

TOXOPLASMA GONDII INFECTION IN PREGNANT WOMEN WITH PREVIOUS ADVERSE PREGNANCY OUTCOME

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SUMMARY: Adverse pregnancy outcome include two or more consecutive spontaneous abortions, intrauterine foetal death, intrauterine growth retardation, low birth weight, stillbirths, early neonatal death and/or congenital anomalies. Maternal infections and infestations play a critical role in adverse pregnancy outcome. In Libya, scarce data exist on the roles of Toxoplasma gondii (T.Gondii) in the etiology of adverse pregnancy outcome. The aim of this study was to evaluate the seroprevalence of T. gondii in relation to previous adverse pregnancy outcome in pregnant women. We also aimed to find out the possible risk factors of acquiring this infection in Libyan women at Benghazi region. We included 143 pregnant women with adverse pregnancy outcome who attended Gynecology and Obstetric Department in Jamhoriya Teaching Hospital over a period of six months to estimate seroprevalences of IgM and IgG antibodies to Toxoplasma gondii. Serum samples were collected and assayed using quantitative ELISA technique. Out of 143 serum sample, 64 (44.8%) were positive for Toxoplasma. IgM seropositivities to Toxoplasma were 8.4%. All of those with high IgG and IgM positive cases gave a positive history of habitual abortion. Further remarks were obtained and will be presented and stressed.

Key words: Toxoplasma gondii, adverse pregnancy outcome, seroepidemiology, Toxoplasma gondii, infestation.

INTRODUCTION

Wide array of organisms, like TORCH agents, Chlamydia trachomatis, Niesseria gonorrhoeae and others can cause recurrent pregnancy wastage due to maternal infections during pregnancy (1). Perinatal infections account for 2% to 3% of all congenital anomalies. TORCH, which includes Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus, and Herpes infections, are of the most

common infections associated with unfavorable outcome of pregnancy (2).

Primary infection with any of the TORCH agents during pregnancy can lead to adverse outcomes, which are initially inapparent or asymptomatic and thus difficult to diagnose on clinical grounds (3). The ultimate consequences of intrauterine infection can be any one of the following (2): no significant problem, asymptomatic chronic infection, spontaneous abortion, intrauterine fetal death, stillbirth or neonatal death, fetal malformation or neonatal infection.

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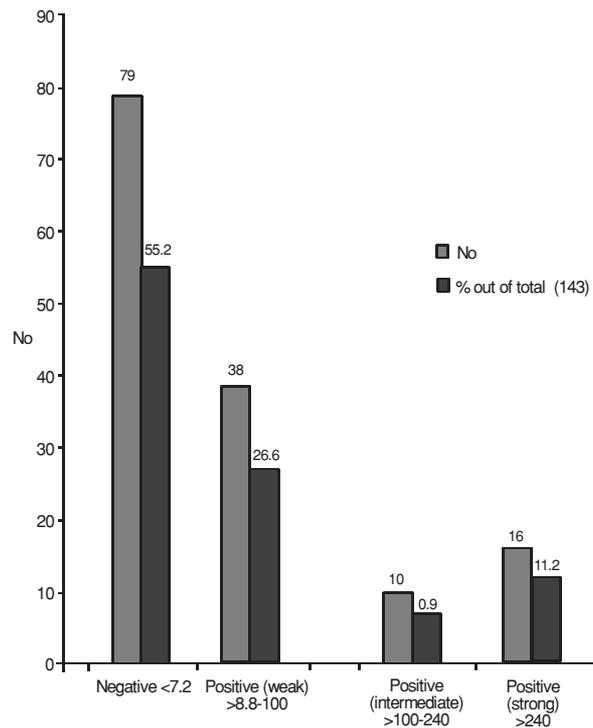


Figure 1: *T.gondii* IgG antibodies expressed quantitatively (IU/ml). Data shown as total no.s and percentages.

Primary maternal *Toxoplasma gondii* (*T.Gondii*) infection during pregnancy is frequently associated with its transmission to the fetus (4). The transmission rate of maternal infection to the fetus is estimated to be about 45%; of these, 60% are sub-clinical infections, 9% resulting in death of the fetus and 30% have severe damages such as hydrocephalus, intracerebral calcification, chorioretinitis and mental retardation (5). Degree of damage depends on the gestational age, since the greatest risk of congenital toxoplasmosis occurs during the first trimester of pregnancy. However, it is during the third trimester that the highest level of transmission occurs which is thought to

be related to the larger size of the uterus but fetal injury is usually much less severe (4).

