of 57 female cases (71.9%). Male cases had higher rates of dysplastic and distichiasis lashes than female cases. However, the difference was not statistically significant.

Table 1 **Age–sex distribution of trachomatous trichiasis cases and age distribution of cases with abnormal eyelashes**

Age (years)	Male No.	Female No.	Total No.	Abnormal eyelashes No.	%
25–49	7	15	22	20	90.9
50+	15	41	56	38	67.9
NR	1	1	2	0	–
Total	23	57	80	58	72.5

NR = not recorded.

Table 2 **Abnormal (distichiasis and/or dysplastic) and normal eyelashes among 80 patients with trachomatous trichiasis**

Condition of eyelashes	No.	%	95% CI
Abnormal eyelashes			
Distichiasis	11	13.8	6.20 to 21.30
Dysplastic eyelashes	27	33.8	23.49 to 44.11
Both of above	20	25.0	15.51 to 34.49
Total	58	72.5	62.72 to 82.28
Normal eyelashes	22	27.5	17.72 to 37.28

n = *total number of cases.*

Analysing the data by age group (Table 1) showed that 20 of 23 cases < 50 years old (90.9%) had abnormal eyelashes (7 distichiasis, 5 dysplastic lashes, 8 both) compared with 38 of 56 cases aged 50+ years (67.9%) (13 distichiasis, 6 dysplastic lashes, 19 both). The relative risk for abnormal eyelashes among those < 50 years was 1.3 (95% CI: 1.03–1.66) compared with those aged 50+ years.

Of 59 cases of trachomatous trichiasis with a history of lid surgery in the past, 46 (78.0%) had abnormal lashes. However, the presence of abnormal eyelashes with past lid surgery was not significant.

The severity of trachomatous trichiasis cases was graded as 'in-turned eyelashes only' (no entropion) or 'in-turned lid margin' (entropion). The distribution of abnormal eyelashes by the severity of trachomatous trichiasis is given in Table 3. Severe distichiasis/dysplastic eyelashes were seen in a significantly larger proportion of cases with entropion than those with trichiasis only.

Table 3 **Abnormal (distichiasis and/or dysplastic) and normal eyelashes among patients with trachomatous trichiasis by severity of trichiasis**

Condition of eyelashes	Severity of trichiasis			
	No entropion (n = 14)		Entropion (n = 66)	
	No.	%	No.	%
Abnormal eyelashes				
Distichiasis	0	0.0	20	30.3
Dysplastic eyelashes	1	7.1	10	15.2
Both of above	6	42.9	21	31.8
Total	7	50.0	51	77.3
Normal eyelashes[a]	7	50.0	15	22.7

[a] *RR = 1.55;* P = *0.05 (2-tailed Fisher exact test).*
n = *total number of cases.*

Discussion

Oman is a trachoma-endemic country (Thylefors B, unpublished report). The national prevalence of trachomatous trichiasis has been recorded as 1.1% [*1*]. The bilateral tarsal rotation procedure for managing trachomatous trichiasis cases has been shown to have a recurrence rate of 23% as a short-term outcome and 60% as a long-term outcome in Oman [*3,4*]. The present study attempted to determine the prevalence of distichiasis and dysplastic eyelashes among trachomatous trichiasis cases.

The trachomatous trichiasis cases seen at the tertiary ophthalmic centre of Oman are likely to be cases in the advanced stages and thus they are not representative of all the trachomatous trichiasis cases of Oman. The oculoplasty surgeon used standard definitions to minimize the chances of misclassification bias. Use of records to determine the past history, and other factors related to exposure, minimized the recall bias. Age–sex stratification enabled us to control for these and other unknown confounders in the study. Thus, the outcome of the study is likely to be true picture of the examined sample.

The study showed that a large proportion of the trachomatous trichiasis cases had dysplastic eyelashes and/or distichiasis. Dysplastic and distichiasis eyelashes were more common in the younger age group as well as those having entropion. Even though male trachomatous trichiasis cases had a higher rate of these conditions, the difference was not statistically significant.

Ocular conditions with chronic irritation, such as Stevens–Johnson syndrome, ocular pemphigoid and the sequel of chemical burns to the eyelids and conjunctiva are known to cause metaplasia of the conjunctiva and skin cells and dysplastic eyelashes [*5*]. *Chlamydia trachomatis* causing chronic conjunctivitis with elements of scarring also could cause similar metaplastic changes resulting in the growth of eyelashes at aberrant places. Thus a causal association of dysplastic and distichiasis to the chronic trachoma infection might be a possibility.

Trachomatous trichiasis as a cause of chronic irritation resulting in dysplastic cyclash could not be established in this study as no temporal relation could be noted because both events occurred at the time of examination.

Oman has a high prevalence of genetic disorders [*6*]. It has a high level of risk factors, such as consanguinity, which are known to be responsible for different genetic disorders [*7*]. The extent and kinetics of genetic change in the outer membrane protein (omp-1) gene of *C. trachomatis* in endemic areas of trachoma suggested a possible association at the molecular level between the trachoma organisms and the host susceptibility [*8,9*]. Thus, genetic and molecular alterations might influence the cellular response to *C. trachomatis* infection in the Omani population.

Different types of human leukocyte antigen (HLA) markers for blinding trachoma in Oman have been observed [*10*]. They could be responsible for the different responses to trachoma organisms at molecular level. The abnormal cell growth resulting in blinding trachoma cases might be linked to different HLA markers.

These observations suggest that acquired factors might be changing the host cellular environment causing an altered response to *Chlamydia* spp. organisms.

The findings of the present study in the presence of the above-mentioned biologically plausible corroborative evidence favours the hypothesis of a causal link between trachomatous trichiasis and dysplastic and distichiasis. Further analytical epidemiological studies with larger sample are needed.

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The preliminary report of first 30 cases was presented in at the Third Meeting of the Global Alliance for the Elimination of Trachoma held at Geneva in December 2000.

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Note from the Editor

We wish to draw the kind attention of our potential authors to the importance of applying the editorial requirements of the EMHJ when preparing their manuscripts for submission for publication. These provisions can be seen in the *Guidelines for Authors*, which are published at the end of every issue of the Journal. We regret that we are unable to accept papers that do not conform to the editorial requirements.

Review

مراجعات

الممارسات الطبية بين خطأ الطبيب ومضاعفات المرض

د. عبد الله محمد منجود

الخلاصة: تتناول هذه الدراسة نبذه تاريخية عن الضوابط التي وضعها الفقهاء المسلمون لتقنين ممارسة الطب، وتشير إلى التقدُّم العلمي والتقني في العصر الحديث، وتنوِّه بحاجة الطبيب المسلم إلى تسلحه بالتقنية والعلم الحديث والتحلي بالضوابط الأخلاقية، وتلقي الضوء على مسؤولية الطبيب، مع التفريق بينها وبين الخطأ الطبي والمضاعفات التي قد تحدث نتيجة للتدخُّل العلاجي. وتوصي الدراسة بتدريس أخلاقيات وفقه الطبيب في كليات الطب في العالم الاسلامي، مع تفعيل دور اللجان الطبية الشرعية في مختلف بلدان العالم الاسلامي.

Medical practice with regard to physicians' mistakes and disease complications

SUMMARY This study gives a historical background on regulations implemented by Islamic scholars to codify medical practice, and highlights the advance of science and technology in the modern era and the need for physicians (along with science and technology) to adhere to religious values. It discusses physicians' responsibilities, the issue of malpractice, and the difference between malpractice and complications. Recommendations are proposed to implement medical ethics in the curriculum of medical colleges around the Islamic world and to promote the role of medical religious committees in Islamic world as is being done in Saudi Arabia.

مقدمة

في عام 319 هجرية أمر الخليفة العباسي (المقتدر) محتسبه (إبراهيم بن بطحا بن أبي أصيبعة) بمنع جميع الأطباء من المعالجة إلا من امتحنه رئيس الأطباء في ذلك العهد وهو (سنان بن ثابت بن قرة) وكتب له رقعة بما يطلق له التصرُّف فيه من الصناعة، وقد امتحن في بغداد وحدها وقتذاك (800 طبيباً) عدا الذين لم يدخلوا الامتحان لشهرتهم وعلو شأنهم في الطب!

حدث هذا بعد أن علم الخليفة أن طبيباً من أطباء بغداد أخطأ في مداواة مريض فمات، وقد غرم هذا الطبيب دية المريض، ومنع من ممارسة الطب [*1*].

وقد فرق الأطباء والفقهاء المسلمون منذ القدم بين من يموت بسبب العلاج وبين من يموت بقصد جنائي، كما فرقوا بين ممارسات الطبيب العادية التي يجري عليها ما يجري على تصرفات بقية الناس، وبين ما يفعله الطبيب مما يُعَدُّ من صميم الممارسة الطبية.

ولم يهمل المشرعون في العهود الإسلامية الأولى ضوابط الإسلام وكلياته. وقد سبقوا بذلك أوربا بقرون طويلة، ولم يقتصر تقدمهم ذلك على التقدُّم العلمي الطبي، بل امتد ليشمل وضع التشريع للممارسات الطبية. لقد أنشأت مراكز ومدارس طبية في بغداد والبصرة والكوفة وقرطبة والقاهرة ودمشق، وظهرت تخصُّصات مثل الكحالة، والجراحة، والنساء والولادة. وكان المتخرج من هذه المدارس يؤدي اختباراً في موضوعه. وكما سبق، فقد منع الخلفاء و الفقهاء والقضاة الطبيب الجاهل الذي يخدع الناس بمظهره ويضرهم بسوء طبه من مزاولة مهنة الطب [*2*]. وفي الحديث الشريف (من تطبب و لم يُعْلَم عنه الطب قبل ذلك فهو ضامن) [*3*].

[1]*A.M. Mangoud. Department of Community and Family Medicine, Faculty of Medicine, King Faisal University Hospital, Al-Khobar, Saudi Arabia.*
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وقد تقدَّم الطب تقدُّماً ملحوظاً في العصر الحديث، وأصبح للممارسات الطبية قواعد يلزم الأخذ بها، تبدأ من الاطّلاع على التاريخ المرضي الشامل، والقيام بالأبحاث اللازمة التي تؤدِّي إلى التشخيص السليم، ومن ثَمَّ العلاج المناسب، وقد تختلف وسائل التشخيص وتتعدَّد باختلاف المكان والإمكانات المتاحة، فقد لا تتوافر أجهزة التشخيص المتقدِّمة ذات التقنية العالية والنفقات الباهظة في كل مكان.

فإن توفرت فلا يجب الالتجاء لها الا عند الضرورة، وإلا أصبح التشخيص فوق طاقة المرضى والمجتمع، وأصبحت الممارسة الطبية مكلفة، وهذا الازدياد في التكاليف يجب أن يحاسب عليه الطبيب. كما تقدَّمت الأدوية والأمصال والمضادات الحيوية في القرن الماضي تقدُّماً ملحوظاً محقّقةً طفرة كبيرة، وأصبح هناك أنواع كثيرة من الادوية، وقد وضعت ضوابط لصرف هذه الأدوية حتى لا تكون مضرة للمريض والمجتمع [4].

والعلاج إما ان يكون دوائياً، أو جراحياً بأنواع الجراحة العديدة من استئصال أو إصلاح وتكميل أو زرع أجسام صناعية أو نقل أعضاء، أو يكون طباً بديلاً لهذين الصنفين من تغيُّر نمط الحياة، والعلاج بالرنين المغناطيسي، والوخز بالإبر. وتطوَّرت الجراحة والتخدير، وأدخلت المناظير الجراحية، والجراحات بالليزر، والجراحات عن بُعْد، والجراحات باستخدام الانسان الآلي.

وقد ظهر مؤخراً مفهوم الطب المُسْتَنِد بالبيِّنات، وهو مبني على أبحاث موثقة أجريت في مراكز متعدِّدة وبشكل تجارب للحالات وللشواهد، مع تقليل للتحيُّز، وزيادة في المصداقية للتجارب السريرية[5،6]. كما ظهر مفهوم الطب الشمولي وطب الاسرة حيث يراعى أن تكون نظرة الطبيب عند عنايته بمريضه نظرة شاملة تأخذ في الاعتبار العوامل النفسية والعضوية والاجتماعية والأسرية، والتعاون مع الفريق الصحي في منظومة متجانسة تحرص على نيلها رضا المريض عن الخدمات المقدَّمة له. وتميَّز القرن الماضي أيضاً بإدخال مفهوم الصحة بدلاً من المرض، ومفهوم جودة الحياة بدلاً من البرء. وأحاطت مفاهيم الجودة الشاملة والإتقان بالممارسات الطبية الحديثة، وأصبحت لازمة من لوازمها، وقد لعبت الشبكة العالمية للمعلومات (الإنترنت) دوراً مميزاً في نشر المعرفة بين الأطباء وزيادة الاتصالات العلمية [7].

ولكي يكون العلاج حسب القواعد يجب أن يكون الداعي له صحيحاً، وأن يكون توقيته صحيحاً، وأن تكون جرعته مضبوطة، وأن يكون الإعداد له إن لزم - قد تم - مع الأخذ في الاعتبار الأمراض الأخرى والعلاجات المصاحبة، وأن يكون مكان تنفيذه مُعَدّاً للعلاج، وأن يكون القائمون عليه مؤهَّلين لذلك علمياً، ومدرَّبين على المهارات اللازمة، كما يجب أن تكون خطة العلاج مُعَدَّة لتقليل احتمال المضاعفات التي قد تحدث برغم كل الاحتياطات السابقة، ولها نسبة معينة معترف بها في كل علاج أو عملية جراحية، ومُدْرَجة في المراجع المُسْتَنِدة بالبيِّنات التي لا تقبل الشك.

ومع كل هذا التقدم في جميع مجالات الطب فإن الطبيب إنسان، والعامل البشري لا يمكن تجاهله، وقد بدأت مراكز طبية عالمية بالاهتمام بأخلاقيات الممارسة الطبية؛ تضع لها الضوابط، وتُسَنُّ لها القوانين، والطبيب المسلم أولى من غيره بالالتزام بهذه الضوابط، وعليه واجب شرعي بأن يتسلّح بفقه يساعده على إنجاز عمله، وخوف من الله ينير له الطريق، كما يجب عليه ان يتعرَّف على الأخطاء الطبية ومسؤوليته تجاهها، وأن يتعرَّف على الفرق بينها وبين المضاعفات التي قد تحدث رغم أخذه بكل ما سبق من تقنية وتدريب ومهارات.

الأخطاء الطبية

الأخطاء الطبية في مضمونها تمس سلامة المرضى، ويُعَدُّ الطبيب مسؤولاً عندما يخل بالتزاماته المهنية، ولا يشترط أن يكون الخطأ المنسوب إليه جسيماً أو بسيطاً، ويكفي أن يكون الخطأ واضحاً. ورغم هذا فان مسؤولية الطبيب عن أخطائه لا تعني أن يؤاخذ بالظن والاحتمال، لأن المسؤولية تترتَّب على خطأ ثابت محقق لا جدال فيه.

الطبيب إذن مسؤول عن كل خطأ يقع فيه شريطة أن يثبت في حقه، بغض النظر عن فداحة الضرر.

وقد يرجع الضرر الذي أصاب المريض إلى خطأ يتحمّله أطباء عدة اشتركوا في علاجه، وهنا يبحث كل عامل على حده، أو تعتبر جميع هذه العوامل متعادلة من حيث تحمل المسؤولية.

ولأن معظم الأخطاء الطبية يمكن التستُّر عليها لعدم دراية المريض بممارسة الطبيب، وجهله بمرضه، ولأن معظم المداخلات العلاجية لها مضاعفات يمكن التذرع بها، أصبحت تقوى الطبيب وخوفه من الله عاصماً للدرء مفسدة الممارسات الخاطئة مع الإفلات من العقوبة [8].

تعريف الخطأ الطبي

تبيَّن مما سبق أن فشل العلاج لا يعتبر في حد ذاته قرينة قاطعة على خطأ الطبيب، فقد يفشل العلاج على الرغم من التزام الطبيب بالقواعد والأصول العلمية.

