

Seroprevalence of Toxoplasma Gondii Among Pregnant Women in Makkah, Saudi Arabia

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نسبة الإصابة بطفيل التوكسوبلازما عند النساء الحوامل في مدينة مكة المكرمة باستخدام الاختبارات المصلية

تهدف هذه الدراسة إلى تقييم نسبة الإصابة بطفيل التوكسوبلازما بين النساء الحوامل بمكة المكرمة وكذا دراسة العوامل البيئية المحيطة وكذلك العادات الشخصية لهؤلاء النسوة والتي قد تساهم في انتشار المرض. تم سحب عينات دم من إجمالي 197 من ماسنلا الحوامل في المرحلة العمرية من 17-45 عاماً واللاتي يترددن على مستشفى النساء بمكة المكرمة لمتابعة الحمل. وقد تم إخضاع كل العينات لاختبار الإليزا للكشف عن الأجسام المضادة لطفيل التوكسوبلازما من النوعين "ج" و "م". وقد تبيّن أن نسبة الإصابة الإحصائية اللازمة لتحليل هذه النتائج وبيان مدى ارتباطها بالمعلومات البيئية والعادات الصحية لاؤها والتي سيقف من خلال نموذج للإستبيان تم إستيفائه من النسوة المشاركات في الدراسة.

وقد أظهرت النتائج أن النسبة المئوية لوجود الأجسام المضادة من النوع "ج"، والتي تدل على الإصابة بالإصابة بطفيل التوكسوبلازما، بين عينة البحث تبلغ 29,4% (58 سيدة من إجمالي 197)، أما نسبة تواجد الأجسام المضادة "م"، والتي تعبر عن الحالة الحادة للمرض، لا تتعدى 5,6%. أما عن نتائج الدراسة أن نسبة تواجد الأجسام المضادة "ج" و "م" كانت الأعلى بين المشاركات من المرحلة العمرية بشكل عام (35-45 عاماً) حيث كانت 42,9% ، 11,4% على التوالي. كذلك لم تكن هناك أي علاقة إحصائية معاد بين نسبة الإصابة بطفيل التوكسوبلازما بين عينة البحث وأي من المتغيرات البيئية التي تم فحصها في نتائجها.

تدل نتائج هذه الدراسة على أن هناك نسبة لا بأس بها من الإصابة بطفيل التوكسوبلازما بين النساء الحوامل بمكة المكرمة وهو ما يدعم الاعتقاد بأن النساء بالمملكة أصبحن معرضات للإصابة بهذا الطفيل ،

مما يحث على البدء في برامج للتثقيف الصحي للنساء الحوامل كملء مخططاً لتباعد الإصابات لتقليلها إلى الحد الأدنى.
التوكسوبلازما أثناء فترة الحمل والتي قد تؤدي إلى مضاعفات وخيمة على الجنين .

Abstract

Toxoplasmosis is a disease caused by the protozoan parasite *Toxoplasma gondii*. Congenital transmission may occur when a pregnant mother acquires *T. gondii* infection for the first time in her life during pregnancy. Detection of anti-*Toxoplasma* immunoglobulin M (IgM) and IgG is essential for the diagnosis of *Toxoplasma* infection in pregnant women. The current study is one of the prime investigations to evaluate the prevalence rate of *T. gondii* among pregnant women in the holy city of Makkah Al-Mukkarmah and to consider some of the environmental and personal factors that may contribute to infection. Serum samples of 197 pregnant women aged 17 to 45 years attending the Maternity hospital in Makkah were tested for anti-*Toxoplasma* IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA). Serological results, reflecting *T. gondii* prevalence rate, were statistically analyzed and linked to epidemiological data collected through a standard questionnaire. The seroprevalence of anti-*Toxoplasma* IgG was 29.4% (58 out of 197), whereas IgM seropositivity was 5.6%. The highest IgG and IgM seroprevalence were among participants aged 35 to 43 years (48.8% and 12% respectively). No statistically significant relation was observed between *T. gondii* seroprevalence and the other variable factors studied. The current study indicates that there is a considerable rate of *Toxoplasma* infection among pregnant women in Makkah and support the concern that Saudi women may be vulnerable to that infection. Moreover, it shows the need to provide health education to pregnant women in order to prevent primary infection during pregnancy.