Considerable confusion and uncertainty exist concerning *T.gondii* as a cause of multiple abortions and other reproductive problems. Even isolation of *T. gondii* from the endometrium several weeks after abortion does not prove congenital toxoplasmosis because *T. gondii* has been found in the uteri of non-pregnant women also. In Austria and France, because of high prevalence of toxoplasmosis, it is compulsory by law to test all pregnant women for *T. gondii*. Women are tested for *T. gondii* antibodies on their first visit to their gynaecologist (6). All seropositive women are also

Table 1: The overall seroprevalences to *T. gondii*, (Total 143).

Microorganism	Immunoglobulin			
	IgG		IgM	
	No	%	No	%
<i>T.gondii</i>	64	44.8	12	8.4

tested every trimester for rising IgG titers; those women who seroconvert during pregnancy are followed clinically and their fetuses are examined for evidence of *T. gondii* infection by ultrasound, amniocentesis and fetal blood (Pinon *et al.*, 2001). Women who have acquired *T. gondii* infection during pregnancy are treated with spiramycin to prevent transmission of *T. gondii* from the placenta to the fetus (amniotic fluid PCR negative) and with sulfadiazine and pyrimethamine to prevent fetal damage if the fetus is found to be infected (amniotic fluid PCR positive) (7).

T. gondii infections in Libyan general populations as well as normal pregnancy women were estimated to be around 50 % (8).

The aims of the present study were to assess seroprevalences (IgG and IgM) of *T. gondii* in pregnant women with previous adverse pregnancy outcome and to find out if there is any significant relationship between this infection and adverse pregnancy outcome. Assessment of the possible risk factors for acquiring this infection in Libyan women is also hoped.

Table 2: *T.gondii* IgG antibodies seropositivities and adverse pregnancy outcome.

Adverse pregnancy outcome	No. tested	Positive		% out of all positive(64)	Negative		Statistical significance
		No.	%		No.	%	
Habitual abortion							
Yes	117	55	47.1	85.9	62	52.9	P>0.05
No	26	9	34.6	14.1	17	65.4	
Total	143	64	44.8	100	79	55.2	
Intrauterine fetal death (IUFD)							
Yes	12	8	66.7	12.5	4	33.34	P>0.05
No	131	56	42.7	87.5	75	57.25	
Total	143	64	44.8	100	79	55.2	
Neonatal death (NND)							
Yes	26	7	26.9	10.94	19	73.1	P>0.05
No	117	57	48.7	89.06	60	51.3	
Total	143	64	44.8	100	79	55.2	
Congenital anomalies (CA)							
Yes	23	9	39.1	14.1	14	60.7	P>0.05
No	120	55	45.8	85.9	65	54.2	
Total	143	64	44.8	100	79	55.2	
Lowbirth weight (LBW)							
Yes	24	9	37.5	14.1	15	62.5	P>0.05
No	119	55	46.2	85.9	64	53.8	
Total	143	64	44.8	100	79	55.2	
Stillbirth (SB)							
Yes	6	3	50	4.7	3	50	P>0.05
No	137	61	44.5	95.3	76	55.47	
Total	143	64	44.8	100	79	55.2	