يعرف الخطأ الطبي بأنه انحراف الطبيب عن السلوك الطبي العادي والمألوف، وما يقتضيه من يقظة وتبصُّر، إلى درجة يهمل معها الاهتمام بمريضه. أو هو إخلال الطبيب بالواجبات الخاصة التي تفرضها عليه مهنته (الإخلال بتنفيذ الالتزام التعاقدي)، وعدم الالتزام بمراعاة الحيطة والحذر والحرص على الحقوق والمصالح التي يحميها المشرع، ويفترض الالتزام بمراعاة الحيطة والحذر أن يكون بمقدور الطبيب الوفاء به، لأنه التزام بقدر ما يكون بقدر الاستطاعة، ذلك بأن القانون والشرع لا يفرضان من أساليب الاحتياط والحذر إلا ما كان مستطاعاً[9]. ولأن التزامات الطبيب ليس منشأها ذلك الواجب القانوني العام بعدم الإضرار بالغير، ولا مناطها ما اتجهت إليه إرادة المريض وقت التعاقد، فإن المرجع فيها يعود إلى القواعد المهنية التي تحدِّد تلك الالتزامات وتبيِّن مداها.

يقوم الخطأ الطبي على توافر العناصر التالية:

- عدم مراعاة الاصول والقواعد العلمية المتعارف عليها في علم الطب.
- الإخلال بواجبات الحيطة والحذر.
- إغفال بذل العناية التي كانت في استطاعته.
- توافر رابطة أو علاقة نفسية بين إرادة الطبيب والنتيجة الخاطئة.

يندرج تحت هذا العنوان

- اتجاه إرادة الطبيب على غير النحو الذي يفرضه أولي الخبرة.
- إغفال الطبيب ما يتمتّع به من معلومات وإمكانات ذهنية كي يدرك الأخطاء المرتبطة بسلوكه.
- عدم توقُّعه النتيجة الخاطئة التي كان في وسعه تجنُّبها لو بذل العناية الكافية.
- الإهمال في إجراءات الحيطة والحذر.

أنواع الأخطاء الطبية

1. الخطأ الفني

خطأ يصدر عن الطبيب يتعلق بأعمال مهنته، ويتحدَّد هذا الخطأ بالرجوع الى الأصول والقواعد العلمية والفنية التي تحدِّد أصول مهنة الطب.

يتولَّد هذا الخطأ إما نتيجة الجهل بهذه القواعد أو بتطبيقها تطبيقاً غير صحيح، أو سوء التقدير فيما تخوله من مجال تقديري.

2. الخطأ المادي

لا شأن له بالقواعد السابقة، وإنما مرده الإخلال بواجبات الحيطة والحذر العامة التي ينبغي أن يلتزم بها الناس كافة، ومنهم الطبيب في نطاق مهنته، باعتباره يلتزم بهذه الواجبات العامة قبل أن يلتزم بالقواعد العلمية أو الفنية المسؤولية الطبية في الشريعة الإسلامية.

يتميَّز التشريع الإسلامي عن غيره بقواعده الشاملة لأمور الدين والدنيا، وهو بذلك قد تفرَّد عن جميع الشرائع السماوية السابقة باحتوائه على تشريعات تصلح لكل زمان ومكان، اعترف بذلك المشرعون المحدثون المنصفون لما جاء به من نظريات قانونية مبنيَّة على المنطق السليم ومحققة للعدالة الاجتماعية، تُعَدُّ بحق مرجعاً خالداً على مر العصور، ومفخرة لتراث الإنسانية، توضِّحه آيات قرآن مُنْزَل من لَدُن خبير عليم، وسنَّة نبوية قولية وفعلية جاء بها الرسول الكريم محمد صلى الله عليه وسلم، واجتهاد على مر العصور، تفتَّقت عنه أذهان أفذاذ الفقهاء المسلمين على مر العصور.

المسؤولية القانونية عن أعمال التطبيب والجراحة

وقد شغل موضوع المسؤولية القانونية عن الممارسات الطبية اهتمام القانون في الغرب، وثار حوله جدل كبير من تعريف ووضع قوانين تنظم حق الطبيب في ممارسة مهنته، وطبيعة هذا الحق، وحدود مسؤوليته، ولو أنصفوا لرجعوا للشريعة الاسلامية التي كان لها السبق والتميُّز في هذا المجال، حيث حدَّدت المسؤولية الطبية بما يكفل حماية الطبيب لحقوق المريض ويشجِّع على تطوير الممارسة الطبية.

إن دراسة الطب في الشريعة الإسلامية فرض من فروض الكفاية، فهي واجب على كل فرد، لا يسقط إلا إذا قام بها غيره، وذلك باعتبار التطبيب ضرورة اجتماعية تحتاج إليه الجماعة [10].

ومن هذا المنطلق فقد اعتبرت الشريعة الإسلامية مزاولة مهنة الطب واجباً، على حين اعتبرتها القوانين الوضعية الحديثة وبعض الشراح حقاً، مثلها مثل سائر المهن الأخرى، ولا شك أن نظرية الشريعة الاسلامية أفضل، وقد سبقت بها أحدث التشريعات الوضعية، لأنها تلزم الطبيب بأن يضع مواهبه في خدمة الجماعة، كما أنها أكثر انسجاماً مع حياتنا الاجتماعية القائمة على التعاون والتكاتف، وتسخير كل القوى لخدمة المجتمع[9].

شروط الضمان في الاسلام

التعدِّي: أي مجاوزة ما ينبغي أن يقتصر عليه شرعاً، أو عُرفاً، أو عادة.

الضرر: أي إلحاق مفسَدة بالغير.

الإفضاء: أي ألا يوجد للضرر سبب آخر غيره (سبب معين أفضى إلى نتيجة محدَّدة).

فإذا تحقَّقت هذه الشروط فإن مسبِّب الخطأ يتحمل المسؤولية، ويضمن تعويض الأضرار التي نتجت عنها، وكما سبق فقد أشار النبيُّ ﷺ إلى المسؤولية الطبية بقوله: (مَنْ تَطَبَّبَ و لم يُعلَم منه طبٌّ فهو ضامنٌ)[3]، ومع أن هذا الحديث النبوي يشير إلى صورة محددة من صور المسؤولية الطبية، وهو مزاولة المهنة دون تأهيل، فإن العبرة كما يقول أهل العلم بعموم النص لا بخصوص السبب، فيؤخذ من قول النبي ﷺ أن كل ممارسة طبية تتحقَّق فيها الشروط التي ذكرناها للضمان فإنها تقع تحت طائلة المسؤولية الشرعية، ويأثم فاعلها، علماً بأن لكل ضرر مقدار من الضمان حددته الشريعة الإسلامية وقد تترتَّب عليه بعض العقوبات (دية، أرش، قصاص).

ويتحمَّل الطبيب ومن في حكمه ممن يزاولون المهن الطبية مسؤولية الأضرار التي تنتج عن أفعالهم، سواء حدثت نتيجة استخدام أدوات ووسائل وأجهزة، أو حدثت بسبب خطأ شخصي، أو تقصير أو إهمال، أو عدم متابعة حالة المريض، أو عدم إجراء ما يلزم إجراؤه في الوقت المناسب، أو عدم استشارة ذوي الخبرة والاختصاص إذا كانت الحالة تستدعي الاستشارة [11].

حالات عدم الضمان:

وقد أدركت المذاهبُ الفقهيَّةُ المختلفةُ طبيعةَ العمل الطبي، وما ينطوي عليه من أخطار ومضاعفات (Complications) لا يستطيع الطبيب أن يتجنَّبها مهما أُوتِيَ من علم وخبرة ومهما بذل من جهد، ولذلك اتجه الفقهاءُ لمراعاة الطبيب، والتخفيف من مسؤولياته عن المضاعفات التي قد تنتج عن عمله، واتفقوا على أنه لا ضمانَ على الطبيب ومَنْ في حكمه من ممرضين وفنيين ونحوهم إذا ما راعى الشروط الآتية:

- أن يكون من ذوي المعرفة في صناعة الطب: أي عارفاً (بالأصول الثابتة والقواعد المتعارف عليها نظرياً وعملياً بين الأطباء، والتي يجب أن يلمَّ بها كل طبيب وقت قيامه بالعمل الطبي)[*12*] فلا ضمان على الطبيب ومن في حكمه ما دام من أهل المعرفة و لم يخطئ، أي أن يكون فعل الطبيب الذي نتج عنه الضرر قد وقع على النحو المعتبر عند أهل الصنعة [*11-8*] وقد صرَّح الحنفيةُ بالحَجْرِ على الطبيب الجاهل الذي لا يُحسن المداواةَ أو لا يعرف أصولَ الطب، وقالوا بمنعه من مزاولة المهنة [*13*].
- أن يُؤْذَنَ له بمزاولة المهنة: أي أن يحصل على ترخيص رسمي بممارسة الطب أو غيره من الاختصاصات الطبية، من الجهة ذات الاختصاص (وزارة الصحة).
- أن يأذن له المريضُ بمداواته: ويشترط أن يكون الإذن معتبراً شرعاً، فإذا كان الإذن معتبراً، وكان الطبيب حاذقاً، و لم يتعمَّد الوقوع في الخطأ، و لم يتجاوز ما أُذن فيه، وسرى التلف إلى المريض، فإن الطبيب لا يضمن، لأنه فَعَلَ فعلاً مباحاً مأذوناً فيه، أما إذا طبَّب بغير إذن، أو بإذن غير معتبر شرعاً، فأدَّى إلى تلف أو عيب فإنه يضمن ما ترتَّب على فعله من أضرار.
- ألا يتجاوز ما ينبغي له في المداواة : فإذا أعطى للمريض جرعة من الدواء أكبر من الجرعة المحددة، أو قطع من العضو أكثر مما ينبغي، أو ما شابه ذلك من تجاوزات، فإنه يتحمَّل مسؤولية فعله، ويلزم بضمان ما نتج عن فعله من أضرار سواء كان فعله عن خطأ أو تقصير أو جهل أو اعتداء، إلا أنه لا يأثم في الخطأ، ويأثم في التقصير والجهل والاعتداء [*11*].

وبوجه عام فإن (التزامات الطبيب مناطُها القواعدُ المهنيَّةُ التي تحدِّدُها وتسُنُّ مداها، فالمخالفة الواضحة للمبادئ المسلَّم بها في الفنِّ الطبيِّ هي وحدها التي يمكن أن تُحَرِّكَ مسؤوليَّةَ الطبيب .. ولا جَرَمَ أن يُعمل حسابٌ لعجز البشر، فالفنُّ الطبيُّ لم يكتمل، وتقتصر محاسبة الأطباء على الأصول العلمية الثابتة، ومؤدَّاهُ أنه لا يصحُّ أن يُسأل الطبيبُ عن أمر مختلَفٍ عليه فنياً، وبمجرد وجود رأي مؤيد لتصرفه يشفع له ويَحُولُ دون مؤاخذته، والرأيُ أنَّ كلَّ من يقوم بوظيفةٍ ذاتِ نفعٍ اجتماعي يجب أن تُرفع عن عاتقه المسؤولية حتى لا يُشَلَّ نشاطُهُ فتضارَّ المصلحةُ العامةُ) [*8*].

شروط انتفاء المسؤولية عن الطبيب في الشريعة الاسلامية

القاعدة الشرعية في المسؤولية الطبية تنص على أن كل من يزاول عملاً أو علماً لم يعرف عنه إتقانه يكون مسؤولا عن الضرر الذي يصيب الغير نتيجة هذه المزاولة، وفي مجال مسؤولية الطبيب الجاهل حديث لرسول الله صلى الله عليه وسلم: «من تطبب و لم يكن بالطب معروفاً، فأصاب نفسا فما دونها، فهم ضامن».

وقد أجمع الفقهاء على وجوب منع الطبيب الجاهل- الذي يخدع الناس بمظهره ويضرهم بجهله- من العمل، ومن القواعد المقرَّرة في الحجر أن ثلاثة يُحْجَر عليهم (أي يمنعون عن العمل): المفتي الماجن، والطبيب الجاهل، والمكاري المفلس، وفي ذلك قول مشهور عند الفقهاء نصه: «إذا قام بأعمال التطبيب شخص غير حاذق في فنه، فان عمله يعتبر عملاً محرماً» [*1*].

ومن القواعد المقررة في الشريعة الإسلامية أن عمل الطبيب عند الإذن بالعلاج أو عند طلبه يُعَدُّ واجباً، والواجب لا يتقيَّد بشرط السلامة، ولو أن واجب الطبيب متروك لاختياره وحده ولاجتهاده العلمي والعملي، فهو أشبه بصاحب الحق لما له من السلطان الواسع والحرية في اختيار العلاج وكيفيته [*14*].

وعلى ذلك إجماع الفقهاء، ولكنهم يختلفون في تعليل انتفاء المسؤولية عن الطبيب. فرأى أبو حنيفة ان العلة ترجع إلى الضرورة الاجتماعية وإذن المجنى عليه أو وليه. ورأى الشافعي وأحمد بن حنبل أن العلة ترجع إلى إذن المجني عليه، وأن الطبيب يقصد صلاح المفعول ولا يقصد الاضرار به [*15*].

ورأى مالك أن العلة هي إذن الحاكم أولاً وإذن المريض ثانياً، وباجتماع هذين الشرطين لا مسؤولية على الطبيب إذا خالف أصول الفن أو أخطأ في فعله [*11*].

وبذلك تتحدَّد شروط انتفاء مسؤولية الطبيب عند علماء الشريعة الإسلامية فما يلي:

(1) إذن الشارع.

(2) رضا المريض.

(3) قصد الشفاء.

(4) عدم وقوع الخطأ من الطبيب.

ومما يلفت الانتباه أن الفقهاء في القانون في العصر الحديث قد توصلوا بعد طول الجدل والبحث إلى نفس هذه الشروط التي قررها علماء الشريعة الإسلامية، كما أن غالبية القوانين الوضعية الحديثة قد تضمنت هذه الشروط لانتفاء المسؤولية عن الأطباء [*16*].

تحديد المسؤولية الطبية قانونياً والتفريق بين الخطأ والمضاعفات

الخطأ الطبي مسألة موضوعية يجب أن يرجع الفصل فيها الى لجنة فقهية طبية، ففي المملكة العربية السعودية، على سبيل المثال، توجد هذه اللجان بشكل دائم في كل منطقة من المناطق الإدارية في المملكة.

تلتزم هذه اللجنة بإثبات توافر الخطأ ليكون حكمها بالإدانة صحيحاً، إذ توجد في كل منطقه من مناطق المملكة لجنة للبحث في هذه الأخطاء، يرأسها قاض يساعده أساتذة من كليات الطب واستشاريين في نفس التخصص، لإظهار عناصر الخطأ المستوجب لمسؤولية الطبيب وعرضه على رأي اهل الخبرة.

من المتعارف عليه أن ثمة قواعد واصولاً مستقرة في علم الطب، لابتعاد فيها وخروج الطبيب عليها يسم سلوكه بالخطأ، وتستوجب مسؤوليته ولا عبرة بكون الخطأ جسيماً أو يسيراً، ولا صعوبة تواجه القاضي في الكشف عن هذه القواعد والاصول، وفي استطاعته أن يعتمد فيها على رأي أهل الخبرة.