Keywords: *Toxoplasma gondii*, Pregnant women, Makkah, Saudi Arabia, Prevalence of toxoplasmosis, ELISA, Serology, IgG antibodies, IgM antibodies, and Congenital toxoplasmosis

INTRODUCTION

Toxoplasma gondii is an obligatory intracellular protozoan parasite which appears to have broad host specificity. Cats and wild Felines are the only definitive host while all other worm-blooded animals including humans are intermediate hosts (Remington *et al.*, 2001). Infection is acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate food or water (Montoya & Liesenfeld, 2004). Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy (Remington *et al.*, 2001). Even though, pregnant women are often asymptomatic or have only mild symptoms, infection may cause spontaneous abortion, still birth, or serious foetal damage. The gestational age

at which the infection is contracted is a key variable affecting the clinical foetal outcome (Ades & Nokes, 1993; Lopez *et al.*, 2000; Martin, 2001). While the prevalence rates of *T. gondii* were up to 50-80% in Central and South American as well as some European populations, primary infection with *T. gondii* in pregnant women occurs all over the world with frequencies between 0.1-1% (Stray-Pedersen, 1993).

Serological testing for anti-*Toxoplasma* antibodies is the mainstay for the diagnosis of toxoplasmosis. Diagnosis of acute maternal infection is mainly based on detection of seroconversion or fourfold rise in IgM antibodies level which appear sooner after infection than IgG antibodies and disappear faster than IgG after recovery (Remington *et al.*, 2001).

The risk factors that are often associated with acute infection in pregnant women were eating raw or undercooked meat and soil contact. Weaker associations were observed for tasting raw meat during preparation of meals, eating salami, drinking unpasteurised milk and animal contact (Lopez, 2000; Remington *et al.*, 2001; Montoya, 2004).

Limited studies have been conducted to explore the seroprevalence of *T. gondii* among pregnant Saudi women (el Hady, 1991; Ghazi *et al.*, 2002). Premature infants in Saudi Arabia, with different clinical presentations, showed an anti-*Toxoplasma* IgM seropositivity of 23.1% (Abdalla *et al.*, 1994). The seroprevalence of *T. gondii* among healthy blood donors was 52.1% in Asir (Al-Amari, 1994) and 37.5% in Al-Hassa area (Yanaza & Kumari, 1994). A prevalence rate of anti-*Toxoplasma* IgG (25%) and IgM (5%) was reported in the Eastern region (Al-Qurashi *et al.*, 2001). A relatively recent study in two rural areas of the Eastern region; Al-Qurain (2004) has reported comparable results regarding the seroprevalence of both IgG and IgM antibodies.

The objective of the current study was to identify the seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies among pregnant women in the holy city of Makkah Al-Mukarramah, and to determine the environmental, personal and behavioural factors associated with infection in these women.

METHODS

Study group

A total number of 197 pregnant women attending the Maternity Hospital in Makkah, participant consent and hospital approval were obtained. The age of the study groups ranged from 17-45 years. Eighty seven (87) women have a history of spontaneous abortion.

Epidemiological assessment

A questionnaire sheet was designed to assess some of the main risk factors which may influence the prevalence of *Toxoplasma* infection among the expecting women volunteers. These data were intended to be completed by interviewing each participant during her hospital visit followed by home visits to validate questions related to environmental factors. However, cultural customs made home visits attempts not applicable. The influential risk factors considered in the study include: maternal age, gestation period, educational level (primary school only; high school; collage; higher education), owning cats, sources of drinking water, finally the nature of cooking and eating habits such as: eating raw or undercooked meat, tasting raw food while cooking, dining in restaurants, and having a domestic helper. The level of knowledge regarding toxoplasmosis and sources of *Toxoplasma* infection was also evaluated.

Samples

A single blood sample was taken from each participant. Samples from all participants were collected over six-month from January to June, 2004. Blood samples were collected in a labeled 5ml tubes. Samples were then stored and delivered at 4°C cool containers. The sera were separated by centrifugation and stored in 0.2 ml aliquots at -20°C till testing.

Serological testing

Serum specimens were tested by ELISA to detect anti-*Toxoplasma* IgG and IgM antibodies using bioelisa TOXO IgG and IgM (biokit, Spain) according to manufacturer's instructions. The cut-off value of the assay was calculated and results were expressed in an index by dividing sample absorbance by the cut-off value. The test was considered negative if the index was <0.9, the result was equivocal when index was from 0.9 to <1.0 while the positive result was if index was ≥ 1.0 . A negative reaction indicates absence of significant *Toxoplasma* antibodies. A positive *Toxoplasma* IgG reaction was interpreted as an indication of either a past or recent infection.

Statistical analysis

A descriptive statistical analysis of the serological data in relation to the epidemiological variables, used in the study, was done. Chi-square test and t-test were used and appropriate p values of <0.05 were considered significant. Data were analyzed using SPSS version 12 software. Data were then presented in tables.

RESULTS

The questionnaire data revealed that 89.3% of participants have never heard or seen information about toxoplasmosis prior to the interview. In addition all women

were unable to identify any of the risk factors associated. Also, all participant's were unaware of being serologically tested or not for toxoplasmosis.

