

Possible role of TORCH agents in congenital malformations in Gorgan, northern Islamic Republic of Iran

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الدور المحتمل لعوامل المقوّسات القنّدية والحصبة الألمانية والفيروس المضعمة للخلايا في التشوهات الخلقية في جرجان، شمال جمهورية إيران الإسلامية
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الخلاصة: أجرى الباحثون هذه الدراسة الوصفية المستعرضة لاستقصاء تكرار العدوى بعوامل المقوّسات القنّدية والحصبة الألمانية والفيروس المضعمة للخلايا، لدى الولدان المصابين بتشوهات خلّقية، في مركز للإحالة في مدينة جرجان في جمهورية إيران الإسلامية. وقد أخذ الباحثون عيّينات الدم من 64 وليداً ومن أمهاتهم خلال مدة 20 شهراً في عاميّ 2003 و2004. وقد أظهرت الاختبارات المصلية أن أربعة من أصل 64 رضيعاً ولدوا بتشوهات خلّقية (6%)، لديهم عبارات لأضداد إيجابية للغلوبولينات المناعية IgM للمقوّسات القنّدية (حالتان)، ولفيروس الحصبة الألمانية (حالة واحدة) وللفيروس المضعمة للخلايا (حالة واحدة). وقد كان الغلوبولين المناعي IgM إيجابياً لدى 9 أمهات من أصل 63 أما (14%)، وكانت الإيجابية فيهن للمقوّسات القنّدية (3 حالات) ولفيروس الحصبة الألمانية (3 حالات) وللفيروس المضعمة للخلايا (3 حالات). ولم يلاحظ الباحثون لدى أي حالة من الحالات فيروسه الهربس البسيط من النمط الثاني ولا اللولبيات الشاحبة.

ABSTRACT This descriptive, cross-sectional study was carried out to explore the frequency of contamination with TORCH agents in neonates with congenital malformations in a referral centre in Gorgan city, Islamic Republic of Iran. Blood samples were taken from 64 neonates and their mothers over a 20-month period in 2003–04. Serologic tests showed that 4/64 infants born with congenital malformations (6%) had positive IgM antibody titres for *Toxoplasma gondii* (2 cases), rubella virus (1 case) and cytomegalovirus (1 case). IgM was positive in 9/63 mothers (14%), also for *T. gondii* (3 cases), rubella virus (3 cases) and cytomegalovirus (3 cases). No cases of herpes simplex virus type II or *Treponema pallidum* were found.

Rôle possible des agents « TORCH » dans les malformations congénitales à Gorgan (nord de la République islamique d'Iran)

RÉSUMÉ Cette étude descriptive transversale a été réalisée afin d'examiner la fréquence de la contamination par les agents TORCH chez les nouveau-nés présentant des malformations congénitales dans un centre spécialisé de Gorgan (République islamique d'Iran). Des prélèvements sanguins ont été effectués sur 64 nouveau-nés et leur mère pendant une période de 20 mois en 2003-2004. Les tests sérologiques ont montré que quatre nourrissons sur 64 nés avec des malformations congénitales (6 %) avaient des taux positifs d'anticorps de type IgM dirigés contre *Toxoplasma gondii* (2 cas), contre le virus de la rubéole (un cas) et contre le cytomégalovirus (un cas). Chez neuf mères sur 63 (14 %), les IgM étaient positifs à *T. gondii* (trois cas), au virus de la rubéole (trois cas) et au cytomégalovirus (trois cas). Aucun cas de virus de l'herpès de type 2 ou de *Treponema pallidum* n'a été détecté.

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Introduction

One of the important goals in medical research is determining the factors that can affect the health of mothers and their fetuses/neonates. Congenital malformations are important causes of infant morbidity, mortality and disability, and are found in approximately 2%–3% of neonates [1]. Congenital malformations have a direct impact on the family, and place a financial burden on medical, educational and support services. Treatment and rehabilitation of children with such malformations is also expensive, and complete recovery is usually impossible [2–5].

The causes of congenital malformations are varied and few studies have evaluated their etiology in neonates [4]. Previous studies have indicated that the prevalence of congenital malformation was 10.00 per 1000 in the north of the Islamic Republic of Iran [5] and the prevalence of neural tube defect was 3.12 per 1000 in this area [6].

Malformations can be divided into broad categories, one being malformations attributed to discrete environmental factors [1, 7]. Infectious agents, as environmental factors, can create intrauterine infections leading to birth defects, abortion and stillbirth [8, 9].

The TORCH agents—toxoplasmosis, other agents, rubella virus, cytomegalovirus (CMV) and herpes simplex virus (HSV)—are the most important infectious agents that can cause congenital malformations. The infections in women are usually asymptomatic and chronic. Prevalence varies from one geographical area to another [10]. The social and reproductive issues—pregnancy wastage, cost of treatment and morbidity to the infant—make TORCH agents a major cause of concern. Many sensitive and specific tests are available for serological diagnosis of these agents. Complex ELISA tests for IgM antibodies against these infections are highly sensitive and specific [11].