إن هذه القواعد ذات مجال تقديري، عندما يعترف العلم بما يدور فيها من خلاف، ويفترض قدراً من الصحة في الآراء المختلفة التي تتنازعها، فلا محل عندئذ للقول بثمة خطأ وقع فيه الطبيب، وعلى ذلك فلو اخذ الطبيب برأي محل خلاف، أو برأي مرجوح، فلا يتوافر الخطأ في حقه طالما أن له في تقدير ما أخذ به السند العلمي القوي، وتأخذ هذه القواعد في اعتبارها أن الصعوبات التي يمكن أن تثار في العمل يصبح أمرها ميسوراً إذا اعتمد القاضي مع اللجنة على المعايير السائدة بين مستوى الطبيب المعتاد، وقدر مدى تقيده بالقواعد العلمية والفنية في الظروف التي أحاطت بالطبيب المسؤول، ولا محل حينئذ للأخذ بمعيار آخر كمعيار الطبيب الشديد الحرص، أو إقامة التفرقة بين الخطأ الجسيم والخطأ اليسير، ولكن قد تكون لهذه التفرقة أهمية في تقدير العقوبة التي تنزلها اللجنة في حدود سلطتها التقديرية، إذ المنطق يقضي أن يكون عقاب من صدر عنه خطأ جسيم أشد من عقاب من كان خطؤه يسيراً، وتقدير جسامه الخطأ هو مسألة موضوعية تخضع لتقدير اللجنة، مستعينة بالظروف التي أحاطت بالخطأ، فإذا كانت اللجنة قد قدرت أن الطبيب قد أخطأ بإجراء الجراحة في العينين معاً، وفي وقت واحد مع عدم الحاجة إلى الإسراع في إجراء الجراحة ودون اتِّخاذ كافة الاحتياطات التامة لتأمين نتيجتها، والتزام الحيطة الواجبة التي تتناسب وطبيعة الأسلوب

الذي اختاره، فعرَّض المريض بذلك لحدوث المضاعفات السيئة في العينين معاً في وقت واحد، الأمر الذي انتهى إلى فقد إبصارهما كلية، فإن هذا القدر الثابت من الخطأ يكفي.

المسئولية الجنائية والمدنية عن الاخطاء الطبية

إن العمل الطبي هو نشاط يتفق في كيفية وظروف أدائه مع القواعد والأصول المقررة في علم الطب، ويتجه في ذاته إلى شفاء المريض، وهو لا يصدر إلا من شخص مرخص له قانوناً بمزاولة مهنة الطب، ومن أهم ما يتطلَّبه القانون لإعطاء هذا الترخيص حصول طالبه على المؤهل الدراسي الذي يؤهِّله لهذه المهنة، اعتباراً بأن الحاصل على هذا المؤهل هو وحده الذي يستطيع أن يباشر العمل الطبي طبقاً للأصول العلمية المتعارف عليها، والأصل في العمل الطبي أن يكون علاجياً أي يستهدف بالدرجة الأولى تخليص المريض من مرض ألمَّ به أو تخفيف حدته أو تخفيف آلامه.

يُعَدُّ كذلك من قبيل الأعمال الطبية ما يستهدف الكشف عن أسباب سوء الصحة، أو مجرد الوقاية من مرض، وأن إباحة عمل الطبيب مشروطة بأن يكون ما يجريه مطابقاً للقواعد والأصول العلمية المقرَّرة، ولا يبقى بعد ذلك إلا الشفاء، فهو من عند الله تعالى. وعلى ذلك يمكن القول إن العمل الطبي هو عمل مشروع حتى ولو ساءت حالة المريض، ولكن إذا اقترن هذا العمل بخطأ ما سئل الطبيب عنه مسؤولية غير عمدية.

تقع المسؤولية على الطبيب:

- إذا فرَّط في الأصول والقواعد العلمية المقرَّرة في علم الطب؛
- إذا قصَّر في أداء عمله و لم يتحرز في أدائه؛
- لا يلتزم الطبيب بتحقيق نتيجة أو غاية هي شفاء المريض ؛
- العناية المطلوبة منه تقتضي أن يبذل لمريضه جهوداً صادقة يقظة، تتفق في غير الظروف الاستثنائية مع الأصول المستقرة في علم الطب وبصرف النظر عن المسائل المختلف عليها والتي تثير جدلاً ونقاشاً لينفتح باب الاجتهاد فيها، فانحراف الطبيب عن أداء هذا الواجب يُعَدُّ خطأً يستوجب مسؤوليته.

أمثلة للتفرقة بين الخطأ الفني والخطأ المادي

إن الطبيب الذي يصف دواء أساء إلى صحة المريض لحساسية خاصة لم يتبينها، أو يغفل عن استدعاء طبيب أخصائي لعدم تقديره خطورة حالة المريض، أو يطبق وسيلة علاج جديدة لم يسبق تجربتها. في كل هذه الأمثلة يعتبر الطبيب مقترفاً خطأً فنياً، أما إذا أجرى الطبيب عملية جراحية وهو في حالة سكر أو بأدوات جراحية غير معقمة أو ترك بعضها في بطن المريض فإنه يكون مقترفاً خطأً مادياً [13].

ضوابط للقياس

المقياس الشخصي

يقاس مسلك الطبيب عند وقوع الخطأ على سلوكه الشخصي المعتاد، فان كان هذا الخطأ قد نتج عن سلوك أقل حيطة وحذراً من سلوكه الذي اعتاده، توافر في حقه الإخلال بواجبات الحيطة والحذر.

المقياس الموضوعي

يقوم على أساس قياس سلوك الشخص الذي يلتزم في سلوكه وتصرفاته قدراً متوسطاً مألوفاً من الحيطة والحذر، فإذا أخلَّ الشخص بواجبات الحيطة والحذر عما يلتزم به الشخص المعتاد توافر في حقه الإخلال بتلك الواجبات.

المضاعفات العلاجية

المعروف أن أي مداخله علاجيه (جراحية أم غير جراحية) تحمل في مضمونها احتمال حدوث مضاعفات بنسبة معينة. إن حدوث مثل هذه المضاعفات لا يعد من قبيل الخطأ الطبي، وفي المجال الجراحي يلزم على الجراح أن يشرح للمريض هذه المضاعفات شرحاً وافياً قبل إجراء العملية، وفي وجود شهود، ثم يوقع على الإقرار الطبي الذي ينص على أن مضاعفات العلمية قد شرحت له، وأنه قد فهمها، وأنه يُقِرُّ بقبوله الطوعي باحتمال حدوثها.

ومضاعفات الجراحة قد تكون عامة، مثل المضاعفات الرئوية المسؤولة عن 25٪ من الوفيات بعد العمليات، وجلطة القلب بعد العمليات الجراحية التي تصيب 6٪ من مرضى القلب، و0.6٪ من المرضى ذوي القلوب السليمة - وإن زادت النسبة إلى 3٪ في مرضى تصلُّب الشرايين، والمضاعفات المخية التي تكثُر في عمليات القلب المفتوح، والمضاعفات النفسية التي تحدث في نصف بالمئة بعد عمليات البطن، ونسبة كبيرة جداً بعد عمليات القلب المفتوح، ومرضى العناية المركزة - والمضاعفات الجنسية التي تكثر بعد عمليات القلب والحوض وشريان الأبهر وكذلك المضاعفات في أوردة الساقين بعد الإصابات وملازمة السرير لفترة طويلة. والمضاعفات الموضعية تختلف باختلاف مكان العملية، وعلى سبيل المثال، إن نسبه عدوى جروح العمليات تتراوح بين 3٪ في الجراحة النظيفة، حيث لا يوجد ميكروبات في مكان العملية إلى 12٪ في العمليات التي تشمل فتح الجهاز الهضمي أو البولي أو التناسلي، وترتفع إلى 16٪ لوجود قيح في مكان العملية[8]. والقابلية لحدوث المضاعفات تزيد حسب ظروف المريض من حيث العمر ووجود الأمراض المزمنة - مثل مرض السكر والأورام الخبيثة والفشل الكلوي والكبدي - والظروف التي رافقت العملية مثل كونها طبيعية أم طارئة أو كونها انتقائية تتم في ظروف مستقرة - وظروف أجرائها وخبرة وعلم القائمين بها - من هنا يتَّضح أن القابلية للإصابة بالمضاعفات تتعلق بعوامل عديدة تشكل منظومة كبيرة - وكلما زادت عوامل الخطورة - كلما حدثت مضاعفات لا يسأل عنها الأطباء - وإنما تعزى إلى الظروف المحيطة - فالشفاء ليس بيد الأطباء، ولكنه بيد الله، والأطباء أداة لا غير[9].

المناقشة

تبيَّن من هذا البحث أن للإسلام منهجاً فريداً في تنظيم الممارسة الطبية، وأن الشريعة الاسلامية كان لها السبق في تقنين تلك الممارسة منذ فجر الإسلام، وهي بذلك قد سبقت أوربا بقرون عدة. جمع هذا المنهج بين الدين والدنيا، وبين النظرية والتطبيق، فلا ضرر فيه ولا ضرار، واتضح له أن ممارسة الطب فرض من فروض الكفاية، ومن هذا المنطلق، فقد اعتبرت الشريعة الاسلامية مزاولة مهنة الطب واجباً، لا يسقط الا بتصدِّي مجموعة للقيام به، وقد تبيَّن أن مزاولة مهنة الطب تحوطها جوانب تتراوح بين السماح والمسؤولية والضمان، ولقد اتَّضحت القاعدة الشرعية في المسئولية الطبية بأن كل من يزاول عملاً أو علماً لا يعرفه يكون مسؤولا عن الضرر الذي يصيب من يعالجه نتيجة هذه المزاولة. وكون الطبيب الغير الحاذق ضامناً يعيد للمجتمع الطمأنينة والأمان، كما أن منع الطبيب الجاهل- الذي يخدع الناس بمظهره ويصرهم بجهله- من العمل، يحمي المجتمع من ضرره وشروره، فهو محجور عليه مثل المفتي الماجن، والمكاري المفلس.

كما بيَّن البحث أن الخطأ الطبي لا يشمل فقط الإضرار الملموس مثل موت المريض أو فقدان عضو من أعضائه، وإنما يمتد ليشمل الإسراف في وصف الأدوية أو طلب إجراء التحاليل، وقد تميز النصف الثاني من القرن العشرين بالتطور الهائل في مجالات التشخيص والعلاج، كما ظهر مفهوم الطب المُسْتَنِد بالبيِّنات والجودة الكلية، الأمر الذي يجب أن يؤخذ في الاعتبار عند وضع ضوابط جديدة لمحاسبة الأطباء على أخطائهم، وهذا سيوسِّع دائرة المحاسبة، وسيجعلها أكثر من ذي قبل، ليصب ذلك في مصلحة المريض ورضائه عن الخدمات المقدَّمة.

ومن ناحية أخرى، فإن المحاذير بدأت تدق ناقوس الخطر في الغرب والولايات المتحدة من الغرامات المالية الباهظة التي يدفعها الأطباء نتيجة للأخطاء في الممارسة الطبية، مما قد يؤثر على أداء الطبيب لعمله دون إفراط أو تفريط. وقد

دلت الدراسات الأخيرة أن الاطباء يلجأون إلى عمل أبحاث غير ضرورية للخوف من المسائلة القانونية والجنائية، مما يشكل عبئاً غير منظور على التكاليف.

وقد سبق الفقهاء المسلمون في الماضي غيرهم في تناولهم لهذه المسألة، حتى لا يكون الضمان سيفاً مسلطاً على رقاب الأطباء، لتزدهر مهنة الطب وتتطور، ويكثر الابتكار، فقد اتفقوا على أن الطبيب الحاذق يجب ألا يسأل عن الضرر الذي يصيب المريض، ولو مات المريض من جراء العلاج، ما دام المريض قد أذن له بعلاجه، وقد قصد ذلك الطبيب شفاء المريض مع عدم تقصير متعمد أو خطأ فاحش. وقد توصل الفقهاء في العصر الحديث بعد طول الجدل والبحث إلى نفس هذه الشروط التي قررها فقهاء المذاهب الاربعة من قبل، مما يدل على سعة فكر الفقهاء الأوائل وتطور العلوم الشرعية في عصرهم.

بيد أن الخطأ الفاحش الذي يكون للطبيب دور فيه لابد وأن يرتب الضمان، وفي هذا عدل وتوازن بين حرية الممارسة الطبية وحقوق المريض والمجتمع.

وعلينا الآن أن نؤكد هذا المعنى، فيكون للمشرّع في هذا العصر موقف وسط بين حماية المريض من خطأ الطبيب وحماية الطبيب من جور القانون، ولن يتم ذلك إلا بالإحسان، وألا ينسى المسلمون الفضل بينهم، مع وجود وازع شرعي وإيماني، ونفس لوامة نابعة من الطبيب تجاه المريض، فيحاسب الطبيب نفسه قبل أن يحاسبه القانون. وهذا هو أصل المراقبة الذاتية التي ظهرت مؤخراً وسيطرت على ممارسة الطب في المجتمع الغربي، بعد أن عانوا من عَنَت القانون وأخطاء الأطباء.

التوصيات

- تدريس مادة الأخلاقيات وفقه الطب في كليات الطب المختلفة بمنطقة شرق المتوسط.
- تشكيل لجان طب شرعية في جميع مناطق العالم الاسلامي بوجه عام، ومنطقة شرق المتوسط بوجه خاص.
- أن تتبنّى منظمة الصحة العالمية ونقابات الأطباء في المنطقة العربية تنوير الأطباء وتعريفهم بحقوقهم وواجباتهم عن طريق عقد ندوات عن أخلاقيات الممارسات الطبية.
- التعميم الطبي المستمر الذي يقلل من الخطأ الطبي.
- تأصيل معنى المراقبة الذاتية Self Auditing في مناهج وممارسات الطبيب.
- تأصيل مفهوم الطب المُستَند بالبيّنات كدليل موثق لمحاسبة الأطباء على الأخطاء الطبية.

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Review

The district health system: a challenge that remains

B.T. Shaikh[1] and F. Rabbani[1]

SUMMARY The health care system in Pakistan has been confronted with problems of inequity, scarcity of resources, inefficient and untrained human resources, gender insensitivity and structural mismanagement. With the precarious health status of the people and poor indicators of health in the region, health care reforms were finally launched by the government in 2001. There are, however, numerous challenges and constraints in the system. The future health of the nation depends on this decentralization initiative. All our efforts should be concerted to support and facilitate the new system, which will mature into institutionalization of the health services at the district level. Most importantly, it will help in strengthening the primary health care services catering to the major fraction of the population. Besides political commitment, we ought to maintain attitudinal, behavioural and cultural conditions conducive to letting this system flourish.

Introduction

Health is a basic human right, and must be available and accessible to all in an affordable framework. To this end, an integrated approach to public health would combine preventive, promotive and curative health at all levels. Promoting good governance and fairness in the health sector through meaningful and consistent emphasis on prompt delivery, equitable and professional services, transparency and accountability must become a cardinal principle of the health sector, where social sector investment is perceived as a mathematical equation

In many parts of the world, progress towards the goal of 'health for all by 2000' has been slow and in some cases unachievable. Whilst most developing countries formulated broad policies, strategies and plans with this goal in mind, the modus operandi has been weak and questionable. In recent years, reforms in Malaysia, the Philippines, South Korea, Spain, Tanzania and Uganda have had mixed results [*1,2*]. The objectives were common: improving 'allocative' and technical efficiency; innovating service delivery, improving quality, transparency and accountability; and achieving greater equity in the distribution of resources [*3*]. All these efforts may also be aimed at adding new resources, circumventing inefficient bureaucracies and assuring empowerment of the people [*4*].

This article is based on a thorough literature review, not only from the local journals but also from some international ones. A few publications of the United Nations Development Programme and the World Bank on the subject of decentralization were consulted to review best practices in

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various countries. Several types of decentralization case studies were included, and the official policy documents of the government of Pakistan were also referred to verify certain details.

