Bacterial infections in suspected cutaneous leishmaniasis lesions*

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In Iran, microscopic examination of skin scrapings from 2202 individuals with clinically diagnosed cutaneous leishmaniasis (CL) lesions revealed the presence of amastigotes in 1123 cases (51.0%).

Bacteriological examinations of the lesions indicated that 788 individuals (35.7%) were also infected with one or more pathogenic bacteria, including coagulase-positive staphylococci (27.8%), beta-haemolytic streptococci (10.6%), and other opportunist pathogenic bacteria (total, 2.5%).

The prevalence of bacterial infections in lesions in which leishmania parasites were detected was 26.5%, while for lesions in which no parasite was found the prevalence of such infections was significantly greater (45%).

The results of this study show that bacterial infections should be considered in diagnosing and treating suspected CL lesions, particularly in areas where there is no facility for carrying out bacteriological examinations. Erythromycin can be used to treat the bacterial infections of the purulent sores.

Introduction

In Iran, both zoonotic and anthroponotic cutaneous leishmaniasis (CL) are endemic in different foci (*1*, *2*). The incidence of CL has increased considerably over the last 10 years in the country, mainly among nonimmune military personnel stationed in the nidi of zoonotic CL, located mostly in Khuzestán province, on the southern borders with Iraq.

When the crust that covers the nodules of CL lesions falls off, an ulcer forms that frequently becomes affected with bacterial or fungal infections. Such secondary infections can reduce the number of amastigotes to submicroscopic levels, and may overshadow the original leishmanial infection. In contrast, in the areas where CL is endemic such bacterial or fungal cutaneous infections may be diagnosed on the basis of their clinical features as leishmaniasis, and be treated with drugs that usually produce side-effects.

Since many of the clinically suspected CL lesions in individuals who had been referred to us for parasitological testing were purulent, we carried out bacteriological examinations as well in order to determine the role of bacterial infections in suspected CL lesions.

Materials and methods

Each of the 2202 individuals with clinically suspected CL lesions who were referred to us for microscopic diagnosis from medical centres and private clinics in Iran from 1981 to 1984 were investigated as outlined below.

Sampling procedures

Both the lesion and the surrounding skin areas were thoroughly cleaned using one or two pieces of cotton wool moistened with alcohol. The indurated edge of the sore was then scratched using a flamed sterilized vaccinostyle, and at least two smears for parasitological examination were prepared from the scratchings. Immediately afterwards the scratched area and the centre of the lesion were thoroughly scraped using a sterile swab and the scrapings were cultivated for bacteria by being inoculated on to blood agar medium inside a disposable plastic plate.

From 461 individuals, one or two smears, mainly of scrapings from purulent lesions, were also prepared on slides for direct microscopic examination. Also, from 98 individuals, who were selected more or less randomly, scrapings of uncleaned nor-

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mal skin were collected, usually from symmetrically distributed areas on their bodies. These scrapings were then cultured on blood agar medium.

Parasitological examinations

The smears that were prepared from scratchings were fixed with methanol, stained with Giemsa, and carefully examined for amastigotes using light microscopy (magnification, × 1000). In those instances where no parasite was found in the smears after at least 30-minutes' observation, but some epidemiological or clinical evidence indicated that the lesions were probably CL, the sampling and microscopic examinations were repeated.

Bacteriological examinations

The inocula were spread on the blood agar medium using the streak method (*3*) and incubated at 37 °C for 24 hours. Smears from each of the morphologically different colonies observed on blood agar were then heat-fixed, treated with Gram stain, and examined microscopically. Bacteria were identified from their form, size, reaction to Gram stain, and colony characteristics on the culture medium. If necessary, differential and selective culture media were used (*3*).

Smears that were prepared directly from lesion scrapings were treated with Gram stain and scrutinized for bacteria. In 441 cases, the susceptibility of the pathogenic bacteria to common antibiotics was assessed according to the method described by Baurer et al. (*4*).

Results

Upon microscopic examination of the Giemsa-stained smears taken from suspected CL lesions, amastigotes were found in 1123 (51%) of the 2202 study individuals.

One or more pathogenic bacteria, including coagulase-positive staphylococci, (27.8%), beta-haemolytic streptococci (10.6%), as well as other opportunist pathogenic bacteria such as *Proteus, Pseudomanas* and *Klebsiella* spp., enterococci, and *Escherichia coli* (total, 2.5%), grew on the blood agar medium from samples of lesions from 788 (35.7%) of the cases (Table 1).