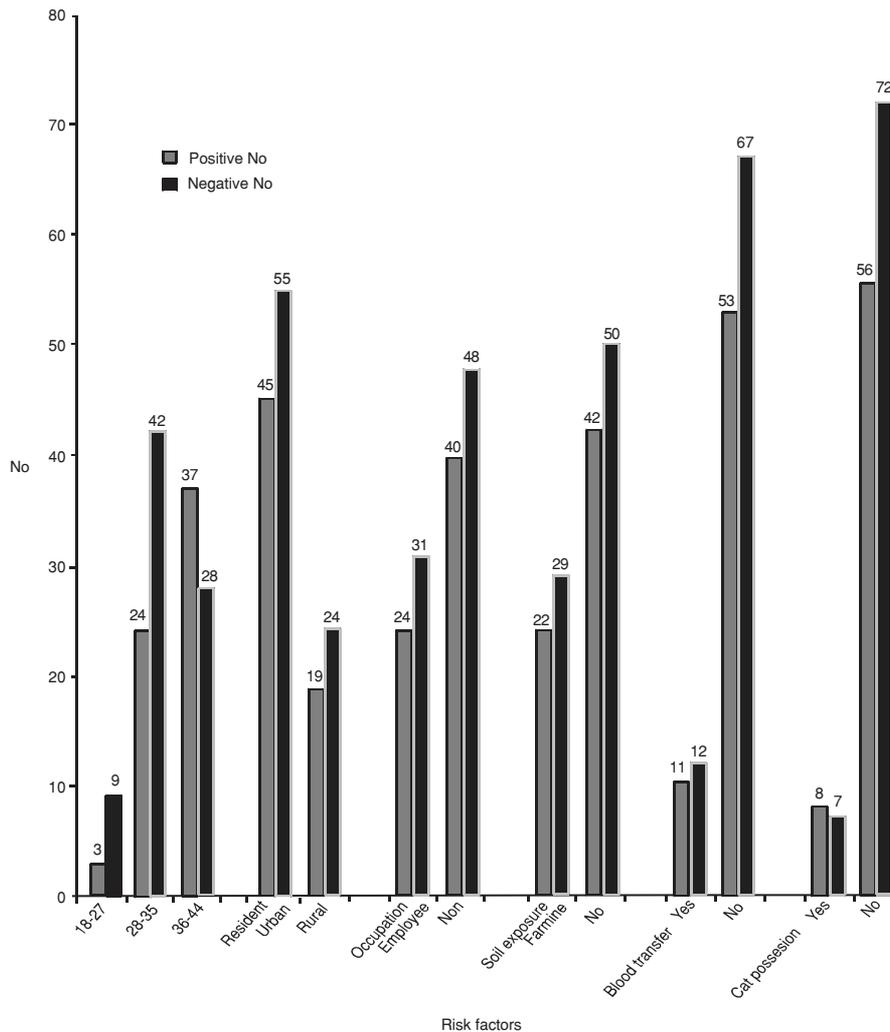


Figure 2: *T. gondii* IgG seropositivities and risk factors.

MATERIAL AND METHODS

A hospital based serological study was conducted in the Department of Obstetric and Gynecology in Jamhoriya hospital-Benghazi (a 300 bed establishment) during the period from 1 April to 30 September 2007 on 143 pregnant women with previous adverse pregnancy outcome. Women were recruited only if they have a history two or more consecutive abortions, intrauterine fetal or neonatal deaths, and stillbirth or low birth weight infants or off springs with multiple congenital anomalies. All women were interviewed for demographic, medical and obstetric informations and data recorded in a specially constructed questionnaire. After taking consent and full history, women were examined thoroughly by a gynecologist to ascertain of the probable factor/ factors

responsible for past reproductive losses.

Blood samples were obtained and serum was separated and stored at -20°C until processing. The following kits were made available:

- A. Toxoplasma IgG ELISA kit from BIOTEC Laboratories Ltd. (UK).
 - B. Toxoplasma IgM ELISA kit from BIOTEC Laboratories Ltd. (UK).
- Instructions supplied by the manufacturers were followed exactly.

Data obtained in this study were analyzed using SPSS statistical package. To determine possible association, every variable was compared to *T. gondii*, Rubella virus or CMV IgG and IgM seroprevalence in 2 x 2 table using chi-square (χ^2) test. A statistically significant difference was considered if a P-value less than 0.05 ($p < 0.05$) was obtained.

Table 3: Strong and weak positive *T.gondii* IgG levels and adverse pregnancy outcome.

<i>T. gondii</i> IgG conc. IU/ml	No. of cases with various adverse pregnancy outcome							
	No.	%	H.A	I.U.F.D	N.N.D	C.A	S.B	L.B.W
>240	16	(11.2%)	16 (100%)	1 (6.3%)	1 (6.3)	4 (25%)	0	1 (6.3%)
>8.8-100	38	(26.6%)	22 (57.9%)	4 (10.5%)	3 (7.9%)	4 (10.5%)	2 (5.3%)	5 (13.2)

Table 4: *T.gondii* IgG concentrations and bad obstetric history in *T.gondii* IgM positive patients (total 12).

IgG concentrations (IU/ml)	No. of cases with different conc.	Previous adverse pregnancy outcome				
		H.A	IUD	NND	C.A	L.B.W
Low (8.8-100)	6	6	0	1	0	0
High >240	6	6	1	0	2	0
Total	12	12	1	1	2	0

RESULTS

Of the 143 pregnant women, 64 (44.8%) were *T.gondii* IgG seropositive (Table 1). Sixteen of them, demonstrated strong responses manifested by high IgG concentrations (>240 IU/ml), whereas the remaining 48 women gave only weak IgG responses (8.8-100 IU/ml), (Figure 1). *T.gondii* IgG seropositivities were found to increase with age (Figure 2). Indeed, in the 18-27 years age group, only three (25%) were *T. gondii* IgG seropositive. In the 28-35 years age group, 24 (36.4%) were seropositive, whereas in the 36-44 years age group, 37(56.9%) were seropositive. Differences in *T.gondii* IgG seropositivities with respect to age were statistically significant ($P<0.05$). IgG seroprevalences in respect to the other risk factors depicted in Figure 2, indicated that residence, occupation, exposure to soil, blood transfusion and cat possession were statistically nonsignificant ($P>0.05$). IgG seroprevalences and history of habitual abortion given in Table 2 showed that out of 117 women (85.9 % of the total recruited) who demonstrated a history of habitual abortion, 55 (47.1%) were IgG seropositive. In contrast, only nine (34.6%) of remaining 26 women with no history

of habitual abortion were seropositive. These differences in IgG seropositivity in relation to history of habitual abortion were, however, statistically insignificant ($P> 0.05$).