Current situation in Pakistan

Healthy public policy covers policy decisions in any sector or at any level of government and is characterized by an explicit concern for health and accountability for health impact [5]. It is an established fact that most of the developing countries are not spending more than 2% of their gross national product (GNP) on health, resulting in poor coverage of public health services. The Government of Pakistan spends about 0.8% of GNP on health care, which is lower than some neighbouring countries such as Bangladesh (1.2%) and Sri Lanka (1.4%) [6]. In Pakistan, only 3.07% of the total annual budget is allocated for economic, social and community services, and 43 out of 100 Pakistan rupees are spent on debt servicing [7]. A further increase in the allocation for the health budget may not be possible for many years to come, therefore, alternative methods of health financing, including cost-sharing, have to be considered. In most of the developing countries of South Asia, out-of-pocket household expenditure on health is at times as much as 80% of medical expenditure [8]. For health expenditure in Pakistan, it is about US$ 17 per head per year, out of which $13 is out-of-pocket private expenditure [9]. Our country spends 80% of its meagre health budget on tertiary care services, which are utilized by only 15% of the population. In contrast, only 15% is spent on primary health care services, used by 80% of the population [10]. Quality of health care is questionable, with considerable expenditure on unnecessary and inappropriate (and sometimes unsafe) care. Despite Pakistan being an advocate of the Alma Ata Declaration and having a huge primary health care infrastructure (set up in the early 1970s, but having declined over the past 2 or 3 decades), there is still a dearth of trained human resources, inequity in financing of health care and a scarcity of reliable information. In this scenario, the devolved district health services are also presented with an opportunity to tackle the health-financing situation. It is obvious that there is willingness to pay for primary health care in the public sector services if users receive improved care. Districts would in this case be able to recover substantial costs and maintain their incomes.

Half a century down the road from independence, social and demographic indicators in Pakistan present a gloomy picture, despite advancement in the economic sector. The current effort of the government to decentralize the system of governance included the health sector, assigning responsibility for health to the newly-created local governments. The district governments, however, still lack the capacity and powers to cope with their new responsibilities.

There are a few questions which arise right away while considering the process of devolution. Are basic data on the characteristics of the population, level of health, major health problems and coverage of essential health care readily available in the district? Have district priorities been appraised? Have targets and objectives for health and health care been set? Does the district have a plan of action for important programmes such as health promotion, maternal and child health, school health, environmental sanitation, occupational health, control of diseases and curative services? Are there effective mechanisms to make communities and the public and pri-

vate health sectors work together? Are there adequate resources, logistics, organizational arrangements and incentives to ensure prompt implementation of programmes? Will activities be monitored regularly? Is there a mechanism for quality assurance? Will periodic evaluation be carried out? Many of these remain unanswered, even after the passage of 2 years.

Devolution of power in the health department

There has been an obvious political, social, economic, demographic and epidemiological need for health sector reform in Pakistan [*11*]. The ultimate goal of any health sector reform is to improve the aggregate health status of the people, whether through de-concentration, devolution or delegation [*12,13*]. It includes packaging of services; the structures and organization of service delivery; financing; and the consumer–provider relationship [*14*]. All these efforts are geared towards the empowerment of the people at grass roots level. The district will be the dominant level for decision-making in the health department. The quantity, quality and access of integrated health care delivery will be improved. This will promote good governance and human resource development for sustainable development. These steps should be the answer to challenges like high infant and maternal mortality rates, low prevalence of contraceptive use and a high population growth rate, along with scarce income and health resources.

The devolution plan in general as well as the health-related section can be analysed in 4 different respects: devolution (political power), decentralization (administrative authority), deconcentration (management functions) and diffusion (power–authority nexus) [*15,16*]. Therefore, the aim is to establish a set of activities that include improved access to and utilization of services; community involvement; local accountability [*17*]; integrated, comprehensive health care delivery; intersectoral collaboration; and a strong 'bottom-up' approach to planning, policy development and management. Hence, attaining equity, effectiveness and efficiency in the health sector should, in principle, lead to sustainability in the system.

Responsibilities/functions at the provincial and district level

The responsibility of the provincial government will cover policy-making and legislation for the province; drug control under the Drug Control Act; monitoring and regulatory functions of medical and allied institutions; health research and related health information gathering; interacting with donors and international agencies; personnel management; provincial procurements; and supervision and monitoring of health programmes [*18*].

Reproductive health and nutrition education; prevention and control of communicable and noncommunicable diseases; food and sanitation; health management information system; environmental and occupational health; hospital referral systems; ambulance services; and financial and personnel management will be the responsibility of the district government. The district government will also look after primary and secondary level health facilities.

Besides these, there will be a district health management team, which will comprise a district health officer, managers from other relevant departments, public and private sector health care providers and community representatives. The district

health management team will adopt a team approach, share and exchange views, and reduce the workload of the district health officer, optimizing the utilization of human resources and improving cooperation and collaboration between stakeholders [*19*]. It is expected that well-defined structures; meaningful partnerships; capacity-building at provincial, district, sub-district and community levels; detailed mapping of resources and services; and integrated approaches towards programme planning will emerge as desired outcomes. The district will get its budget share according to population size, socioeconomic development, health infrastructure, health needs and problems, and indicator-based performance evaluation. This will contribute to and maintain equitable allocation of health resources between different districts with different priorities.

Challenges and constraints

The new actors in the health system are looking for motivation and incentives for their new assignments. Defining their administrative roles and jurisdiction still remains a challenge. The distribution of financial power between provincial and district representatives is mandatory. The new political government of Pakistan is still in the early stages of dealing with this dilemma. Though this is the phase of transformative learning and transition, the jurisdiction of the Public Service Commission; medical colleges; tertiary hospitals; and federal initiatives like the programmes for AIDS, malaria, tuberculosis and lady health workers, and the Expanded Programme on Immunization need to be defined. The crucial step, however is building awareness in the general public.

In countries where the administrative machinery has been decentralized to district level successfully, the development of the district health systems has been remarkable, despite an initial decrease in productivity, reluctance of the centre to share power and instability of the political framework [*20*]. Decentralization without delegation of appropriate financial and administrative powers does not work. There is also a need to ensure political commitment inside the district to create a fiscally and socially responsible management. The process requires trained health managers at district level, a team approach and planning support from the centre in the form of clear job descriptions, guidelines and advisory staff [*21*]. Devolution to the district level is imperative, and crucial if primary health care is to be improved. The advantages are a manageable size at district level, easily obtainable information and smooth communication between different stakeholders [*22*]. Moreover, decentralized programmes can be designed with a knowledge of the local culture and circumstances, thereby improving technical and allocative efficiency with appropriate local institutional capacity building [*23*].

District health managers need to be trained in areas like HIV/AIDS, control of diarrhoeal disease/acute respiratory infection, the Expanded Programme on Immunization, maternal and child health/family planning, health education, health management information system, nutrition, environment and sanitation, community mobilization, personnel and project management, etc. [*24*]. Information and data regarding population, health indicators, deployment of funds and coverage will be needed to appraise the district priorities and to set future objectives and targets. It will only then be possible to ascertain the

strengths and weaknesses of the existing district system, and take measures to improve it. The district health system has officially been with us for more than 2 years. The transformation of the fragmented and inefficient apartheid health system into a coherent health system capable of addressing the health needs of the vulnerable and marginalized population was, and still is, a massive challenge.

At the same time as adopting this new system, we cannot ignore ambitious targets like gender equality, empowerment of women, reduction in mortality rates for infants and children, reducing maternal mortality, improving primary health care and reproductive health services and poverty reduction. In order to meet such challenges, the decentralized system has to focus on equity, efficiency and good governance [*4,25*] and on developing tools to monitor and assess health system needs, especially in rural areas [*26,27*]. Today, about 67% of Pakistani people live in rural areas [*28*], but they have been completely neglected and disenfranchised in the decision-making processes that affect their daily lives. Lessons could be learned from the best practices of Brazil, Columbia, Morocco, South Africa and Chile [*29,30*].

Conclusion

With the transfer of administrative and financial powers to district authorities/local bodies, programmes relevant to the local needs and priorities will be facilitated [*21*], resources will be mobilized and greater community participation will be ensured [*30*]. There will be continuous monitoring and surveillance of continuity and quality of services, thus ensuring sustainability. This will create motivation, confidence and a sense of ownership. The strengthening of the first level care facility will bring an improvement in various health indices. The services and programmes could be designed on the concept "by the people, for the people, of the people" through more meaningful community participation, mobilization and empowerment. Of course, health system research should be part of every plan now. It will assist the newly-created district health system in defining clear goals and objectives, formulating strategies and providing quality services to the most underserved groups in the population. Only this approach can provide evidence-based services and address the needs of the community.

Decentralization has considerable promise. The empowerment of the decentralized bodies will lead to institutionalization of democracy, which is the key to progress. It will promote greater community participation, responsiveness of government institutions and increased flow of information between people and government [*31*]. This will not only make development programmes more flexible in catering to local needs, but also transparent and sustainable.

Health care is a right and not a privilege. Today, we are living in an epoch of urbanization, industrialization, demographic transition and globalization and their repercussions. Pakistan needs increased political freedom, economic facilities, social opportunities and transparency to break the vicious cycle of corruption and underdevelopment. This devolution plan, if granted full legitimacy, presents an opportunity to usher in a more democratic system of governance and effective and transparent health care delivery and management. For that to happen the citizens of Pakistan will need to participate proactively in the construction and functioning of the new institutions, and take advantage of the new environment for self-empowerment.

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Report

Development of the Regional Malaria Training Centre in Bandar-e Abbas, Islamic Republic of Iran

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SUMMARY The resurgence of malaria has highlighted the need for training health professionals in malaria control planning. The course described here was organized jointly by the World Health Organization, the Ministry of Health and Medical Education and the School of Public Health in Iran. The first course was held in 1997 and the fifth WHO-approved course is now in progress. The course focuses on dynamic, interactive, practical and problem-solving learning methods. It provides the participants with the knowledge, skills, competence and confidence to be able to analyse the malaria problem. The course fulfils the requirements of the Roll Back Malaria campaign. In the 8-week training period subjects such as basic bio-statistics and epidemiology, microcomputing, malaria parasitology, malaria entomology, vector control, case management, epidemiological approach to malaria control, field work and planning for malaria control are taught. Each participant is evaluated in each subject. A total of 71 participants from 17 countries in the WHO African and Eastern Mediterranean Regions, mainly those with a malaria problem, have graduated.

Introduction

The WHO Regional Malaria Training Centre in Bandar-e Abbas, southern Iran, was inaugurated in 1996 and held its first international training course in 1997. Since that time there have been courses annually, the fourth of which is presently in progress. The development of the centre, and of the international course, is a collaboration between the Ministry of Health and Medical Education, the School of Public Health of the Tehran University of Medical Sciences, the WHO Regional Office for the Eastern Mediterranean and WHO Headquarters.

The purpose of this report is to put into perspective and on record, the development of the Regional Malaria Training Centre and the series of 4 international training courses that have been held there and our vision for the future.

The Regional Malaria Training Centre

Historical evolution

In February 1992, the first Iranian Congress of Malaria was held in Zahedan, Sistan and Baluchistan province. This coincided with a revamping of the national malaria control programme to accelerate control of this disease in the 3 most affected provinces in the country, Hormozgan,

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Kerman and Sistan and Baluchistan. Advantage was taken of the presence of WHO to organize rapidly and conduct a short workshop on planning and management of malaria control programmes for senior staff of the Ministry of Health and Medical Education. The value of this for the realization of the accelerated control programme was very soon recognized and discussions were held between the Ministry of Health and Medical Education, the dean of the School of Public Health and WHO on the possibility of developing a training programme in the Islamic Republic of Iran for national staff at the district level.

A technical review of global malaria control efforts was carried out during the 1980s by WHO through the expert committee meetings on malaria (18th and 19th) and the scientific working groups. This culminated in the endorsement of the Global Malaria Control Strategy by the Ministers of Health of more than 90 countries at the WHO Ministerial Conference on Malaria held in Amsterdam in October 1992. The strategy was also endorsed in subsequent years by the Economic and Social Development Committee of the United Nations. This political endorsement of the way forward opened up opportunities for a wider global recognition of the malaria problem and its impact on the global economy. It also raised awareness among the peoples of the world of the plight of the millions of persons affected, especially children, in malaria-endemic parts of the globe.

In 1997 and 1998, WHO provided US$ 10 million each year for accelerated malaria control in Africa, much of it spent on programme planning and human resources development. Building on the momentum created, WHO established the Roll Back Malaria campaign in 1998, directed principally, though not exclusively, to the problem in Africa.

Spurred on by these events, the Islamic Republic of Iran began developing a national training programme for district health centre managers to plan and manage malaria control activities. The need for this was recognized following the revamping of the malaria control programme in the 3 southern provinces of the country and implementation through the primary health care system.

In 1996, the WHO Regional Committee for the Eastern Mediterranean Region discussed the malaria problem in the Region and the approach to control. A resolution was passed fully supporting the establishment of a regional malaria training centre in the Islamic Republic of Iran.

This fitted in well with the development of the training programme in Iran. The Centre for Health Education and Research in the Tehran School of Public Health, situated in Bandar-e Abbas, Hormozgan province, was considered ideal for the purpose. It was, therefore, designated as the WHO Regional Malaria Training Centre in 1996.

Relevance to the local health care structure

In Iran, each province has a University of Medical Sciences as part of the structure of the Ministry of Health and Medical Education. The vice-chancellor for health affairs of each of these universities is the person responsible for the delivery of health services to the population. During 1996, discussions were intensified between WHO Headquarters, the Regional Office for the Eastern Mediterranean, the Ministry of Health and Medical Education and the Dean of the School of Public Health regarding the development of the Regional Training Centre. A decision was made to hold the first international course at the centre in 1997 as a means of precipitating its further development. A 12-week course was designed on

malaria and other tropical diseases and the planning and control of these diseases.

The curriculum was developed during 1996 and 1997, and faculty members of the School of Public Health were identified as tutors and facilitators to cover as many subjects as possible. The close collaboration between the provincial universities, the training centre in Bandar-e Abbas and the Tehran School of Public Health was crucial for the success of this activity and was, therefore, cultivated from the very beginning to facilitate the smooth running of the course and especially for the development of the field training sites and the field exercises.

Principal objectives of the training centre

From the very outset, the authorities in Iran identified the need for training in planning and management for malaria control. The academic structure of the Tehran University of Medical Sciences School of Public Health, with satellite research stations distributed in 10 of the 30 provinces in the country, is such that basic subjects such as parasitology, medical entomology, vector control, general epidemiology and simple statistics could be adequately covered. However, the more malaria-specific subjects such as the epidemiological approach to malaria control, clinical management of the severely ill patient and planning and management were not areas for which experienced and knowledgeable teaching staff were readily available. In addition, with the revamping of the national malaria control programme by the Ministry of Health and Medical Education and planning the accelerated control programme for the 3 southern provinces of Hormozgan, Kerman and Sistan and Baluchistan, the need for well-trained health professionals to plan and manage the programme at district level was recognized. Thus, it was primarily to meet this national need and to comply with the WHO Regional Committee resolution that the Bandar-e Abbas centre was developed for international training courses in planning and management. The preparatory period was the latter part of 1996 and the first 7–8 months of 1997. During that time the physical facility was set up, the tutors and facilitators prepared themselves, the evaluation instruments were developed, the training materials completed and printed, supplies and equipment procured, books ordered and delivered for the library and trainees and the course advertised within the Region and world-wide to recruit trainees.

The training courses

The first course was for 12 weeks, from 27 September to 18 December 1997. It was on "Malaria and other tropical diseases and planning their control" and included, besides malaria, leishmaniasis, schistosomiasis and filariasis. There were 17 participants, of whom 10 were from Iran and the remainder from Afghanistan (2), Sudan (3), Yemen (1) and Zambia (1). Field training was for only 3 days in Siahu district of Hormozgan province. Two teams were formed; one stayed and worked in the village of Sagaz Ahmadi and the other in Siahu town. A joint WHO, Ministry of Health and Medical Education and School of Public Health certificate of achievement was awarded to those reaching the required standard.

The second course was held at the Regional Training Centre in 1998 from 22 August to 24 October. This was the first part of an 8-week course, which did not include other tropical diseases. It was on "Malaria and planning its control" and all 21 places on the course were filled. Participants came from Egypt (1), Islamic Republic of

Iran (13), Namibia (1), Somalia (3), South Africa (1) and Yemen (2). This was the first course which had a female participant.

The third course was for 8 weeks, from 22 September to 18 November 1999. A total of 20 participants attended, of whom 8 were Iranian and the remainder from Botswana (1), Egypt (1), Iraq (1), Jordan (1), Saudi Arabia (1), Somalia (2), Sudan (1), Syrian Arab Republic (1), Yemen (2) and Zimbabwe (1). This was the first course where the international participants outnumbered the nationals.