One or more pathogenic bacteria grew in the blood agar medium with samples obtained from 302 (26.8%) of 1123 individuals whose CL lesions exhibited amastigotes upon parasitological examination, as well as with samples from 486 (45%) of the 1079 individuals for whom no parasite was found (Table 2). A few Gram-positive cocci were usually observed in 125 (27.1%) of the 461 smears prepared directly from lesions. In two cases, a few Gram-negative cocci were also detected and *Proteus* spp. were isolated on the blood agar medium. Bacteria grew on the blood agar medium from scrapings from 249 (54%) of the above-mentioned 461 individuals.

For the 98 individuals from whom scrapings were taken from suspected CL lesions and from normal skin, cultivation of these samples on blood agar medium indicated that pathogenic bacteria grew in 44 cases (44.9%) from lesion scrapings and in six cases (6.1%) from those from normal skin. In the

Table 1: **Pathogenic bacteria isolated from suspected cutaneous leishmaniasis lesions of 2202 individuals in the study**

Bacteria	No. of lesions with leishmania parasites (L^+)	No. of lesions with no parasite (L^-)	Total
Beta-haemolytic streptococci + CP staphylococci[a]	33 (33.1)[b]	73 (66.9)	106
Beta-haemolytic streptococci	15 (11.9)	111 (88.0)	126
CP staphylococci	228 (45.6)	272 (54.4)	500
Beta-streptococci + *Proteus* spp.	—	1 (100.0)	1
CP staphylococci + *Proteus* spp.	1(33.3)	2 (66.6)	3
Proteus spp.	13 (68.4)	6 (31.6)	19
CP staphylococci + *Pseudomonas* spp.	—	1 (100.0)	1
Pseudomonas spp.	5 (55.5)	4 (44.4)	9
Beta-haemolytic streptococci + *Klebsiella* spp.	—	1 (100.0)	1
CP staphylococci + *Klebsiella* spp.	—	1 (100.0)	1
Klebsiella spp.	2 (33.3)	4 (66.6)	6
Beta-haemolytic streptococci + *Escherichia coli*	1 (100.0)	—	1
CP staphylococci + *E. coli*	2 (66.6)	1 (33.3)	3
E. coli	2 (66.6)	1 (33.3)	3
Enterococci	—	8 (100.0)	8
Total	302 (38.3)	486 (61.6)	788

[a] CP = coagulase positive.
[b] Figures in parentheses are percentages.

Table 2: **Distribution of pathogenic bacteria in suspended cutaneous leishmaniasis lesions in the study individuals**[a]

Age group (years)	No. tested	No. with leishmania parasites	No. of lesions with coagulase-positive staphylococci			No. of lesions with beta-haemolytic streptococci			No. of lesions with any pathogenic bacteria		
			L^+	L^-	Total	L^+	L^-	Total	L^+	L^-	Total
0–10	395	148 (37.4)[b]	33 (22.3)	72 (29.1)	105 (26.5)	6 (4.0)	57 (23.0)	63 (15.9)	37 (25.0)	116 (46.9)	153 (38.7)
11–20	586	283 (48.2)	77 (27.2)	116 (38.2)	193 (32.9)	22 (7.7)	65 (21.4)	87 (14.8)	85 (30.0)	156 (51.4)	241 (41.1)
21–30	800	537 (67.1)	126 (23.4)	86 (32.7)	212 (26.5)	18 (3.3)	43 (16.3)	61 (7.6)	145 (27.0)	119 (45.2)	264 (33.0)
31–40	167	73 (43.7)	12 (16.4)	28 (29.7)	40 (23.9)	2 (2.7)	7 (7.4)	9 (5.3)	14 (19.1)	35 (37.2)	49 (29.3)
41–50	116	47 (40.5)	11 (23.4)	14 (20.2)	25 (21.5)	0 (0.0)	7 (10.4)	7 (6.0)	13 (26.7)	21 (30.4)	34 (29.3)
>50	138	35 (25.3)	5 (14.2)	34 (33.0)	39 (28.2)	1 (2.8)	7 (6.7)	8 (5.8)	8 (22.6)	39 (37.8)	47 (34.0)
Total	2202	1123 (51.0)	264 (23.5)	350 (32.4)	614 (27.8)	49 (4.3)	186 (17.2)	235 (10.6)	302 (26.8)	486 (45.0)	788 (35.7)

[a] L^+ = lesions with leishmania parasites; L^- = lesions with no leishmania parasites.
[b] Figures in parentheses are percentages.

cultures of normal skin scrapings a few colonies of coagulase-positive staphylocci were observed, and higher densities of such organisms were found in the samples taken from the lesions of these individuals.