Out of 197 sera tested, 58 samples were IgG positive (29.4%), while 11 were IgM positive (5.6%). Only 4 serum samples were positive for both (IgG and IgM). A history of spontaneous abortion was reported in 30% of the IgG positive cases and 2.3% of the IgM cases.

Seroprevalence in relation to gestational age

There was no statistical significant correlation between IgG or IgM seropositivity and the gestational age of the fetus. Comparable results of seropositivity of both IgG and IgM were obtained in both first trimester (30.17% & 5.33% respectively) and second trimester (33.33% & 9.52% respectively), while the number of women in their third trimester was too low to predict (Table 1). However seropositivity of both antibody types (especially IgM) tended to be higher in the 2nd trimester.

Seroprevalence in relation to women age

The seroprevalence of *Toxoplasma* IgM level according to maternal age was 5.6% with no significant difference among age groups, whereas, the IgG level was significantly higher ($p < 0.05$) in older age groups. Among expectant females aged 26-35 year old was 31% and 48.8% in 35-45 year old compared to only 19.5% among the younger age group 17-26 years old. While, the seropositivity of IgM increased with age reaching its highest level (12.2%) in the oldest age group (35-45 year old.), its correlation to the different age groups was not statistically significant (table 2).

Seroprevalence in relation to educational background

Results showed that IgG seroprevalence tends to be lower in educated participants (from primary school education up to university graduates) (27.8%) compared to the uneducated (not exposed to formal or informal education) group (42.8%). However, this tendency falls short of being statistically significant (table 3). On the other hand, the IgM seropositivity levels were comparable between educated and uneducated groups (5.7% and 4.7% respectively) (table 3).

Seroprevalence in relation to other influence factors

There were no significant associations between seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies and other risk factors considered in the study. These influential factors include: water supply, cat exposure, consumption of undercooked meat, tasting food while cooking, dining out doors in restaurants, having a domestic helper, being exposed to any kind of knowledge about toxoplasmosis and history of previous abortion (table 4).

DISCUSSION

The current study is one of few studies in Saudi Arabia to explore the prevalence of *T. gondii* infection among one of the most important clinical categories of toxoplasmosis in immunocompetent hosts who are pregnant women. If a woman gets infected with *T. gondii* for the first time in her life during pregnancy, she may pass infection to her fetus; a situation that ultimately could lead to a very serious fetal damage. Most previous studies in Saudi Arabia have concentrated on the prevalence of *Toxoplasma* infection among general population. The current study is also one of the leading studies that evaluate some environmental and behavioural factors that may influence the infection rate of *T. gondii* in the kingdom. The *Toxoplasma* seroprevalence obtained in this study among pregnant women in the holy city of Makkah was 29.4% which is comparable to results previously reported in Abha 31.6% (el Hady, 1991) and in Makkah 35.6% (Ghazi *et al.*, 2002). A comparable seroprevalence data was also obtained from healthy blood donors from two rural areas in the Eastern Region 25% to 26.36% (Al-Qurashi *et al.*, 2001; Al-Qurashi, 2004). A higher *T. gondii* seroprevalence 52.1% was reported among blood donors in Asir (Al-Amari, 1994). The 29.4% seroprevalence rate obtained lies within the range of the average prevalence rate of *T. gondii* in most parts of the world which is 20-30% (Wilson & McAuley, 1991). Low prevalence rates of 10% were reported in the United Kingdom (Allain *et al.*, 1998) and Norway (Jenum *et al.*, 1998), and rates as high as around 55% were reported in France (Ancelle *et al.*, 1996) and Greece (Decavalas *et al.*, 1990). Higher prevalence rates were also reported in some neighbouring Arab countries like Kuwait (58.2%) (Al-Nakib *et al.*, 1983) and Jordan (37%) (Morsey & Michael, 1980)

Regional variations in the incidence of *Toxoplasma* infection rates from one country to another or even within the same country, has been well documented. This variation has been attributed to climate, cultural differences regarding hygienic and feeding habits (Jenum *et al.*, 1998; Remington *et al.*, 2001; Dupouy-Camet *et al.*, 2003). The frequency of stray cats in a humid rainy climate favouring the survival of oocysts has contributed to the high *Toxoplasma* prevalence in Central America (Remington *et al.*, 2001). Stray cats are widely spread in Makkah city, however, the hot and dry weather conditions are not ideal for oocyst survival, compared to cooler and more rainfall environmental conditions in the Eastern and Southern regions of the kingdom, which are in favor of a higher prevalence. Farming and animal rearing are also common.