The fourth course is presently under way, running for 8 weeks from 6 September to 2 November 2000. Unfortunately there are only 13 participants, 9 Iranians and the remainder from Afghanistan (1), Egypt (1), Pakistan (1) and Saudi Arabia (1). There are 2 women on the course, 1 from Iran and 1 from Pakistan, making a total of 3 since these courses began.

During these courses, 71 participants have been trained, a total of 40 (56.3%) were from the Islamic Republic of Iran and 55 (77.5%) were medical doctors.

The learning approach

Since the inception of the training course, a participatory approach to learning has been adopted, with the learning being dynamic, interactive, practical and problem-solving. Emphasis has been placed on practicals, small group discussions, individual projects and team fieldwork to develop the skills and competence necessary to plan and manage a malaria control programme.

Lectures have been kept to a minimum with a few guest speakers only being invited. Tutors have been encouraged to reduce lectures to short introductions to the subject with discussions and exchange of experience and ideas predominating the sessions. This approach to learning has taken time to be accepted by all the tutors involved in the course. All tuition is in the English language.

The faculty

To ensure the sustainability of the course and the potential of the training centre, attention has been directed towards developing a national faculty to teach all 8 disciplines presently in the course curriculum. This has been a gradual process. Since the beginning, external tutors have been required for clinical malaria, epidemiological approach to malaria control and planning and management. All other subjects were taught by national specialists in the field.

As an approach to strengthening the national capacity to manage the whole curriculum "shadow tutors" were appointed to teach alongside the external tutors. It was difficult at first to find suitable young staff for this purpose, to ensure continuity and sustainability. However by this year's course (2000), the difficulty has been solved. A national clinician working in the private medical sector, who is very knowledgeable in clinical malaria, managed the teaching of those sessions with competence. An epidemiologist from the School of Public Health, who has been appointed Director of the Training Centre, and a PhD graduate are gradually assuming responsibility for the course in epidemiological approach to malaria control. For the first time 2 national tutors have been assigned as "shadow tutors" for planning and management, an MD and the PhD graduate who is also taking over the epidemiological approach to malaria control. These 2 are graduates from the first international course held in 1997. Thus, in a year or two all subjects will be adequately taught entirely by Iranian tutors.

Curriculum development

The curriculum has been evolving over these past 4 years. Changes have been made as a result of feedback from the participants themselves and from the tutors. The content has been updated in line with the WHO general approach to malaria control, in particular the Roll Back Malaria objectives and main thrust.

Twelve weeks was clearly too long for senior managers to be away from their duty stations. The training period has, therefore, been reduced to 8 weeks. Even this is felt to be too long by some; it is, however, difficult to reduce the time further without a large investment of time and money.

There are 3 objectives to be achieved by the end of the course, when the participants should have gained sufficient knowledge and skills to be able to:

- analyse the malaria situation and problems and find solutions,
- plan, implement, manage and evaluate antimalaria programmes,
- develop a training programme for capacity building for malaria control.

The basic subjects are oriented towards malaria control and have been found to be necessary in view of the heterogeneity among the participants on entering the training programme. The course is aimed at providing district level, responsible, health professionals with the skills and competence to be able to plan, manage, evaluate and replan malaria control programmes with the resources available and as an integral part of the health system of the country.

The course is not designed to train the participants in research methodology and its implementation, nor is it intended to produce microscopists, parasitologists, entomologists or vector control specialists. The time spent on the basic subjects is justified to bring the participants up-to-date. In addition, it provides them with the skills, competence and confidence to supervise the diagnostic, entomological, vector control and other services under their authority. Furthermore, a thorough knowledge of these subjects is essential to be able to understand the epidemiological approach to malaria control and for programme planning purposes.

The structure and content of the course can best be illustrated diagrammatically (Figure 1).

Field training

The field experience is a critical part of this programme. This has taken longer to develop for a variety of reasons. The experience in the year 2000 has been the best to date but improvement is still required.

The first course in 1997 provided only a 3-day field experience in 2 health districts of Hormozgan province, Ahmadi and Siahu. There had been no preparation ahead of time and thus the experience was very limited. Accommodation could not be found and participants travelled daily to the fieldwork sites.

In 1998 the field experience was for 5 working days in 3 districts of Hormozgan province, Minab, Roudan and Siahu. Owing to the absence of suitable accommodation, the participants travelled to the sites from the training centre in Bandar-e Abbas by road on a daily basis, a 1½-hour journey.

In 1999 WHO provided a consultant from 9 to 28 May to help select and develop 3 field-training sites for the 1999 course. These were the health districts of Minab in Hormozgan province, Kahnuj in Kerman province and Iranshar in Sistan and Baluchistan province. They are accessible by road from the training centre in Bandar-e Abbas with the exception of Iranshar. The

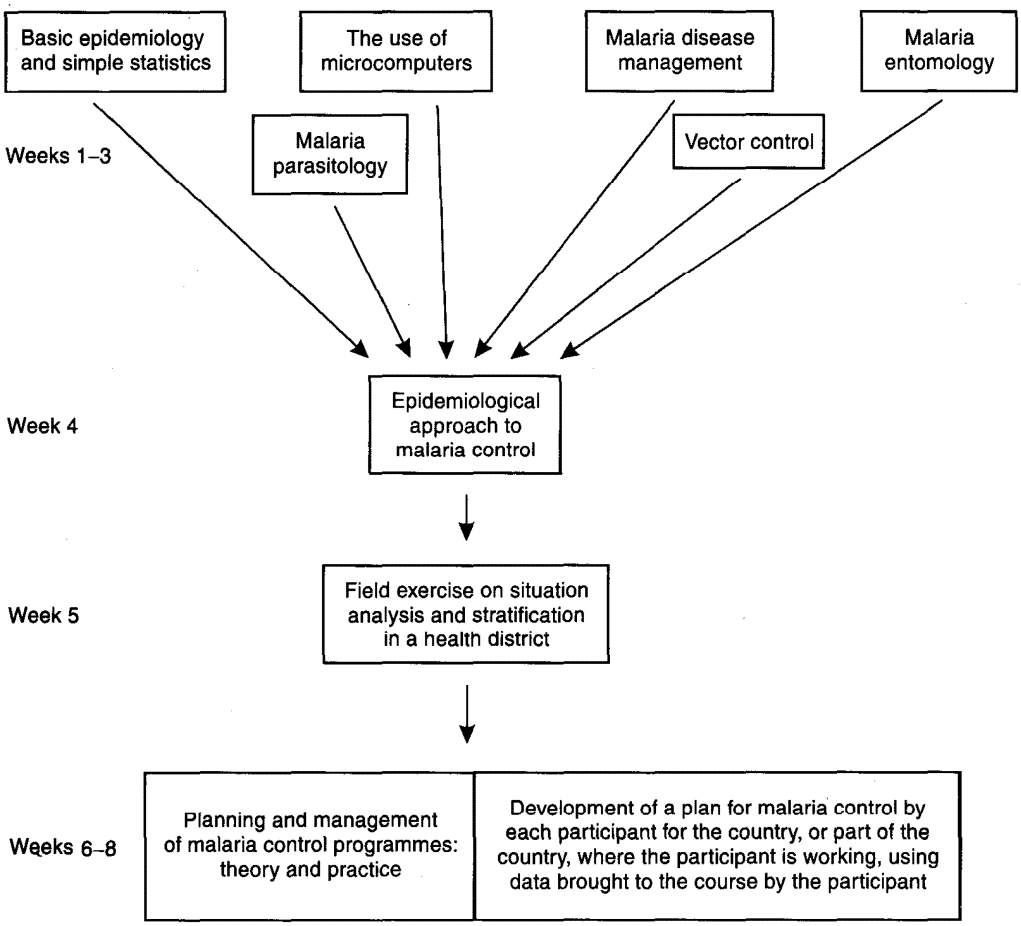

Figure 1 **Course structure**

team of participants travelling to Iranshar will first have to fly to Chabahar and then travel by road for 5 hours to Iranshar. Nevertheless, they represent excellent examples of the different epidemiological types of malaria in Iran and thus can be expected to provide the participants with the best possible experience. A list of data that needed to be compiled and translated into English well before the course commenced was drawn up and left with the heads of the district health centres. During the remainder of 1999 this data was collected, compiled and translated. Unfortunately, at the time of conducting the 1999 training course, owing to security issues, the participants were not permitted to travel to Kahnuj and Iranshar. Thus, they were assigned to the same 3 areas in Hormozgan province as in the 3 previous courses, but only the statistics for Minab were readily available as planned. The outcome was al-

most the same as in previous years: accommodation in Siahu was very poor and the participants had difficulties. The exercise was nevertheless useful but not implemented as planned.

In 2000 there were only 13 participants, which was not enough for 3 field teams, so only 2 field sites were needed. The course manager and coordinator decided to select Minab health district in Hormozgan province, which had been used in the 3 previous courses, and Chabahar city and health district in Sistan and Baluchistan province. The former was accessible by road and the latter by a 50-minute flight from Bandar-e Abbas. The experience was much improved and appreciated by all participants.

Accreditation

Since the inception of the course, 2 certificates have been awarded jointly by WHO and the School of Public Health, a certificate of achievement for those reaching the required standard and a certificate of attendance for those who satisfied the attendance requirement but who did not come up to standard otherwise.

Following long-standing discussions with the university, the course has now been accredited as a diploma course of Tehran University of Medical Sciences. The tutor/trainee contact is 350 hours and the standard of tuition has reached an acceptable level for the award of a diploma. The diploma is in "Malaria and planning its control," designated DMPC. The year 2000 class will be the first to graduate with this university diploma.

This is a major step forward in the development of the course and is expected to increase the attractiveness of this course to overseas participants as well as nationals and contribute to its sustainability.

Course management

The course is organized and managed jointly by WHO (WHO Representative, Islamic Republic of Iran; the Regional Office for the Eastern Mediterranean; and Headquarters), the School of Public Health of Tehran University of Medical Sciences and the Ministry of Health and Medical Education. The course director is the dean of the School of Public Health; the course manager is the head of the Entomology Department in the School of Public Health in Tehran.

The management of the course has also evolved over the 4 years that it has been running. The long experience that WHO has in planning, organizing and implementing international courses on malaria has been fully exploited in establishing the Regional Malaria Training Centre and the international course. For the first 3 courses, WHO provided the technical coordinator for the whole period of the course, and the School of Public Health a national counterpart. From the present course, the course administration will be carried out by the course manager and the role of technical course coordinator has been shared between the course manager and the director of the training centre in Bandar-e Abbas.

Financial management and accountability is the responsibility of the course director. Requests for allocations are made to the course director by the course manager. The actual accounts are maintained with the assistance of the School of Public Health, Tehran University of Medical Sciences.

Multisectoral collaboration

The structure of the health system in the Islamic Republic of Iran greatly facilitates the possibility for close multisectoral col-

laboration. The establishment and development of the Regional Training Centre, and in particular the malaria training course, have fostered close collaboration with the provincial universities of medical sciences of Hormozgan, Kerman and Sistan and Baluchistan. Working closely with the vice chancellors for health affairs of these universities, good collaboration has been established with the district health authorities, especially in the districts where the field training exercises have been carried out. The chancellors of the universities of medical sciences in each of these provinces and the vice chancellors for health affairs have welcomed the extension of this international training activity into these provinces and pledged their full support. Benefits are expected to accrue by having the national and international participants carry out a situation analysis in the selected health districts. It was agreed that, for the period of the field training, a field facilitator (who will be a past participant on this course) from the School of Public Health and a field coordinator appointed by the chancellor of the provincial university would be provided in each province. The terms of reference of these two key professionals were agreed upon. Thus, in 1999 and 2000 the heads of the district health centres were appointed as the field coordinators.

Further close cooperation was received from the Hormozgan University of Medical Sciences in 2000 in the form of permission to use their server to provide internet access for the participants. This has proved to be an excellent arrangement, and more cost effective with only a local telephone call instead of long distance to Tehran as in the previous year.

Since 1997, close collaboration has been promoted with the Vice Chancellor for Health Affairs of Hormozgan University of Medical Sciences, which is situated in Bandar-e Abbas, and with the director in charge of the provincial malaria programme. Both were past participants on the WHO Italy–Thailand course.

Sustainability of this course is to a large extent dependent upon a commitment on the part of, and close practical collaboration between, the School of Public Health in Tehran; the Ministry of Health and Medical Education; the universities of medical sciences of Sistan and Baluchistan, Kerman and Hormozgan provinces; and the provincial health authorities. This collaboration will be seen and felt by the international and national participants of this course. It will demonstrate that multisectoral collaboration is a key element to sustaining capacity-building for the control of communicable diseases. Participants will return to their places of work as ambassadors for the course and with a model of how this could be made to work well. The other strength in running the course in the Islamic Republic of Iran is the excellent primary health care system and through that, the delivery of malaria control activities.

Outcome to date

In summary, the Regional Malaria Training Centre has been developed from the Centre for Health Education and Research situated in Bandar-e Abbas, which is one of the field stations belonging to the School of Public Health in Tehran. The government has been fully committed to developing the centre and the training activities. Over the past 4 years, the facilities have been renovated and further developed, equipped and staffed for the courses. The School of Public Health has provided members of its faculty as tutors and facilitators.

The World Health Organization has provided full support in the form of fellowships for participants; staff members as

tutors; consultants as technical coordinators and tutors; and books, equipment, training materials and evaluation instruments.

The courses have a built-in system for evaluation of both participant and tutor performance and for feedback from the participants and tutors on the organization and functioning of the course. It is through this mechanism that improvements have been made each year to facilities, training materials, evaluation instruments and choice of tutors and facilitators.

Relevance to Roll Back Malaria

The training being offered in the Regional Malaria Training Centre in Bandar-e Abbas provides the participants with the knowledge, skills, competence and confidence to be able to analyse the malaria problem and develop from first principles approaches to controlling the disease according to the prevailing epidemiological situation.

Participants are taught how to conduct knowledge, attitude and practices studies and have the opportunity to carry out such studies themselves in order to understand the communities in which they are working. During the situation analysis they identify the at-risk population and devise approaches to controlling the disease and to protecting people from malaria. Their training includes the use of comparatively new technologies, in particular the use of a geographic information system for stratification and surveillance.

The experience in the Islamic Republic of Iran provides participants with the opportunity to examine the primary health care system and how it functions to accomplish malaria control; community mobilization is very much to the forefront. The educational process follows the 6 key elements of Roll Back Malaria, in particular, effective case management; detection and control of, and preparedness for, epidemics; early diagnosis and prompt treatment of cases and monitoring of treatment failures; multiple and cost-effective approaches to preventing infection; sector-wide capacity-building and intensified community efforts; partnerships for action, especially with the provincial universities, the health system and the private sector; and a research and development approach along with the in-depth analysis and critique of published articles.

The training is all about effective management, and in the implementation of the learning experience participants are given the responsibility to manage their own resources, including time, and to organize their own teamwork. Thus, the process of education at the training centre embodies team building, wide collaboration, effective management practices and modern approaches to learning.

Vision for the future

In the first 4 years of the Regional Malaria Training Centre there has been remarkable progress in all aspects: administrative, constructive and educational. There are still several areas for improvement and these are in the medium-term plan for the centre and the diploma course. They include further improvement of the physical facilities, strengthening the library, improving the field experience and the field training sites and improving the evaluation instruments.

The course has reached the point of curriculum development whereby very little can be cut or added within the same time frame. However, there is one way in which learning can be improved and made more enjoyable, and possibly reduce the length of the course, and that is through computer assisted learning. The basic modules used during the first 4 weeks of the course could be developed as computer assisted learning

modules. This would mean that participants could bring themselves up to date faster by using these learning tools under tutor guidance and learning at their own rate, depending on their basic knowledge of the subject. Practical work and group discussions would continue and eventually the length of the course could be slightly reduced without damaging the learning experience. Consideration would, however, still need to be given to the minimum number of contact hours needed to maintain the accreditation.

The potential value of this course to the national malaria control programme in the Islamic Republic of Iran and to the health system has not yet been fully exploited, nor has the capacity of the Iranian health system to improve the learning experience. The future augurs well for both.