The number of positive cases and the prevalence of leishmania as well as of coagulase-positive staphylococci, beta-haemolytic streptococci, and other pathogenic bacteria found in the suspected CL lesions, together with the age distribution of the individuals with these infections are shown in Table 2 and in Fig. 1 and 2.

The prevalence of resistance to 14 common antibodies of six of the species of pathogenic bacteria is given in Table 3.

Fig. 1. **Positivity rate for leishmania, coagulase-positive (CP) staphylococci, beta-haemolytic (β-) streptococci, and all pathogenic bacteria isolated from the lesions on 2202 suspected cases of cutaneous leishmaniasis in Iran.**

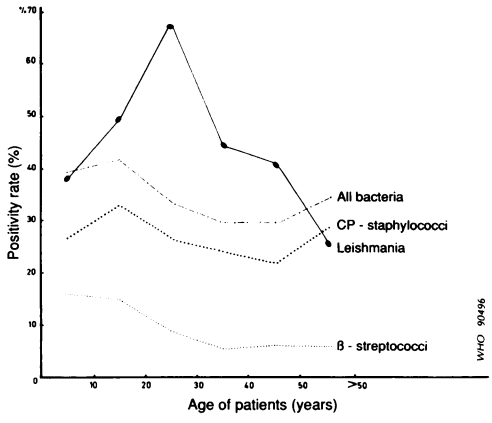
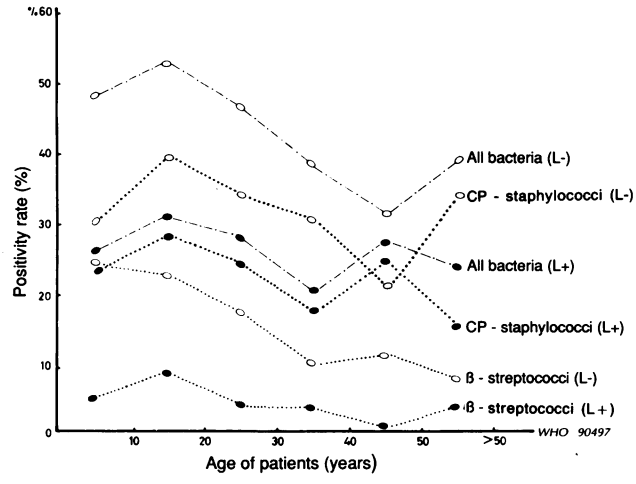


Fig. 2. **Positivity rate for coagulase-positive (CP) staphylococci, beta-haemolytic (β-) streptococci, and all pathogenic bacteria in lesions that were positive (L^+) or negative (L^-) for leishmania parasites on 2202 suspected cases of cutaneous leishmaniasis in Iran.**



Discussion

The results of this study, which is probably the first to investigate the role of bacterial infections in clinically suspected cutaneous leishmaniasis lesions, showed that the difference in the prevalence of bacterial infections in lesions in which amastigotes were detected (26.8%) and in those in which no parasite was found (45.6%) was statistically highly significant ($\chi^2 = 50.6$, $P < 0.001$). Although it is possible in few cases of CL, particularly those involving purulent sores, that amastigotes were not detected microscopically,

Table 3: **Prevalence of resistance to 14 common antibiotics of strains of pathogenic bacteria isolated from suspected cutaneous leishmaniasis lesions in the study**