The significant relation showed in the current study between *Toxoplasma* prevalence rate and the mother's age confirms the fact that seroprevalence of *Toxoplasma* is well known to increase with age; the greater the prevalence, the earlier the rise (Remington *et al.*, 2001; Dupouy-Camet *et al.*, 2003) This association does not mean that older age is a risk factor predisposing to infection but might be

explained by the older the person the longer time being exposed to the causing agent and may retain a steady level of anti-*Toxoplasma* IgG in serum for years. A contradictory result was reported in the Eastern Region where seropositivity declined with age (Al-Qurashi *et al.*, 2001). The present data showed the highest level of seroconversion was among 35-45 age group (12% IgM seropositivity); a result that does disagree with the common finding which supports most frequent seroconversion in 15-35 years age groups (Jackson & Hutchison, 1989).

The current results suggested that the educational level of pregnant women, in spite of being not statistically significant, may present possible protective measures against *Toxoplasma* infection. High education level may reduce risk exposure and increase awareness to adopt appropriate hygienic measures regarding food and cooking habits such washing chopping boards with soap or bleach, using different chopping board for meats and vegetables, the frequent washing of knives and hands while cooking and avoiding contamination of food by protecting it from flies and dust.

Many of the risk factors examined, such as cat contact, handling or eating raw or undercooked meat and drinking unfiltered water have been documented to have an influence on *Toxoplasma* transmission in different parts of the world. (Wilson & McAuley, 1991; Remington *et al.*, 2001; Dupouy-Camet *et al.*, 2003; Montoya & Liesenfeld, 2004). The absence of a statistically significant relationship between the prevalence of *Toxoplasma* infection among pregnant women in Makkah and many of the factors explored in the study, does not indicate that these factors have no influence on the transmission of toxoplasmosis. However, it may suggest that such factors play a limited role in this region due to religious beliefs and cultural habits. Furthermore, weather conditions in Makkah do not enable these factors to exert their full influence on *T. gondii* transmission.

This study may also be informative and useful to the public health community. It revealed that only around 10 % of participants were aware of toxoplasmosis and its association with congenital disease. Furthermore, all pregnant women were not aware of being previously tested for *Toxoplasma* nor were able to identify any risk factor by their gynecologist. This suggests that there is a need to launch an awareness program for the pregnant women and additional studies of this type should be encouraged to add to the knowledge of the community about the risks of exposure to *T gondii* by pregnant women in Saudi Arabia.

Antibody		Gestational age			Total
		1 st Trimester	2 nd Trimester	3 rd Trimester	
IgG	POSITIVE	51 (30.17%)	7 (33.33%)	0	58
	NEGATIVE	118	14	7	139
IgM	POSITIVE	9 (5.33%)	2 (9.52%)	0	11
	NEGATIVE	160	19	7	186
IgG and IgM		4			4
Total		169	21	7	197

Table 1: Seropositivity of Anti-*Toxoplasma* IgG (A) and IgM (B) in relation to gestational age

Age group	IgG		IgM		Total
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	
17-26	69	23 (25%)	89	3 (3%)	92
26-35	50	20 (29%)	66	4 (6%)	70
35-45	20	15 (43%)	31	4 (11%)	35
TOTAL	139	58	186	11	197

Table 2: Seropositivity of Anti-*Toxoplasma gondii* IgG and IgM in relation to participant's age

Educational background	IgG		IgM		Total
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
Educated	49 (27.8%)	127	10 (5.7%)	166	176
Non educated	9 (42.8%)	12	1 (4.7%)	20	21
Total	58 (29.4%)	139	11 (5.6%)	186	197

Table 3: Seropositivity of Anti-*Toxoplasma gondii* IgG and IgM in relation to Educational background

Influence Factors		IgG			IgM	
		Total	No. Of Positive Cases	Percent	No. Of Positive Cases	Percent
Water Sources	Treated	139	43	30.9 %	8	5.8 %
	Untreated	58	15	25.8 %	3	5.2 %
Cat Exposure	Yes	27	6	22.2 %	0	0 %
	No	170	52	30 %	11	6.4 %
Uncooked meet	Yes	31	11	35.5 %	1	3.2 %
	No	166	47	28.3 %	10	6.00 %
Resturant dependents	Yes	85	24	28.2 %	5	5.9 %
	No	112	34	30.4 %	6	5.4 %
Test food during cooking	Yes	170	49	28.8 %	8	4.7 %
	No	27	9	33.3 %	3	11.1 %
Domestic helper	Yes	75	23	30.7 %	6	8.0 %
	No	122	35	28.7 %	5	4.1 %
Pervious Abortion	Yes	87	26	29.9 %	2	2.3 %
	No	110	32	29.1 %	9	8.1 %
Toxo knowledge	Yes	20	9	45.00	1	5.00 %
	No	177	49	27.7	10	5.6 %

Table 4: Seropositivity of Anti-*Toxoplasma gondii* IgG and IgM in relation to studied influence factors

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