Any gains achieved in malaria control with the full support of the Roll Back Malaria campaign will not be sustained without continuing development of human resources and improved management effectiveness throughout the health systems. Strengthening and supporting the management of health systems and services is vital to the success of communicable disease control programmes, especially those as complicated as malaria. The opportunity in the Islamic Republic of Iran for a pilot training course in malaria control through primary health care, supported by Roll Back Malaria, and improved health systems, supported by the Management Effectiveness Programme, provide evidence that the strategy is sound and can provide an ideal training ground for participants on the diploma course and, through them, subsequent dissemination to other countries of the Region and to other WHO Regions.

Case report

Rothmund–Thomson syndrome in a young man without cataract involvement

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Introduction

Rothmund–Thomson syndrome is a rare disorder that is inherited as an autosomal recessive trait occurring predominantly in females at the age of 3–6 months or later [*1*]. It is characterized by light sensitivity and some degrees of poikiloderma. The disease has a worldwide distribution. One review of the literature showed 200 cases up to 1992 [*2*].

Rothmund–Thomson syndrome was probably first described by Rothmund, an ophthalmologist, in 1868. All reported cases suffered from premature cataracts [*3*]. Later, Thomson described sisters with similar skin changes in 1923 and subsequently the disorder was labelled as poikiloderma congenita [*4*]. Cataracts were not a fundamental finding in them. Taylor reviewed all the literature and proposed the eponym of Rothmund–Thomson syndrome [*5*].

We report a case of the syndrome in a young man without any problems with vision or premature cataracts or malignant transformation. Facial photosensitivity and involvement of his skin, such as sparse scalp and eyebrow hair, with involvement of the fingernails were also noted. We review some of the relevant literature.

Case report

An 18-year-old man was seen with photosensitivity of the face, neck and ears, with warty papules on his extremities. A bird-like face and poikilodermic skin were prominent signs in this case. In the physical examination, he was slightly short in stature, but small hands and feet were noted.

Pigmentation, telangiectasia and reticulate erythema were seen all over the face except on the upper lids, under the nose, lower lip and dorsum of both ears. Erythema had appeared when he was 6 months old. The eyebrows were thin with loss of bilateral end parts. Hypo- and hyperpigmentation areas, with partial plaques of keratosis pilaris, were seen on the neck, trunk and distal parts of the limbs. Bilateral partial ill defined brown scaly plaques were found in both axillae. Warty hyperkeratotic papules were seen on the palms, soles, and the back of his hands and feet. Papyrus scars were present on his knees.

He had a history of right forearm fracture at the age of 16 years that fused after 2 years. No history of ocular problems or convulsion was reported. Genital examination revealed normal development, and hypopigmented macules were seen on the

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penile shaft. His fingernails were thin, but mucous membranes were normal in appearance except for mild gingivitis.

In the laboratory evaluation, fasting blood sugar, complete blood cell count, and liver and renal function tests were normal. The serum levels of sodium, calcium and phosphorus were normal. The serum alkaline phosphatase level was raised at 368 IU/L (normal range 100–290 IU/L).

Radiographic reports of the pelvis, hands, skull, chest, forearms and the lower extremities were normal, without any patchy sclerosis, cystic changes or osteolytic lesions except mild osteoporosis in the bones of the right hand.

Discussion

Rothmund–Thomson syndrome is a rare, inherited disease [*1*]. The disease is an autosomal recessive disorder and a worldwide review of literature in 1992 showed that 200 cases had been published up to that time [*2*].

Heterozygous carriers are virtually normal but may be identifiable by a minor sign such as light sensitivity. The parents of our case suffer from mild photosensitivity without any other signs of the syndrome. The genodermatosis of Rothmund–Thomson syndrome can be diagnosed by clinical findings such as short stature, especially in the limbs, light sensitivity and poikiloderma. Children are usually normal at birth and have minimal findings in the first 3 months of life [*6*]. Cataract was not seen in this case. Bilateral cataracts can develop between the fourth and seventh year, usually in about 40% of reported cases, but they are more frequent in some families than in others [*2,5*].

The diagnosis of Rothmund–Thomson syndrome is made on clinical grounds as no consistent laboratory test has been identified [*7*]. The essential features in the differential diagnosis are the age of onset, the distribution of the lesions, and the combination of atrophy, telangiectasia and mottled pigmentation, most intense on light exposed skin but not necessarily confined to it [*8*]. Short stature with photosensitivity and radiodermatitis of the facial skin were seen; skin signs such as loss of scalp and eyebrow hair with nail involvement were seen in this case. Scalp hair is often sparse and fine, and may be absent. Eyebrows, eyelashes, and pubic and axillary hairs are often sparse or absent. Nails are normal, or small and dystrophic.

Teeth are often normal, but microdontia and early caries have been reported [*3,7*]. Short limbs without any malignant transformation were prominent features in our case. Squamous cell carcinoma may develop in the keratosis or in the surrounding atrophic skin. After cutaneous epithelioma, osteosarcoma is the most frequent malignancy [*1*].

Thus patients with Rothmund–Thomson syndrome need a careful survey [*3*]. Physical development is frequently retarded; most patients are of small stature and some are dwarfs. The dwarfism is proportionate, with slender delicate limbs, small hands and feet, and short stubby fingers. The skull may be small and bird like, sometimes with a saddle nose. Aminoaciduria has occasionally been reported but has not been a consistent finding [*1*]. One report described osteogenesis imperfecta in a patient [*9*].

Hypogonadism and the incidence of hyperparathyroidism also appear to be increased [*10*]. Mental development is usually normal, but may be retarded. Neurological examination of our case showed no abnormality, and genital organs also had

normal development, but hypopigmented macules were detected on his penile shaft. In excised biopsy specimens, flattening of the epidermis with dermoepidermal junction oedema were seen. Acrogeria, Kindler syndrome, dyskeratosis congenita, xeroderma pigmentosa, and Bloom and Cockayne syndromes must be differentiated from Rothmund–Thomson syndrome [*1*].

Light sensitivity is a common feature in patients with Rothmund–Thomson syndrome [*1,2,3,8*]. High sun protective sunscreen is thus recommended for all patients with this syndrome.

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Case report

Molecular basis of RhD-positive/D-negative chimerism in two patients

S.S. Eid[1]

SUMMARY This study investigated two patients with Rh chimerism: patient A, a healthy individual, and patient B with myelofibrosis. Flow cytometry studies showed two red blood cell populations of Rh phenotypes R,r and rr at percentages of about 25% and 75% respectively. Normal RhD transcript sequences were found following RT-PCR. Genomic DNA (gDNA) showed normal exon, intron, GATA regions and exon/intron boundary sequences except for a single base change in intron 7 (C→A) of exon 7 in patient A. The major change found in both patients was the absence of *RHD* exon 9 DNA in gDNA isolated from peripheral blood. These findings suggest a somatic mutation, probably in a stem cell common to the myeloid lineage of both patients, and indicate that patient A may undergo malignant transformation in the future.

Introduction

Rh blood group and gene complex

The Rhesus (Rh) blood group system plays a key role in immunohaematology and transfusion medicine. The Rh antigens are the most immunogenic red blood cell protein antigens in humans. Antigens of the Rh blood group system are carried on two proteins encoded by genes denoted *RHD* and *RHCE*. Recently, it has been established that the Rh locus on chromosome 1p34.3-p36.1 comprises at least two distinct but highly homologous genes, a D gene and a CcEe gene (Figure 1) [*1*].

The D and CE polypeptides both consist of 417 amino acids, which differ by 35 amino acids as a result of 44 nucleotide substitutions in the coding sequence [*2*]. Cherif-Zahar et al. first described the intron-exon organization of the 10-exon *RHCE* gene. The organization of the closely linked and highly homologous *RHD* gene appears to be similar [*3*].

Genetic basis of the RH locus polymorphism

The RH locus is highly polymorphic. The structure of the RH locus was first established by studying blood samples collected from the Caucasian population where the *RHD* gene is completely deleted in a D-negative phenotype [*1*]. *RHD* gene deletion accounts for almost all D-negative phenotypes [*1,4*]. An intact but dysfunctional *RHD* gene was reported in a small number of phenotypically D-negative Caucasians. Two examples of such individuals have been studied at the molecular level. Avent et al. [*4*] reported a nonsense mutation in the *RHD* gene, while Andrews et al. [*5*] reported a four-nucleotide deletion in exon 4 of the *RHD* gene. In the African population a significantly higher proportion (up to 60%) of serologically D-negative individuals carry *RHD* genes compared with Europeans [*6*]. Among Japanese people that are typed as D-negative by standard serology, two different *RH* genotypes can be defined.

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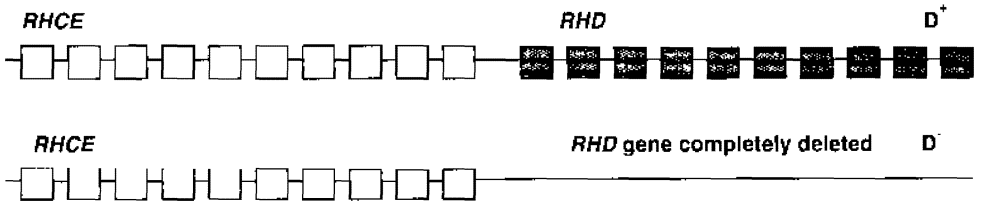


Figure 1 **Proposed molecular organization of the Rh genes**

The first group of individuals lack *RHD* genes (that is, are genotypically similar to Caucasian D-negatives) and the second group possesses *RHD* genes. Two groups of workers reported that this second Japanese D-negative allele appears to be of D_{el} (D-elute) phenotype, which can only be identified by complicated adsorption and elution tests [*7,8*]. However, Okuda et al. [*9*] stated that this group does not correspond to the D_{el} phenotype, and concluded that the *RHD* gene is highly detectable among Japanese D-negative individuals. D_{el} has recently been correlated with a 1013 bp deletion, including exon 9 [*10*], in the *RHD* gene.

Rh mosaicism and myeloproliferative disorders

Disease-related abnormal expression of blood group antigens has been recognized for a long time. Rh group changes characterized by the presence of two populations of red cells with different phenotypes (Rh mosaicism) have been reported in some patients suffering from acute or chronic myelogenous leukaemia, myeloid metaplasia, polycythaemia and myelofibrosis [*11–14*]. The myeloproliferative disorders are thought to have a clonal origin arising from a mutation in the haematopoietic pluripotential stem cell [*15*]. Occasionally the clone has an associated chromosome anomaly or a change in antigenic characteristics. Cooper et al. thought that these changes might also have a clonal origin [*16*].

Although in some cases there was an association of Rh loss with chromosome aberrations [*16–18*], no detectable abnormality of chromosome 1, where the RH locus is located (1p34-p36), has been noticed in other cases. In these examples the Rh mosaicism most probably resulted in the expression of an abnormal clone of stem cells (somatic mutation), which occasionally disappeared during clinical remission with a return to a normal Rh phenotype [*18,19*]. However, it is not clear whether the leukaemic process itself causes these changes in Rh blood group expression or not. Rh mosaicism was also found in apparently healthy individuals in whom chimerism could be eliminated as a possible explanation [*13,20,21*]. In one case a somatic mutation affecting only one of monozygotic twins was suspected [*22*]. In a healthy donor and a patient suffering from a non-haematological disease (prolapse of an invertebral disc) a mosaicism for the blood group RH and FY locus (chromosome 1q) was noticed [*23,24*].

In these studies, serological Rh typing established that persons who had initially typed D$^-$ subsequently had mixed field reactions indicating RhD chimerism. Methods for direct detection of the *RHD* gene were not available when these studies were

reported. The subsequent availability of polymerase chain reaction (PCR) for detecting genes encoding Rh proteins has made it possible to demonstrate the *RHD* gene even when conventional serological methods do not detect D antigen. Although the molecular basis of *RH* genes has been largely clarified [*25*], there is currently no information available regarding the molecular alterations causing Rh blood group changes in malignant diseases, except for one report [*14*] which studied the molecular basis of RH chimerism in two patients who were about 75% RhD-negative and 25% RhD-positive. One patient suffered from chronic myeloid leukaemia and the other was a normal patient whose Rh chimerism was detected on preoperative blood typing. Both patients were found to have *RHCE* and not *RHD* at exon 9.

Methods

Patients

Patient A was a woman aged 25 years old with no haematological disorders or other malignancies. She was found to have Rh chimerism after preoperative (laminectomy) blood group typing. She had not been transfused and does not have a twin.

Patient B was a 79-year-old Caucasian woman, referred by her general practitioner to the haematology outpatients clinic at Norfolk and Norwich hospital for investigation of persistent mild anaemia and leukocytosis. Her blood film and bone marrow aspirate suggested a diagnosis of myelofibrosis.

Blood samples

Blood samples were sent to the International Blood Group Reference Laboratory, Bristol, by the University of Cambridge Division of Transfusion Medicine, where serological tests and flow cytometry were performed and both patients were diagnosed with Rh chimarism. The International Blood Group Reference Laboratory supplied DNA and cDNA from common RhD-positive and RhD-negative phenotypes.

Genomic DNA extraction and analysis

Genomic DNA (gDNA) was extracted from peripheral blood as described by Avent and Martin [*26*]. PCR reactions were carried out using gDNA templates derived as previously described. Each PCR reaction mix had a final volume of 50 µL consisting of 2.5 mmol/L $MgCl_2$, 10 mmol/L Tris pH 8.3, 1.25 mmol/L dNTPs, 25 µmol/L diluted stocks of primers, 100 ng gDNA and 2.6 U Expand™ High Fidelity enzyme mix. The PCR reactions were carried out on a Perkin Elmer-Cetus DNA thermal cycler TC1. The PCR conditions and the sets of primers used in the amplification of exons 1–10 are shown in Table 1 and Table 2 respectively. The PCR products were gel-purified using a Qiaex II kit (Qiagen) following the manufacturer's instructions. Purified DNA was sequenced using dye-labelled terminator cycle sequencing chemistry on an Applied Biosystems 373A DNA sequencer.

PCR amplification of Rh transcripts

Rh transcripts from two overlapping fragments (exon 1–7 and exon 7–10) were isolated, following RT-PCR on total RNA from peripheral blood reticulocytes using Dynabeads Oligo $(dT)_{25}$. cDNA was prepared as described by Sambrook et al. [*27*].

Two sets of primers were used to amplify the Rh transcripts. The first set of primers was used to amplify the region from exon 1 to exon 7 and had the following sequences.

- Exon 1 *RHD* forward (sense) amplimer: 5′-TCCCCATCATAGTCCCTCTG-3′

Table 1 **PCR conditions for amplifying genomic DNA**

Exon no.	Initial denaturation (°C)	Denaturation (°C)	Annealing (°C)	Elongation (extension) (°C)	No. of cycles
1	94 for 5 min	94 for 1 min	60 for 1 min	72 for 2 min	30
2	94 for 5 min	94 for 1 min	58 for 1 min	72 for 2 min	30
3	94 for 5 min	94 for 1 min	65 for 1 min	72 for 2 min	30
4	94 for 5 min	94 for 1 min	60 for 1 min	72 for 2 min	30
5	94 for 5 min	94 for 1 min	60 for 1 min	72 for 2 min	30
6	94 for 5 min	94 for 1 min	65 for 1 min	72 for 2 min	30
10	94 for 5 min	94 for 1 min	60 for 1 min	72 for 2 min	30

Long PCR conditions were used to amplify exons 7 and 8/9.
For exons 8/9, initial denaturation 4 min at 94 °C, denaturation 20 s at 94 °C, annealing 30 s at 60 °C, extension 8 min at 68 °C for 10 cycles, then another 20 cycles under the following conditions: denaturation 20 s at 94 °C, annealing 30 s at 60 °C, extension 8 min and 20 s at 68 °C.
For exon 7 the same conditions were used, except the annealing temperature was 58 °C.