There are a few reports about the role of infectious agents in birth defects [10–13] and there are different recommendations about screening with serology tests during pregnancy. This study was done to determine the frequency of contamination with infectious agents in neonates with congenital malformations born in a referral centre in Gorgan city (northern Islamic Republic of Iran) and their mothers.

Methods

This cross-sectional study was done on all neonates born with major congenital malformations during a 20-month period in Dezyani hospital, a referral gynaecology centre in Gorgan, in the north of the Islamic Republic of Iran during 2003–04.

All neonates delivered alive in this hospital during the study period were screened for congenital malformations including neural tube defects, cleft lip and palate and limb defects. The charts of neonates with birth defects were subsequently extracted for detailed study. Variables recorded included: date of birth, sex and type of malformation.

Blood samples were taken from the affected neonates and their mothers after obtaining permission from the parents and the hospital directors. Then a questionnaire on demographic characteristics, including age and parents' residence, was completed by interview for each case. Ethical approval was obtained from the Health Research Ethics Review Board at the University of Golestan, Gorgan.

Serum was prepared from the samples and serologic tests were done to determine IgM and IgG levels against rubella virus, *T. gondii*, CMV and HSV type II with ELISA tests (StarFax 303 ELISA reader and kits, Radiom Company). The cut-off values used were as follows: rubella IgG positive > 20 IU/mL, rubella IgM positive > 10 IU/mL;

T. gondii IgG and IgM positive > 50 IU/mL; CMV IgG positive > 1.1, CMV IgM positive > 15 IU/mL; HSV IgG positive > 1.1 IU/mL, HSV IgM positive > 1.1 IU/mL

For *Treponema pallidum* the rapid plasma reagin (RPR) test was used (Omega Co., Scotland). Flocculation of samples was considered a positive result.

Results

This study included 64 neonates (46% males) and 63 mothers (1 mother delivered twins). Mean and standard deviation (SD) of maternal and paternal ages were 26.4 (SD 5.0) and 29.6 (SD 5.0) years respectively.

The frequency of IgM and IgG antibodies against TORCH agents in the sera of the neonates and mothers is shown in Tables 1 and 2. Of the 64 neonates with birth defects, 4 (6%) were IgM positive against TORCH agents: 1 for rubella virus, 2 for *T. gondii* and 1 for CMV.

Of the 63 mothers, 9 (14%) had positive IgM antibody titres for TORCH agents: 3 for *T. gondii*, 3 for rubella virus and 3 for CMV.

IgM against rubella virus was observed in 1 neonate and 3 mothers.

Tables 1 and 2 show that 62.5% of neonates with congenital malformations and 58.7% of mothers were positive for CMV IgG antibody. IgG antibody against *T. gondii* was positive in 38.1% of mothers and 35.9% of neonates, but 3.2% of neonates and 4.8% of mothers had positive IgM antibody for *T. gondii*. IgG and IgM against HSV type II in neonates and mothers were negative in all cases. The RPR test for *Treponema pallidum* was also negative in all mothers.

Table 3 shows the distribution of positive infections according to type of defect. Of the 4 neonates with major malformations who were positive for TORCH agents, 2 had neural tube defects, 1 had cleft plate and 1 had limb defects.

Discussion

In this study, positive IgM antibody against TORCH agents was found in 6% of neonates with congenital malformations. This is lower than in a study in Tabriz in the north-

Table 1 Distribution and percentage of IgM and IgG antibody TORCH agents in serum samples of neonates with major congenital malformations (n = 64)

Test	Positive		Borderline		Negative	
	No.	%	No.	%	No.	%
<i>Herpes simplex virus</i>						
IgG	0	0.0	0	0.0	64	100.0
IgM	0	0.0	0	0.0	64	100.0
<i>Rubella virus</i>						
IgG	51	79.7	8	12.5	5	7.8
IgM	1	1.6	0	0.0	63	98.4
<i>Cytomegalovirus</i>						
IgG	40	62.5	5	7.8	19	29.7
IgM	1	1.6	0	0.0	63	98.4
<i>Toxoplasma gondii</i>						
IgG	23	35.9	3	4.7	38	59.4
IgM	2	3.2	0	0.0	62	96.9

Table 2 Distribution and percentage of IgM and IgG antibody TORCH agents in serum samples of the mothers of neonates with major congenital malformations (n = 63)

Test	Positive		Borderline		Negative	
	No.	%	No.	%	No.	%
<i>Herpes simplex virus</i>						
IgG	0	0.0	0	0.0	63	100.0
IgM	0	0.0	0	0.0	63	100.0
<i>Rubella virus</i>						
IgG	53	84.1	4	6.3	6	9.5
IgM	3	4.8	5	7.9	55	87.3
<i>Cytomegalovirus</i>						
IgG	37	58.7	7	11.1	19	30.2
IgM	3	4.8	7	11.1	53	84.1
<i>Toxoplasma gondii</i>						
IgG	24	38.1	5	7.9	34	54.0
IgM	3	4.8	4	6.3	56	88.9
<i>Treponema pallidum</i>						
IgG	0	0.0	0	0.0	63	100.0
IgM	0	0.0	0	0.0	63	100.0

west of the Islamic Republic of Iran with 32.5% [12]. This difference may be due to a higher prevalence of TORCH agents in pregnant mothers in Tabriz or to differences in sampling and study design.

