

Follow-up of pregnant women with active cytomegalovirus infection

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متابعة الحوامل المصابات بعدوى نشيطة بالفيروس المضخم للخلايا

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خلاصة: تمت متابعة ستين حاملاً توجد أو لا توجد بهن بينات مصلية على الإصابة بعدوى نشيطة بالفيروس المضخم للخلايا. واستمرت المتابعة إلى أن وضعن أحاملهن، من أجل اكتشاف معدل ورمح العدوى الخلقية الظاهرة بهذا الفيروس بين المواليد في الموصل بالعراق، وأنماط الإصابة. فتم تشخيص العدوى عن طريق اكتشاف الغلوبولين المناعي الميمي IgM للفيروس باستعمال اختبار إليزا. ولقد اكتشف هذا الغلوبولين المناعي في عينات من دم الحبل السري لستة (10%) من الأطفال ذوي المرض الظاهر (من لديهم تشوهات خلقية مختلفة) والمولودين لأمهات مصابات بالعدوى النشيطة بالفيروس. واكتشفت شذوذات في الجهاز العصبي في هذه الحالات الست جميعها (حالتان مصابتان بصغر الرأس وأربعة بها استسقاء في الرأس). وينبغي الاشتباه في وجود عدوى خلقية بهذا الفيروس، في الأطفال الذين يولدون بتشوهات خلقية، ولاسيما تشوهات الجهاز العصبي المركزي. وجددير بالذكر أننا اكتشفنا في دراستنا هذه عددا كبيرا من حالات استسقاء الرأس.

ABSTRACT Pregnant women (60) with and without serological evidence of active cytomegalovirus (CMV) infection were followed until delivery to detect the incidence and types of overt congenital CMV infection in neonates in Mosul, Iraq. Infection was diagnosed by the detection of CMV-IgM, using ELISA. CMV-IgM was detected in cord blood samples of six (10%) overtly sick infants (with different congenital malformations) born to mothers with active CMV infection. Central nervous system abnormalities were detected in all six cases (two with microcephaly and four with hydrocephaly). Congenital CMV infection should be suspected in infants born with congenital malformations, especially those of the central nervous system. The detection of a significant number of hydrocephalus cases in our study is notable.

Suivi des femmes enceintes atteintes d'une infection à cytomegalovirus évolutive

RESUME Des femmes enceintes (60) avec ou sans preuve sérologique d'infection évolutive par le cytomegalovirus (CMV) ont été suivies jusqu'à l'accouchement pour déceler l'incidence et les types d'infection congénitale à cytomegalovirus patente chez des nouveau-nés à Mossoul (Iraq). L'infection a été diagnostiquée par la recherche d'IgM anti-CMV en utilisant la méthode ELISA. L'IgM anti-CMV a été détectée dans les prélèvements de sang du cordon ombilical de six (10%) nourrissons manifestement malades (avec différentes malformations congénitales) nés de mères atteintes d'une infection à cytomegalovirus évolutive. Des anomalies du système nerveux ont été détectées chez les six cas (deux présentaient une microcéphalie et quatre une hydrocéphalie). L'infection congénitale à cytomegalovirus devrait être suspectée chez les nourrissons nés avec des malformations congénitales, en particulier celles du système nerveux central. La détection d'un nombre considérable de cas d'hydrocéphalie dans notre étude est notable.

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Introduction

Cytomegalovirus (CMV) can cross the placenta and cause both fetal and placental infections. Most congenital infections are asymptomatic; only 10% of fetuses infected *in utero* will develop clinical signs of CMV infection. Studies have shown that infants congenitally infected with CMV as a result of primary infection of their mothers are more likely to have overt sequelae than those infected from reactivated infections of the mothers [1,2].

Transmission of CMV infection to the fetus has been identified in all trimesters of pregnancy. Abortion can result from ascending CMV endometritis and the virus has been isolated from post-abortion uterine discharge [3]. The usual manifestations of overt CMV infection at birth are hepatosplenomegaly, jaundice, thrombocytopenia and various congenital malformations, especially those involving the central nervous system [4,5].