Antibiotic	CP staphylococci[a]	*Pseudomonas* spp.	*Proteus* spp.	*Escherichia coli*	Enterococci	*Klebsiella* spp.
	No.	No.	No.	No.	No.	No.
Ampicillin	406 (99.7)[b]	— —	7 (100.0)	7 (100.0)	3 (42.8)	3 (100.0)
Sulfamethoxazole/ trimethoprim	7 (1.7)	9 (90.0)	5 (71.4)	1 (14.4)	4 (57.1)	0 (0)
Carbenicillin	— —	1 (10.0)	— —	5 (71.4)	— —	— —
Cefaloridine	5 (1.2)	— —	3 (42.9)	4 (57.1)	0 (0)	2 (66.6)
Chloramphenicol	16 (3.9)	10 (100.0)	0 (0)	1 (14.4)	2 (28.6)	0 (0)
Cloxacillin	0 0	— —	— —	— —	— —	— —
Colistin	— —	6 (60.0)	5 (71.4)	— —	— —	2 (66.6)
Erythromycin	13 (3.1)	10 (100.0)	— —	6 (85.7)	2 (28.6)	3 (100.0)
Gentamicin	— —	6 (60.0)	— —	0 (0)	— —	0 (0)
Kanamycin	— —	7 (70.0)	3 (42.9)	— —	— —	0 (0)
Novobiocin	4 (0.9)	— —	— —	— —	— —	— —
Penicillin	406 (99.7)	10 (100.0)	— —	7 (100.0)	7 (100.0)	— —
Polymyxin B	— —	5 (50.0)	2 (28.6)	2 (28.6)	— —	0 (0)
Tetracycline	319 (78.3)	10 (100)	4 (57.1)	3 (42.8)	5 (71.4)	— —
No. of lesions tested	407	10	7	7	7	3

[a] CP = coagulase positive.
[b] Figures in parentheses are percentages.

such large differences indicate that in endemic areas a large proportion of bacterial wounds are clinically diagnosed as CL.

As reported by Zaini in 1984, fungal infections such as sporotrichosis have been diagnosed as CL and treated unsuccessfully with meglumine antimonate in Iran (*5*).

Among the bacterial infections found, staphylococcal were the most prevalent among all age groups, with significantly higher rates for lesions in which no parasites were found (Fig. 1 and 2). From about 6% of subjects with suspected CL lesions, coagulase-positive staphylococci were isolated upon cultivation of scrapings of normal skin. This might have originated from infection of normal skin with staphylococci from the lesions.

Beta-haemolytic streptococcal infections were also frequently observed in suspected CL lesions, particularly among individuals from the younger age groups whose lesions did not exhibit parasites. (Fig. 1 and 2).

These findings indicate that lesions with streptococcal infections have been more frequently misdiagnosed clinically as cutaneous leishmaniasis. Since beta-haemolytic streptococcal infections are medically important especially in children, they have to be considered in diagnosing and treating suspected CL lesions in endemic areas.

The maximum prevalence of cutaneous leishmaniasis (67.1%) occurred among 21–30-year-olds, who usually had been on military missions in the south-west of the country, particularly in Khuzestán province.

Although we employed only aerobic cultivation methods in the study, microscopic examinations of Gram-stained smears from purulent lesions did not reveal any important anaerobic bacteria, and it therefore does not appear that such bacteria were prevalent to any great extent in the suspected CL lesions.

In general, the results indicate that aerobic bacteria, principally coagulase-positive staphylococci and beta-haemolytic streptococci are important pathogenic bacteria in suspected CL lesions in Iran. This should therefore be borne in mind in diagnosing and treating such lesions. In areas where there is no facility for carrying out bacteriological examinations, erythromycin is a suitable drug for the treatment of such bacterial infections of purulent suspected cutaneous leishmaniasis lesions.

Résumé

Infections bactériennes dans des lésions de leishmaniose cutanée possibles

En Iran, l'examen microscopique de raclages cutanés provenant de 2202 individus pour lesquels avait été posé le diagnostic clinique de leishmaniose cutanée (LC), a montré la présence d'amastigotes dans 1123 cas (51%).

Les examens bactériologiques de ces lésions ont montré que 788 individus (35,7%) étaient également infectés par une ou plusieurs bactéries pathogènes, y compris des staphylocoques à coagulase positive (27,8%), des streptocoques bêta-hémolytiques (10,6%), et d'autres bactéries pathogènes opportunistes (total, 2,5%).

La prévalence des infections bactériennes dans les lésions où des leishmanies avaient été décelées était de 26,5%, alors que pour les lésions dans lesquelles aucun parasite n'avait été trouvé, la prévalence de ces infections était nettement plus élevée (45%).

Les résultats de cette étude montrent que des infections bactériennes peuvent être envisagées dans le diagnostic et le traitement de lésions de LC possibles, particulièrement dans les régions où il n'existe pas de moyens permettant d'effectuer des examens bactériologiques. L'érythromycine peut être utilisée pour traiter les infections bactériennes des lésions cutanées purulentes.

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