Our findings showed that 41.3% of mothers were negative or borderline for antibodies against CMV which means that they are susceptible to CMV infection during pregnancy. Intrauterine CMV infection and prenatal CMV infection occur in 9.5%–22% of neonates [14]. CMV infection in pregnancy continues to be the leading problem

as most primary maternal infections are missed due to the paucity of distinct clinical findings. Furthermore, it seems that there is an increasing role of reinfection with CMV (that also usually passes unnoticed) as an important etiologic factor in congenital CMV [15].

In this study, 62.5% of neonates had CMV IgG antibodies. Previous studies showed that fetal infection occurs in 5% of pregnancies if primary CMV infection exists and 10%–15% of these infected fetuses will be delivered with important congenital

Table 3 Distribution of type of malformations and IgM antibody in neonates and mothers

Defect	IgM-positive neonates				IgM-positive mothers				Total no. of anomalies
	Toxo	Rubella virus	CMV	No.	Toxo	Rubella virus	CMV	No.	
Neural tube defect	+	–	+	2	3+	2+	2+	7	32
Cleft palate	–	+	–	1	–	+	+	2	14
Limb anomaly	+	–	–	1	–	–	–	0	6

Toxo = *Toxoplasma gondii*; CMV = *cytomegalovirus*.

malformations [16,17]. Blackburn et al. reported CMV IgG antibody in up to 96% of pregnant women [18] but in our study 58.7% of the mothers tested positive.

In a study by Ebrahimpour et al. in Tabriz, 3.08% of neonates with congenital malformations were positive for CMV IgM antibody [12], but in the present study just 1 case (1.6%) with congenital malformations was positive, perhaps due to a higher frequency of CMV infections in Tabriz. All mothers in the Tabriz study were positive for CMV IgG antibody, but in our study less than 60% of mothers tested positive for CMV IgG antibody.

In a study in a Turkish hospital on 128 pregnant women, 87.5% were IgG positive for CMV [19]. As Chiodo et al. reported, it is very hard to discriminate between primary infection and recurrent infection in pregnancy [20]. CMV infection may occur in the first trimester and cause congenital malformations, but at the time of delivery, due to the switching of IgM to IgG, it is difficult to determine the relationship between congenital malformations and CMV infection.

Sever et al. established a matched case-control study to evaluate the relationship between congenital malformations and serologic evidence of TORCH in mothers. However, they could not find any significant association [21].

In a study in the United States of America, all neonates with intrauterine growth disorder had CMV IgM negative results [22]. It was concluded that serologic screening for TORCH agents is not cost-effective. In the present study, 38.1% of mothers and 35.9% of neonates tested positive for IgG antibody against *T. gondii*, but 3.2% of neonates with congenital malformations and 4.8% of mothers tested positive for IgM antibody for *T. gondii*. Toxoplasmosis can be transmitted to the fetus up to 6 months after primary infection. The transmission

rate is higher in the 3rd trimester, which can explain the higher rate of *T. gondii* IgM in neonates in the present study.

Ebrahimpour et al. in Tabriz reported anti-*Toxoplasma* IgG in 100% of mothers and neonates [12]. This difference from our study is likely due to geographical differences in the prevalence of toxoplasmosis. Kaur et al. in India reported positive IgG for *T. gondii* in 11.6% of mothers and recommended that screening tests for TORCH include toxoplasmosis [23]. However, an Irish study detected very few positive tests in screened samples and considered screening tests were not feasible [24].

Cao et al. studied TORCH serology in 2 groups, healthy mothers and mothers with abnormal conception, and reported *Toxoplasma* antibody in 16.6% and 11.1% respectively. They considered TORCH screening feasible [25].

No positive results for HSV type II were seen in our study compared with Ebrahimpour's team in Tabriz, who showed positivity for HSV type I and II IgG antibodies in all (40 cases) of mothers [12]. This difference could be due to different laboratory methods and social differences. Also, Kaur et al. reported a prevalence of 7% for HSV type II antibody IgG in India [23]. These differences need further investigation.

Conclusion

In this study IgM antibody against toxoplasmosis, rubella virus and CMV was positive in 14% of mothers and 6% of neonates. Screening tests for TORCH agents in pregnant mothers without a history of abnormal infants are not feasible, but in pregnant mothers with a history of abnormal conception or babies, serologic testing of both mothers and neonates could be useful for detecting malformations.

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