In Iraq, studies have revealed that the majority of women of childbearing age are seropositive for CMV and that they contract the infection either through prenatal or postnatal transmission or during early childhood [6,7]. Sexual transmission and blood transfusion are other sources of infection. Primary CMV infection has been found to be more prevalent in pregnant women than in non-pregnant women. This difference may be attributed to the susceptibility of seronegative women, at the onset of pregnancy, to their first CMV infection [1,2]. CMV infection is endemic in Iraq; the prevalence rates of cytomegalovirus IgM and IgG antibodies in non-pregnant women have been reported to be 1% and 84% respectively, and 2.5% and 90% in pregnant women [6].

Our study was designed to follow up a number of pregnant women with serological evidence of active CMV infection until

delivery in order to detect the incidence and types of symptomatic congenital CMV infection among the neonates.

We were unable to isolate CMV from the urine and from the placental tissues and post-abortion uterine discharge due to a lack of facilities for viral isolation. Instead, active CMV infection was diagnosed using an enzyme-linked immunosorbent assay (ELISA) to detect the presence of specific CMV-IgM markers in the blood of the pregnant women and in the cord blood of the infants.

Subjects and methods

Sixty (60) pregnant women with serological evidence of active CMV infection and 50 pregnant women without such evidence were enrolled in the study. The cases and controls were chosen during a large-scale study of the prevalence of CMV infection among women of childbearing age (pregnant and non-pregnant) who regularly attended antenatal and infertility care units in Mosul [6]. Information on age, parity, residence, telephone number and socioeconomic status was recorded using a questionnaire. Both groups were followed up until delivery through the antenatal and infertility care units and through personal communication. The outcome of the pregnancies were reported (abortion, stillbirth, normal delivery and delivery with congenital malformations). Neonates with severe symptomatic anomalies were admitted to neonatal care units and cord blood samples were taken to test for the presence of CMV-IgM antibodies. The women of both groups were nondiabetic and of moderate socioeconomic status. The age and parity of the women and the gestational age at which CMV infection was detected are shown in Table 1.

The ELISA technique was performed using kits intended for estimating concentration of specific CMV-IgM markers. The kits were purchased from Sigma Diagnostics (USA), the technique was performed according to the manufacturer's instructions and each sample was tested in duplicate. The kits were designed to eliminate errors introduced by the rheumatoid factor (absorption with heat-aggregated human IgG). Using the microplate reader, the concentration of CMV-IgM antibodies in a sample was calculated as follows:

$$\text{CMV-IgM concentration as \% of calibrator} = \frac{\text{sample absorbance}}{\text{calibrator absorbance}} \times 100$$

A CMV-IgM percentage of 40 or more is indicative of active infection. Detection of the specific CMV-IgM marker is a reliable method and is likely to be positive in infected infants with viruria and helpful in the diagnosis of congenital infection in 95% of symptomatic neonates [8,9].

Results

The majority of pregnant women with active CMV infection delivered apparently

normal infants. The exceptions were two cases of abortion, one case of stillbirth and six cases of severely sick infants born with symptomatic manifestations of congenital CMV infection. The clinical and serological findings of the women whose infants were infected are listed in Table 2. CMV-IgM marker was detected in cord blood samples of the infected neonates. The pregnant women without active CMV infection delivered normal infants except in two cases of abortion and one case of stillbirth (of unknown causes).

The prevalence of overt congenital CMV infection in our study was 10% (6 out of 60). Central nervous system anomalies were observed in all 6 cases, and hydrocephalus was detected in 4 of the cases. The age and parity of the women and the gestational age at which CMV infection was detected were found to have no significant correlation with the clinical findings of the infected infants.

Discussion and conclusions

The most important clinical effects of congenital CMV infection are on the central nervous system. Asymptomatic infections

Table 1 Comparison of age, parity and the gestational age at which cytomegalovirus (CMV) infection was detected in the pregnant women

| Variable | Pregnant women with active CMV infection | Pregnant women without active CMV infection |
|--|--|---|
| Mean age \pm s (years) | 30.2 \pm 6.4 | 30.6 \pm 5.6* |
| Average parity | 3.2 | 3.3 |
| Gestational age at which active CMV detected (weeks) | | |
| <12 | 14 | |
| 12-24 | 22 | |
| >24 | 24 | |