- Exon 7 RHD reverse (antisense) amplimer: 5′-AAGGTAGGGGCTGGACAG-3′

The second set of primers was used to amplify the region from exon 7 to exon 10 and had the following sequences:

- Exon 7 RHD forward (sense) amplimer: 5′-TGGTGCTTGATACCGCGGAG-3′
- Exon 10 RHD reverse (antisense) amplimer: 5′-AGTGCATAATAAATGGTGAG-3′

PCR reactions were carried out using the following conditions: 94 °C for 1 min, 55 °C for 1 min and 72 °C for 3 min for 35 cycles in a 50 µL reaction mix composed of: 10 mmol/L Tris-HCl pH 8.3, 2.5 mmol/L $MgCl_2$, 1 µm/L each primer, 1.25 mmol/L each dNTP, 100 ng cDNA, and 2.6 U Expand™ High Fidelity enzyme mix. PCR products were gel purified on 1.5% agarose gel using a Qiaex unit (Qiagen) and cloned into a PCR™ II vector following the manufacturer's instructions. Sequence analysis of cloned PCR products was performed using dye-labelled terminator cycle sequencing chemistry on an Applied Biosystems 373A DNA sequencer with 0.5 to 1.0 µg plasmid DNA as template. Both strands of DNA were sequenced.

Results

Rh transcript analysis

Reticulocyte RNA isolated from the patient was reverse transcribed and transcripts arising from the *RHD* gene were amplified using two overlapping sets of primers (exons 1–7 and 7–10). PCR products of the expected sizes (1200 and 387 bp for 1–7, 7–10 respectively) (Figure 2) were cloned into PCR™ II plasmid as described in the Methods. Six clones of each transcript (1–7, 7–10) were isolated and fully sequenced on both strands. The results revealed that all these clones' sequences are identical to the *RHD* gene sequence in both patients.

Table 2 **Sets of primers used and their product sizes**

Exon	Primer name	Direction	Specificity	Primer position	Sequence (5′ to 3′)	Product size (bp)
1	[a]RH1F	F	CDE	Promoter -675 to -652	CTAGAGCCAAACCCACATCTCCTT	952
	[a]RH1R	R	CDE	Intron 1 129 to 106	AGAAGATGGGGGAATCTTTTTCCT	
	[a]RHD 1F	F	D	Promoter -149 to -132	ATAGAGAGGCCAGCACAA	428
	[b]RHD IN 1R	R	CDE	Intron 1 84 to 64	TCTGTGCCCCTGGAGAACCAC	
2	[a]RH EX2 IN 1F	F	CDE	Intron1 -72 to -53	ACTCTAATTTCATACCACCC	480
	[b]RHDC 2R (M)	R	DC	Intron 2 225 to 205	TGGATTCCTTGTGATACTCG	
3	[a]RH3F	F	CDE	Intron 2 2823 to 2842	GTGCCACTTGACTTGGGACT	3890
	[a]RH3R	R	CDE	Intron 3 28 to 11	AGGTCCCTCCTCCAGCAC	
4	[b]RHD IN 3F	F	D	Intron 3 -36 to -16	GCCGACACTCACTGCTCTTAC	390
	[a]RH4R	R	D	Intron 4 198 to 175	TCCTGAACCTGCTCTGTGAAGTGC	
5	[a]RH5F	F	D	Intron 4 -267 to -243	TACCTTTGAATTAAGCACTTCACAG	507
	[c]RHD IN 5R	R	CDE	Intron 5 73 to 56	GTGGGGAGGGGCATAAAT	
6	[a]RH6F	F	CDE	Intron 5 -332 to -314	CAAAAACCCATTCTTCCCG	511
	[c]RHD IN 6R	R	D	Intron 6 41 to 21	CTTCAGCCAAAGCAGAGGAGG	
7	[a]RHD IN 6F	F	D	Intron 6 -102 to -85	CATCCCCCTTTGGTGGCC	400
	[a]RHD IN 7R	R	D	Intron 7 169 to 152	AAGGTAGGGGCTGGACAG	
	[c]EX7 FOR2	F	D	Exon 7 949 to 967	AACCGAGTGCTGGGGATTC	3500
	[a]RH7R	R	CDE	Intron 7 ~ 3000	GCTTGAAATAGAAGGGAAATGGGAGG	
	[a]RHD EX 7R	R	D	Exon 7 1008 to 985	ACCCAGCAAGCTGAAGTTGTAGCC	3600
	RH 3F	F	CDE	Intron 5 -332 to -314	CAAAAACCCATTCTTCCCG	
8 and 9	[a]RH8/9R	R	CDE	Intron 9 ~ 300	CACCCGCATGTCAGACTATTTGGC	580
	[b]EX9 IN 8F	F	CDE	Intron 8 -165 to -144	GCTGGTCCAGGAATGACAGGGC	
	[b]RH IN 8F	F	CDE	Intron 8 69 to 91	CGTGTGGCTGGATGTCTATGTGC	5000
	[c]Ex 9R	R	D	Exon 9 1219 to 1193	AAA CTT GGT CAT CAA AAT ATT TAA CCT	

Table 2 **Sets of primers used and their product sizes** *(concluded)*

Exon	Primer name	Direction	Specificity	Primer position		Sequence (5' to 3')	Product size (bp)
	[a]RHCDE IN 7F	F	CDE	Intron 7	-67 to -48	CCTTTTTGTCCCTGATGACC	3511
	[b]RH IN 8R	R	CDE	Intron 8	131 to 109	CCAATTCTGAAATTATCTGATCC	
10	[a]RH10F	F	CDE	Intron 9	~ -40	CAAGAGATCAAGCCAAAATCAGT	380
	[a]RH10R	R	D	3' UTR	1541 to 1522	AGCTTACTGGATGACCACCA	

[a]*Primers adapted from Wagner et al. [28]*
[b]*Primers designed by Dr B. Singleton, International Blood Group Reference Laboratory.*
[c]*Primers designed by Dr N. Avent, International Blood Group Reference Laboratory.*
F = forward (sense).
IN = intron.
R = reverse (antisense).
EX = exon.

PCR amplification of the gDNA

All gDNA PCR products (Figure 3) were excised, gel-purified, and the two DNA strands sequenced using the same sets of primers used in the amplification. Almost all exons, introns, GATA regions within the promoter region, and exon-intron splicing boundaries were found to be identical to the normal *RHD* gene. The single exception was intron 7: the primers *RHD* IN 6F, *RHD* IN 7R gave no product for patient A, while patient B and the control gave a product with a size of 400 bp (Figures 3 and 4b). When primers *RHD* EX 7R and *RHD* 6F were used, there was a product of 3600 bp,

Figures 2a and 2b **PCR products of cDNA obtained by the amplification of 2 overlapping fragments: exon 1–7 and exon 7–10**

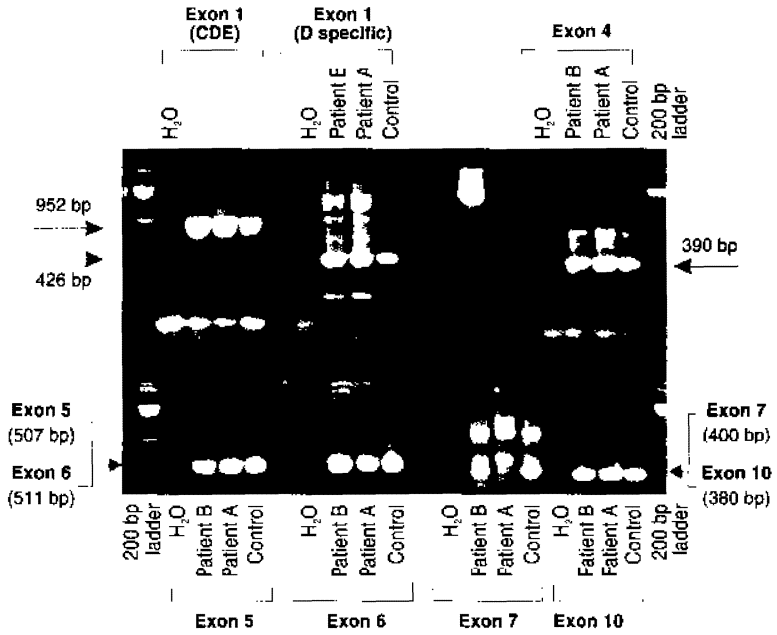
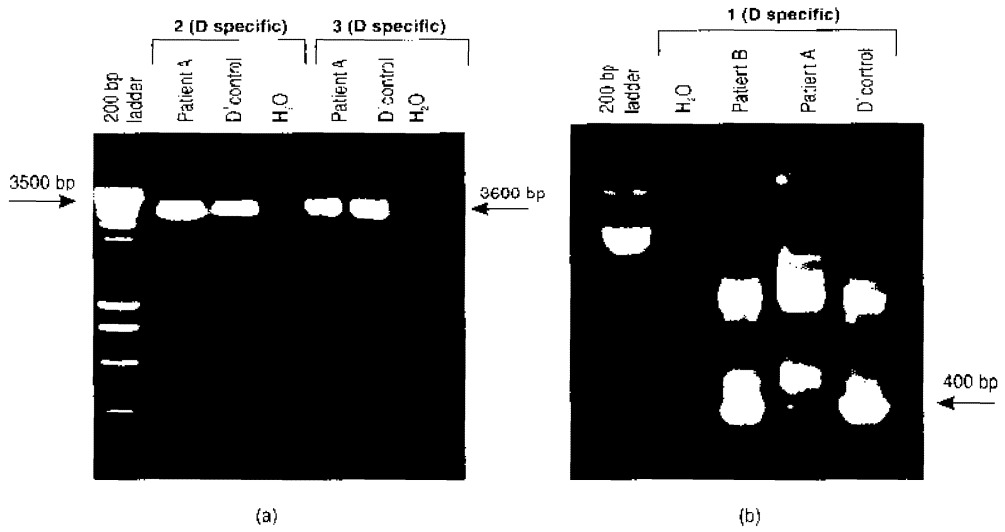


Figure 3 **PCR products, stained with ethidium bromide, include exon 1 (CDE), exon 1 (D specific), exon 4, exon 5, exon 6, exon 7 and exon 10 for both patients (no product is seen for patient A in exon 7)**

which indicates that at least the 5′ half of exon 7D is present. When primers EX 7FOR2 and RH 7R were used to amplify the 3′ regions of exon 7 and intron 7 PCR gave a 3500 bp product (Figure 4a). When sequenced this gave A instead of C (151 exon 7 position) at the 3′ end of primer *RHD* IN 7R which is located on intron 7 (Figure 5), that is, the CE sequence not the D sequence. This explains why PCR with D IN 6F and D IN 7R failed. When RH CDE IN 7F and RH IN 8R primers were used to amplify exon 8, the PCR gave one product of about 3511 bp for both patients. An interesting finding was that on one occasion, patient A gave two products (Figure 6).

This raises the possibility of a mutation, deletion or insertion in intron 8. When purified, these products gave very poor quality DNA and therefore no sequencing was carried out. The same PCR was repeated more than once. Each time, a single product was obtained (Figure 7a). When EX 9 IN 8F and RH 8/9R primers were used, the PCR gave a 580 bp product (Figure 7b). Patient A and patient B both gave CE-specific sequences only, that is, C at nucleotide 1170 and T at nucleotide 1193 (Figure 8).



Figures 4a and 4b **Amplification products of exon 7 in patient A, using a control and 3 primers: (1) D IN 6F, D IN 7R (400 bp) (2) EX 7 FOR 2, RH 7R (3511 bp) (3) *RHD* 7R, *RHD* 6F (3511 bp)**

Discussion

This study presents the results from two patients, one suffering from a myeloproliferative disorder and the other a healthy individual. Both were females having a mixture of R_1r, rr cell populations as demonstrated by serological tests and flow cytometry, which has become a valuable tool in the detection of minority red cell populations.

Loss of RhD antigen in malignant haemopathies and in healthy individuals is extremely rare and molecular information on the blood group changes of these patients is lacking. The molecular basis of Rh chimerism in both patients was studied to find out whether the 2 patients had the same molecular basis, and compare them with those described by Cherif-Zahar et al. (del G600) [*14*].

The *RHD* transcripts were studied using two overlapping fragments, exon 1–7 and exon 7–10. The PCR products of both fragments were cloned and sequenced for both patients. As the results indicated, no change was detected in the coding region. However, since the D antigen was not detectable on about 75% of native red cells, either a mutant transcript or truncated protein might be degraded within the cells so that the 7–10 fragment PCR might not amplify immature fragments and pick up only the normal transcript. As a result, no mutant *RHD* was found because no transcription from the mutant gene occurs. When these PCR amplifications were carried out for all 10 exons of the *RHD* gene in patients A and B, all exon/intron boundaries, exon, intron, and GATA regions were found to be normal, except for intron 7 in patient A where a single base change was found (C→A) at the 151 exon 7 position, which is at the 3′ end of primer *RHD* IN 7R, that is, in the CE and not the D sequence. This explains why PCR with *RHD* IN 6F and *RHD* IN 7 failed (Figures 3 and 4b). This minor change does not

Figure 5 **DNA sequences of exon/intron 7 showing sequence match between the 3´ ends of the primer. Sequence shows a comparison the between exon/intron 7 sequences of patient A and the *RHD* and *RHCE* controls.**

Shows the sequence difference between *RHD* and *RHCE

↑ Shows the mutation found in intron 7 at 151 exon position (c→a).

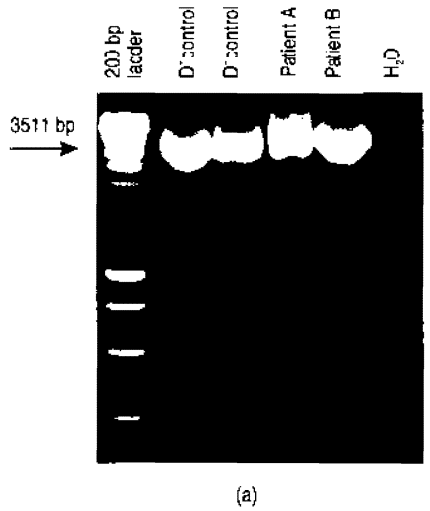


Figure 6a **Amplification products of exon 8 using primer RH CDE IN 7F, RH IN 8R (CDE)**

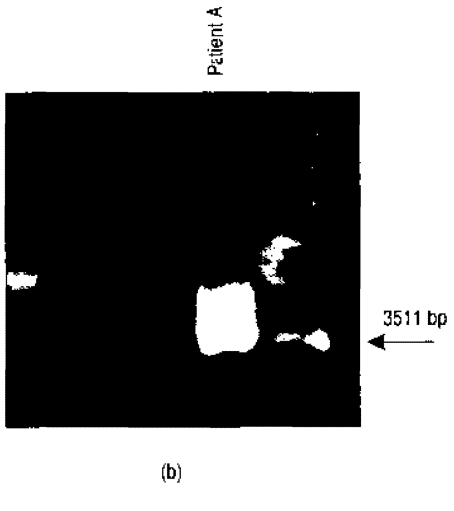
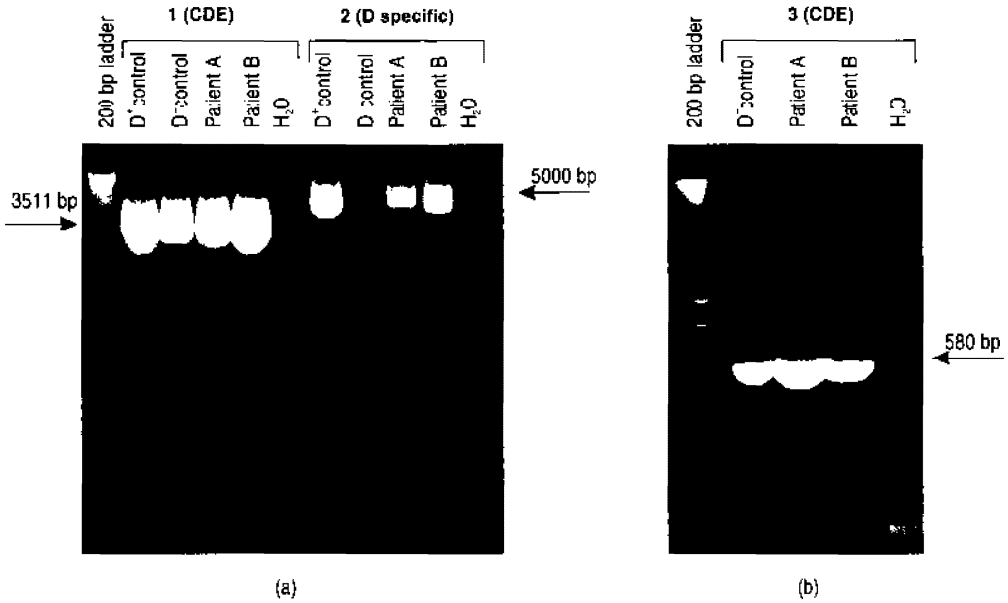


Figure 6b **Further migration for patient A (two products)**



Figures 7a and 7b **Amplification products of exon 8/9, using thee primers: (1) RH CDE IN 7F, RH IN 8R (2) RH IN 8F, EX 9R (3) RH 8/9 R, EX 9 IN 8F**

Figure 8 **DNA sequences of exon/intron 9 showing sequence match between the 3' ends of the primer. Sequence shows a comparison the between exon/intron 9 sequences of patient A and the *RHD* and *RHCE* controls.**

***Shows the sequence difference between *RHD* and *RHCE*.**

↓ Shows that both patients have *RHCE* sequences not *RHD*.

provide an explanation for the patient chimerism, but the presence of CE-specific bases in intron 7 of *RHD* may suggest that part of the *RHD* gene in patient A has been replaced by *RHCE*, resulting in an *RHD-CE-D* hybrid gene. However, more experiments are needed to see if this is the case.