On the basis of IgG concentrations (depicted in Table 3), 16 women responded strongly by showing high IgG titers of more than 240 IU/ml, all of them (100%) had the diagnosis of habitual abortion. There were 38 women with low IgG concentrations and of them only 57.9% complained a history of habitual abortion. Association between high levels *T.gondii* IgG and the habitual abortion seemed to exist ($P \leq 0.05$).

In respect to IgM profiles, depicted in (Table 1 and Table 4), twelve (8.4%) of the recruited women were *T.gondii* IgM seropositive. All of them were *T.gondii* IgG positive with different concentrations.

IgM seroprevalences in relation to age indicated that three (25%) out of the 12 positive women were between 18-27 years. Another three (25%) ranged between 28-35 years, whereas the other six (50%) belonged to the age group of 36-44 years. Differences in *T.gondii* IgM seropositivity in relation to age were, however, statistically insignificant ($P>0.05$).

All of the 12 *T.gondii* IgM seropositive women gave history of two or more consecutive abortions ($P>0.05$). All of them were IgG positive; six showing low IgG titers whereas the other six were strong responders as shown in Table 4.

DISCUSSION

Toxoplasma gondii is one of the few protozoan parasites that cross the placenta and infect the fetus. Consequences of congenital infection range from spontaneous abortion or prematurity to asymptomatic or overt congenital toxoplasmosis (8).

Fetuses are at risk during primary infection or even after reactivation of chronic ones (9). Antenatal screening for toxoplasma infection is now as important as VDRL, HIV, and HBV and HCV screenings in endemic areas, because toxoplasmosis is a preventable disease. Even when primary infection occurs during pregnancy, early diagnosis and treatment can reduce the frequency and severity of the disease in the neonates (10). However, the cost effectiveness of such screening programs in developing countries is still not known despite the high frequency of abortions, congenital anomalies, premature deliveries and intrauterine and neonatal deaths in these countries (7). The role of toxoplasma infection in these manifestations remains to be clarified.

In Benghazi toxoplasmosis seroprevalences during normal pregnancy is between 48.9% and 50.1% as shown Swalem and Fituri (11). In the present study, a comparable non significantly different overall IgG seropositivity rates of 44.8% in women with previous adverse pregnancy outcome was detected. Figures lower than these were shown by (12) (63.9% among women with previous complicated pregnancy) and Dar *et al.* (13) who reported a level of 62.3% in women with previous adverse pregnancy outcome. Differences might be due to small number of women with previous adverse pregnancy outcome included in their studies (86, 41 women respectively). Furthermore, both used indirect hemagglutination and indirect immunofluorescent tests in their assessment. All of these tests are of much lower sensitivities and specificities compared to the quantitative IgG- and IgM-ELISA

tests employed in the current study that have sensitivity of 99% and specificity of 97% as documented by the manufacturer.

The current results as well as those cited more recently in Benghazi fit within the ranges of reported seroprevalences in most of Arab countries. Comparable figures in both pregnant and women with previous adverse pregnancy outcome had been shown by (14) in Egypt and Qublan *et al.* (15) in Jordan (prevalences of 43% and 47% respectively). Our results are also not much different from that given by (16) in Iraq (49.2%), Abbas *et al.* (6) in Saudi Arabia and Elmansouri *et al.* (17) in Morocco, all of them showed a rate of 51.2% and 50.6% respectively. On the other hand, Al- Nakib (18) in Kuwait and Nimri *et al.* (4) in Jordan gave somehow higher levels of 58%, 54% respectively. Moreover, lower figures were reported in Saudi pregnant women by Ghazi *et al.* (19) and in UAE by Singh (20) (35.6%, 24.2%,) respectively. In summary, the present Libyan data are within the reported figures in the majority of Arab countries due to the similar social and health habits. Furthermore, population based studies are in deep need to ascertain of the exact picture of toxoplasmosis in different Arab countries.