*Unpaired t-test not significant

s - standard deviation

Table 2 Clinical and serological findings of women with active cytomegalovirus (CMV) infection and their infected infants

| Mother | | | | Infant | | | |
|-------------|--------|--|----------------------------------|-------------------|-------------------|--|----------------------------------|
| Age (years) | Parity | Gestational age CMV-IgM detected (weeks) | CMV-IgM ELISA* (% of calibrator) | Gestation (weeks) | Birth weight (kg) | Clinical findings | CMV-IgM ELISA* (% of calibrator) |
| 25 | 2 | 6 | 76 | 38 | 2.6 | Microcephaly, meningocele, hepatosplenomegaly, petechiae | 70 |
| 20 | 0 | 22 | 66 | 40 | 3.6 | Hydrocephalus, thrombocytopenia, jaundice | 80 |
| 28 | 4 | 28 | 58 | 36 | 2.7 | Hydrocephalus, hepatosplenomegaly, jaundice, petechiae | 66 |
| 30 | 1 | 30 | 74 | 42 | 3.1 | Hydrocephalus, hepatosplenomegaly, dextrocardia | 85 |
| 32 | 3 | 10 | 60 | 26 | 1.2 | Microcephaly, respiratory distress, thrombocytopenia, jaundice | 65 |
| 24 | 0 | 24 | 68 | 29 | 1.6 | Hydrocephalus, respiratory distress, hypothermia | 74 |
| 36 | 3 | 18 | 74 | 22 | 1.2 | Maceration (stillborn) | ♯ |

* % of calibrator of ≥ 40 was indicative of active infection.

♯ Blood sample was not available.

at birth are the major cause of central nervous system anomalies, which carry a high incidence of intellectual impairment. Microcephaly is found in many severely affected symptomatic infants [10,11]. Retrospective studies have shown a higher incidence of CMV antibodies and CMV excretion in microcephalic children than in normal children or children with other neurological defects [12]. Ventricular dilatation is a common finding in the brains of symptomatic infants and is often associated with microcephaly. Head enlargement due to hy-

drocephalus has been less commonly reported than microcephaly [12,13].

The incidence of the symptomatic sequelae of congenital CMV infection found in our study (10%) and the observed clinical manifestations are similar to those reported in other studies [1,2,4,5]. The infants in our study, however, showed poor prognosis, a high mortality rate and major neurological handicaps. Results from our study and from a previous study performed in Iraq (Al-Ali HYM, Yaseen SA, Al-Rawi S, unpublished data, 1995) indicate that the central nervous

system is affected in all cases of symptomatic congenital CMV infection and that hydrocephalus is present in a significant number of infected infants born with congenital anomalies.

Both live attenuated (Towne strain) and CMV subunit vaccines have been evaluated, although the use of these vaccines remains controversial because of safety concerns. Several prophylactic measures are useful for the prevention of CMV infection in patients at high risk. Improvements in standards of living and hygiene may decrease the incidence of infection and the use of blood from seronegative donors decreases the rate of transfusion-associated transmission. Also, matching organs or bone marrow transplants by CMV serology reduces rates of primary infection following transplantation. The isolation of neonates with generalized cytomegalic inclusion disease from other neonates is advisable. Three drugs, ganciclovir, foscarnet sodium and cidofovir, are currently licensed for systemic treatment of CMV infection but have had promising results only in some life-threatening patients [14-16]. These antiviral drugs are untried in congenital infection, and the use of ganciclovir is not recommended except in very ill babies because of the risk of gonadal toxicity.

In conclusion, CMV can cause a wide and varying pattern of neonatal infections,

and it is reasonable for obstetricians and paediatricians to consider CMV infection in dealing with infants born with congenital anomalies, especially those of the central nervous system such as microcephaly and hydrocephalus. Such infants and their mothers should be screened for evidence of active CMV infection.

Universal screening of pregnant women for CMV infection during an early prenatal visit is not yet recommended worldwide. With the absence of a safe vaccine, seronegative women at the onset of pregnancy should be advised to practise careful hygiene and to minimize contact with carriers and other sources of infection in order to decrease the chances of infection. Since no treatment is available for pregnant women with active primary CMV infection, and the effect of the infection on pregnancy is unpredictable, regular follow-up of these women is advised. Data are insufficient to recommend therapeutic abortion if fetal infection is discovered in early pregnancy, and 90% of infected babies are asymptomatic at birth [17].

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