An alternative explanation is that this change may affect the end part of RHD gene, which results in the loss of RhD antigen. This is supported by the finding that the RHD exon 9 is absent in both patients, with no *RHD* exon 9 isolated from peripheral blood gDNA. This may be explained by an insertion of DNA (possibly a replacement with part of *RHCE*) or the deletion of a segment of DNA in intron 8 including exon 9. Any alteration in the amino acid sequence can impair stability, resulting in an unstable molecule that degrades almost as quickly as it is synthesized. As a result no *RHD* gene is expressed. Possible support for this hypothesis comes from the observation that another primer (D 8/9F, RH IN 8R) produced a large product in high yield (Figure 6a). In patient A, further migration gave two bands in one occasion (Figure 6b), but on other occasions both patients A and B gave only one large product which could not be resolved into discrete bands (Figure 7a). This product needs to be cloned and sequenced to see if any mutation is present in intron 8 which may cause defective processing or splicing of the primary mRNA transcript, resulting in improper translation and the absence of *RHD* exon 9. When D-specific primers were used (Figure 7a) weak PCR products for *RHD* exon 9 were produced in patients A and B, stronger in patient B. This may be explained by the high leukocyte count. The presence of these products in both patients when D-specific primers were used may be due to amplification of exon 9 from the minority of cells that are *RHD*-positive. The high ratio of myeloid cells to reticulocytes may explain why this change could be detected more easily in the gDNA than in the Rh transcripts in patient B, which is not the case for patient A.

Weak D phenotypes are associated with severely depressed D expression. Wagner et al. [*28*] detected two changes in exon 9: a substitution at nucleotide 1177 (T→G) changing tryptophan to arginine and giving rise to a weak D type 9 phenotype, and another at nucleotide 1154 (G→C) which changes glycine to alanine, and gives rise to a weak D type 2 phenotype. In our patient this is not the case, since the whole *RHD* exon 9 is absence. In a Japanese population a deletion in 1013 bp in the *RHD* gene that includes exon 9 has been reported [*10*]. This deletion is correlated with the D_{el} (D-elute) phenotype (which can only be defined by sophisticated adsorption and elution tests), whereas in our case D antigen expression is severely depressed. Any *RHD* alteration to exon 9 affects D antigen expression. These findings differ from Cherif-Zahar et al.'s finding [*14*] of a CML patient whose RhD-positive phenotype shifted to RhD-negative, where sequence analysis of Rh transcripts amplified from reticulocytes revealed a single nucleotide deletion (del G600) localized in a region encoded by exon 4 of the *RHD* gene.

Comparing the two patients with some of the D-negative phenotypes, it is most likely that the two cases showed a genuine D-negative phenotype caused by clonal changes accompanied by absence of *RHD* exon 9. More analysis is needed to define the precise mechanism of RhD chimerism, but our results indicate that the defect is within the region of exon 9 in both patients. Northern blotting is helpful in detecting any changes in the RNA level. The 8/9 PCR product (Figure 7a), using primer 1, should be cloned and sequenced. Clinically, the

healthy patient has been advised to have regular check-ups to rule out any clonal changes that may develop over time. In the case of the myelofibrosis patient, it may be that during the myelodyplastic process a downregulating gene is activated, inhibiting *RHD* gene expression.

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Genetics in developing countries

Low- to middle-income countries vary in their capacities in medical genetics. Some may not have the resources to set up appropriate genetic services. Others provide genetic services but need assistance to improve equity of access to these services. The World Health Organization is supporting country capacity building by constructing educational modules and pilot studies to develop national community genetics, including the ethical, legal and societal implications (ELSI).

Source: WHO Fact sheet: genetics and health (http://www.who.int/genomics/en/E_hgn- final.pdf)

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Al Hamza B, Smith A. *The fifth sign of identity*. Cairo, American University Press, 1990.

Journal article:
Jones A et al. One day in Tibet. *Journal of tautology*, 1993, 13(5):23–7.

Document:
Al-Itneen M, ed. *The principles of uncertainty*. Geneva, World Health Organization, 1985 (document WHO/DOC/537).

12. Figures and tables with appropriate captions should each be on a separate sheet, numbered sequentially with arabic numerals and attached to the end of the paper. Each figure and table should be referred to in the text and its placement in the text should be clearly indicated where appropriate. Where appropriate, sources should be given for each figure or table. If any figures, tables or other materials have been copied from other sources, authors have the sole responsibility for securing the necessary permission. In order to avoid layout problems in final production tables and figures should be limited as far as possible. Not more than one table or figure per 1000 words is preferable. Figures derived from data must be accompanied by those data to enable redrawing if necessary.

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المجلة الصحية لشرق المتوسط
دلائل إرشادية للمؤلفين

١. ينبغي أن لا تكون الورقات المقدَّمة للنشر، قد نشرت أو قبلت للنشر في أي مكان آخر. ويحتفظ المكتب الإقليمي لمنظمة الصحة العالمية لشرق المتوسط بجميع حقوق استنساخ أو إعادة نشر المواد التي تنشر في *المجلة الصحية لشرق المتوسط*.

٢. يمكن أن ترسل الورقات الأصلية، المكتوبة بالعربية، أو الإنكليزية، أو الفرنسية، للنظر فيها من قِبَل رئيس تحرير *المجلة الصحية لشرق المتوسط*، بالمكتب الإقليمي لمنظمة الصحة العالمية لشرق المتوسط، ص. ب. (٧٦٠٨)، بمدينة نصر (١١٣٧١)، بالقاهرة، في مصر. ويتم تقديم خلاصات للورقات، باللغات الثلاث.

٣. ينبغي أن يكون موضوع الورقات منتمياً لمجال الصحة العمومية، أو أي ميدان تقني وعلمي آخر، له صلة بالمجالات ذات الأهمية لمنظمة الصحة العالمية، مع الإشارة بشكل خاص إلى إقليم شرق المتوسط.

٤. ينبغي تقديم ثلاث نسخ من كل مخطوطة أو مطبوعة. كما ينبغي أن لا يتعدى النص، مع الجداول، والرسومات المرافقة، ١٥ صفحة مطبوعة على الآلة الكاتبة مع ترك فاصلين بين كل سطر، من القطع A4 (٤٥٠٠ كلمة)، وأن تكون الطباعة على وجه واحد فقط من الصفحة. وعندما يتم إعلان المؤلف بأن المطبوعة التي قدَّمها قد تم قبولها من دون شرط، أو قبولها بشروط، ينبغي أن يقدِّم قرص حاسوبي (٣,٥ بوصة)، يتضمن النص، والجداول، والرسوم البيانية والتوضيحية. وبالنسبة للورقات المقدَّمة باللغتين الإنكليزية والفرنسية، يرجى، بناءً على طلب رئيس التحرير، أن يتم تقديم النص، في كلٍّ من، صيغة معالجة الكلمات (وحبَّذا لو أمكن استخدام برنامج الكلمات اللينة الدقيقة Microsoft Word، بالنسبة للحاسوب الشخصي، غير أننا يمكن أن نترجم غالبية الصيغ الأخرى)، وفي شكل محفوظ كنص/ملف الكود الأمريكي القياسي لتبادل المعلومات ASCII (أسكي). وينبغي اتباع نفس الإرشادات في ما يتعلق بالورقات المقدمة باللغة العربية. وإذا كانت الورقة المقدمة، هي ترجمة كلية أو جزئية لعمل آخر لم ينشر، فينبغي تقديم نسخة من هذا العمل، في لغته الأصلية. وحيثما أمكن، يفضل أن تكون الرسوم البيانية في شكل رسوم هارفارد البيانية، مع استخدام برنامج النوافذ Windows أو إكسل Excel، وتقديم الرسوم التوضيحية والصور الفوتوغرافية في صيغة EPS أو TIFF. كما أنه من الضروري تقديم ثلاث مجموعات من الصور الفوتوغرافية والرسومات الأصلية، مع المعطيات الأساسية. وفي حالة وجود أي نص أو حروف مكتوبة على الصور، فينبغي تقديم نسخة إضافية خالية من أي نص مطبوع أوأي حروف مكتوبة .

٥. يتم مراجعة جميع الورقات المقدَّمة مراجعة دقيقة من قِبَل الزملاء، وفي ضوء هذه المراجعة، تحتفظ هيئة التحرير بحق قبول أو رفض أي ورقة. ومن المتفق عليه أن جميع الورقات التي يتم قبولها، تخضع للمراجعة الإحصائية والتحريرية، بحسب ما يلزم، بما في ذلك اختصار النص، أو حذف بعض الجداول أو الرسوم البيانية.

٦. ينبغي أن يكون عنوان الورقة مختصراً على قدر المستطاع، وحبَّذا لو كان حوالي ١٠ كلمات، وأن يكتب على ورقة منفصلة، مع تحديد اسم المؤلف (أو أسماء المؤلفين)، وعضويتهم في المؤسسات المختلفة، وأعلى الدرجات العلمية التي حصلوا عليها. كذلك، ينبغي ذكر العنوان البريدي، والمعلومات الأخرى اللازمة للاتصال بالمؤلف (بريد إلكتروني، فاكس، هاتف). ويجب أن لا يزيد عدد المؤلفين على خمسة. ولابد أن يكونوا قد ساهموا جميعاً في تصميم البحث أو تحليل نتائجه أو كتابته، وأن يكونوا قد وافقوا، جميعاً على النسخة النهائية المقدَّمة. وقد يطلب من المؤلفين إثبات الإسهام الذي قدَّموه. ويمكن إدراج أسماء أخرى إلى عبارات الشكر التي تكون في مقدِّمة الورقة.

٧. ومن أجل تيسير ترجمة الخلاصات وأسماء المؤلفين، على المؤلفين الذين تكون لغتهم الأم تكتب بحروف عربية، ويكتبون مؤلفاتهم بالإنكليزية أو الفرنسية، أن يزودوا رئيسي التحرير بأسمائهم كاملة، مكتوبة بالحروف العربية، ثم بالحروف اللاتينية.

٨. الورقات التي تمثّل تقارير حول نتائج البحوث الجديدة، ينبغي أن تكتب بالترتيب التالي: المقدمة؛ المواد (المواضيع) والطرق؛ النتائج؛ التحليل؛ والمناقشة. وينبغي أن تشفع هذه الورقات بخلاصة لكل منها، لا تزيد على ١٠٠ كلمة، تبيّن بوضوح، وبإيجاز، الأهداف، والسياق، والنتائج، والاستنتاجات.

٩. ينبغي أن يثبت المؤلفون، بحسب ما يلزم، أن جميع الأشخاص الذين أجري عليهم البحث، قد وافقوا موافقة واعية على ذلك، وفي حالة تعذر الحصول على موافقة المشاركين (أحياء أو أموات)، ينبغي أن يثبت المؤلفون أنه قد تم الحصول على موافقة وكلائهم أو ورثتهم.

١٠. ينبغي أن تتناول مقالات الاستعراض والمراجعة الماضية، النقاط التالية: الأهداف، المصادر، طرق الانتقاء، تجميع المعطيات وتفسيرها والاستنتاجات.

١١. ينبغي أن يقتصر الاستشهاد من أي أعمال منشورة، في النص، على المراجع الحديثة الأساسية. ولا ينصح بزيادة المراجع على ٢٥ مرجعاً على الأكثر، باستثناء المقالات النقدية. ويلزم ترقيم المراجع، كلما ظهرت في النص، وأن يليها أعداد عربية بين أقواس [أقواس مربعة]. كما ينبغي تدوين هذه المراجع في قائمة مرقمة، في صفحة منفصلة، في نهاية الورقة، وأن تتضمن المعلومات التالية، إن أمكن: اسم المؤلف أو أسماء المؤلفين، والحروف الأولى من أسمائهم، وعنوان الورقة أو الكتاب في اللغة الأصلية، إضافة إلى ترجمته؛ واسم المجلة بالكامل، مع رقم المجلد، وعدد الصفحات؛ واسم الناشر (التجاري أو المؤسسي)؛ ومكان النشر (المدينة والبلد)؛ وتاريخ النشر. وسوف يتم إعادة الورقات التي تكون فيها المراجع غير كاملة، أو غير مرتبة بحسب هذه المبادئ، إلى المؤلف، لتصحيحها. وفي ما يلي أمثلة للأسلوب الذي تفضل المجلة الصحية لشرق المتوسط أن يتبع:

كتاب:

Al Hamza B, Smith A. *The fifth sign of identity*. Cairo, American University Press, 1990.

مقالة في مجلة:

Jones A et al. One day in Tibet. *Journal Of tautology*, 1993,13(5): 23-7.

وثيقة:

Al-Itneen M, ed. *The principles of uncertainty*. Geneva, World Health Organization, 1985 (document WHO/DOC/537).

١٢. وفي ما يتعلق بالرسومات والجداول، المشفوعة بالشروح الملائمة، فإنه ينبغي أن ترد كل منها في صفحة منفصلة، ومرقمة على التوالي بالأعداد العربية، وملحقة في نهاية الورقة. كما ينبغي الإشارة إلى كل رسم وكل جدول يشار إليه في النص، وتحديد مكانه بوضوح، بحسب ما يلزم، وحبّذا لو أمكن تحديد مصدر كل رسم وكل جدول. وفي حالة نقل أي رسومات أو جداول من مواد أخرى، فإنه تقع على عاتق المؤلف، أو المؤلفين، المسؤولية الكاملة عن الحصول على الأذون اللازمة. وبُغْيَةَ تجنُّب أي مشكلات في طريقة تنسيق المنتج النهائي، فإنه ينبغي الاقتصار على قدر الإمكان في إدراج الجداول والرسومات. وحبّذا لو أمكن الاقتصار على جدول واحد أو رسم واحد لكل ١٠٠٠ كلمة. علماً بأن الرسومات المتعلقة ببعض المعطيات، ينبغي أن تصاحب هذه المعطيات، وأن يتسنّى إعادة رسمها، إذا تطلّب الأمر.

١٣. لا ترد الورقات والقريصات الأصلية، إلا بناءً على طلب من المؤلف الرئيسي.

١٤. بعد النشر، يحصل المؤلفون على نسخة من العدد الذي ترد فيه المقالة، بينما يحصل المؤلف الرئيسي على ٥٠ نسخة من البحث المنشور. وتقدَّم الطلبات للحصول على المزيد من النسخ، أو على معلومات حول الأسعار، إلى رئيس التحرير.

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