This work tried to investigate the various risk factors for acquiring toxoplasmosis. It is indicated that specific IgG antibodies significantly increase with age (56.9 % among the eldest age group of 35-44years compared to 25% in the younger women. This is similar to many earlier results (5,6, 15). With increasing age, risk of acquiring toxoplasmosis as well its harmful pathological sequelae become higher and more possible, probably mandating the implementation of *T. gondii* serological testing for advanced age pregnant ladies.

Cat possession appeared not to affect the prevalence of toxoplasmosis IgG, which is in agreement with (12) and (21) data but in disagreement with (8) for Libyan pregnant women and (16) for Iraqi complicated pregnancy women. Presence of cats appear to be of primary importance in the transmission of infection in many areas of the world. In Libya, harboring cats is not a frequent practice and most cats are straying. These cats obtain their food from remains of raw meat thrown in the street garbage.

Children are often seen playing with cats and a large number of families frequently uses farms for picnics where food may be consumed and food remnants are thrown. In our region, cats may play indirect role in transmission of toxoplasma through contaminated meat, vegetables and fruits.

Sources of *T. gondii* infection for Libyan women are yet unknown. Most possible way of transmission is probably through handling of raw contaminated meat, vegetables or fruits during food preparation. Watani meat, either sheep, goat or camel meat might become contaminated with oocysts due to poor hygiene observed in Libya during handling of meat from slaughter house to kitchen. In addition, in Libya, consumption of lamb is greater than that of beef. These trends may have increased exposure to toxoplasma because lamb has a higher risk of infection than beef or poultry.

Causal relationships between toxoplasmosis and adverse pregnancy outcome were widely investigated in number of studies (3, 4, 22). The present work tried to find out if there was an association between *T. gondii* IgG seropositivity and habitual abortion, intrauterine fetal demise, neonatal death, congenital anomalies, low birth weight and stillbirth (Table 2). Considerable confusion and uncertainty exist concerning *T. gondii* as a cause of habitual abortion and other reproductive failures. A number of investigators succeeded in linking some of adverse pregnancy outcome with toxoplasmosis (4, 24, 25). These studies stressed on the requirement of routine screening for toxoplasma for all antenatal cases as early diagnosis and appropriate intervention help in proper management and fetal outcome (3, 23).

The present research by employing quantitative ELISA assay supply reliable evidences on the role of toxoplasmosis in bad obstetric history. In Figure 1, 38 (26.6%) women presented with weak IgG responses. Low IgG titers indicated either an old infection or waned anti-toxoplasma immune responses. The 16 (11.2%) women who showed high IgG seropositivity, probably because a recent or a chronic illness reactivated infection. Of the high responders, six women were IgM positive indicating that the infections were indeed recent ones (all of them

were admitted to hospital because of abortion). The rest women which were IgM negative probably indicating a chronic infections, although, the exact significance of these responses is yet not exactly determined because IgG avidity tests were not available. Within the weak responders, only 57.9% suffered of HA, compared to all (100%) of the strong responders suffered of HA. Few patients in both of these groups demonstrated the rest of bad obstetric manifestations. High frequency repeated abortions in those with strong titers might indicate a re-exposure or reactivation during pregnancy due to a reason or another which need a deep investigation. A second sample for rising IgG or IgM or IgG avidity test is needed to establish if the infection is recent or not. Toxoplasma IgG seroprevalences according to the current attempt increased only with age whereas, association with other proposed risk factors were negative. Role of toxoplasmosis, estimated by the overall IgG seroprevalences, was not demonstrated for all the bad obstetric manifestations examined. A link between habitual abortion and high IgG quantities was evident, indicating the need for use of quantitative serologic tests, such as ELISA as we did in this work.

In the present study, an overall *T. gondii* IgM seropositivity of 8.4% was reported. Much higher rates were reported for adverse pregnancy outcomes (22, 3, 25, 26) (13.1%, 10.5%, 20%, 11.6%, and 10.38%). Shashi *et al.* (23) reported an outstanding greatly high seropositivity of 42.5%. All of the IgM positive women of the present study demonstrated history of habitual abortion. This is in accordance with data shown by (1, 22) on the positive correlation between an IgM positive result and abortion. Six of them are showing high IgG titers, while the other six demonstrated low titers. In summary, these observations indicate an active toxoplasmosis irrespective of being primary or reactivated. Confirmation of primary *T. gondii* infection serologically is usually possible by the following findings: rising IgG titer, positive IgM test or a positive IgG avidity test (26). High IgM seropositivity (8.8%) necessitates systematic measures to prevent acute toxoplasma infection during pregnancy in Benghazi, Libya.

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