Original Article

SEROPREVALENCE OF TOXOPLASMOSIS AMONG PALESTINIAN ABORTED WOMEN IN GAZA.

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Abstract:
Aims: The microbial pathogen *Toxoplasma gondii* is commonly associated with congenital infections that are not clinically apparent. Primary *T. gondii* infection in the first trimester pregnancy may cause severe congenital anomalies or even foetal loss**.** The present study is aimed to determine the seroprevalence of toxoplasmosis among aborted women in Gaza, to study the relationship between animal rearing and the presence of Toxoplasmosis and to assess the relation of some abnormalities and infection with Toxoplasmosis.
Materials and Methods: A hospital-based study was implemented to detect, describe and analyze toxoplasmosis among women with abortion attending Al-shifa hospital in Gaza strip. Quantitative investigation of each blood sample for the levels of the specific IgG and IgM of *T.gondii* was done from 312 aborted women.
Results: The overall prevalence of Toxoplasma IgG was found 17.9% and Toxoplasma IgM was found to be 12.8%. The age of the participant woman ranged from 16 to 45 years old; 216 came from Gaza city (69.2%) and the others reside in near villages 96 (30.8%). Most of the participants (59.0%) had secondary education level. Animal breading was reported for 33.3% of the aborted women.
Conclusions: Toxoplasmosis still exists and considered one of the risk factors for pregnancy miscarriage. Both IgG and IgM tests are recommended as routine tests among pregnant women in Gaza.

Key words: Abortion, Gaza Governorate, Prevalence, and Toxoplasmosis.

تحديد الانتشار المصلى لداء المقوسات بين النساء المجهضات فى غزة

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الخلاصة:
هدفت الدراسة الحالية الى تحديد الانتشار المصلى لداء المقوسات بين النساء المجهضات فى غزة، ودراسة العلاقة بين تربية الحوانات ووجود المرض وتقييم العلاقة لبعض الحالات غير الطبيعية والاصابة بداء المقوسات. طبقت الدراسة فى المستشفى للكشف ووصف وتحليل داء المقوسات بين النساء المجهضات الذين يترددن على مستشفى الشفاء فى قطاع غزة. وقد تم اجراء فحص كمى لكل عينة دم لقياس مستويات محددة لكل من أضداد الغلوبين المناعى G و M من 312 امرأة مجهضة.
وقد أظهرت النتائج أن معدل انتشار اضداد من الغلوبولينات المناعية G و M كانت 17.9% و 12.8% على التوالى حيث كانت أعمار النساء المشاركات تتراوح بين 16-45 سنة. وجدت الدراسة أن 69.2% من النساء يقطن مدينة غزة، بينما 30.8% من المشاركات يقطن القرى القريبة. كذلك بينت الدراسة أن معظم المشاركات 59.0% تلقوا التعليم الثانوى. سجلت تربية الحيوانات 33.3% من النساء المجهضات.
تدل نتائج هذه الدراسة أن داء المقوسات لا زال موجودا ويعتبر أحد عوامل الخطر المسببة للاجهاض. أوصت الدراسة بأن يعتبر فحص الغلوبولينات المناعية G و M كأحد الفحوصات الروتينية للنساء الحوامل فى غزة.

Introduction:

Toxoplasma gondii is a single-cell protozoan that belongs to the family *Coccidia.* It is an obligatory intracellular protozoan with a heterogeneous life cycle in humans and other vertebrates [1]. Human infection with *T.gondii* causes toxoplasmosis. Postnatal toxoplasmosis is usually an asymptomatic disease, but often takes a severe course in immunocompremised hosts [2]. Congenital toxoplasmosis is acquired through vertical transmission of *T.gondii* to the foetus by transplacental transfer from the mother usually following acute maternal infection. If congenital toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion [3].

Sporadic abortion is defined as the termination of pregnancy by any means before the foetus is sufficiently developed to survive. While habitual abortion is defined as three or more consecutive spontaneous abortions. Habitual abortion is one of the most distressing problems in obstetrics, particularly in those women who have no successful pregnancies [4].
The seroprevalence of toxoplasmosis infection in women with bad obstetric history (including sporadic or habitual abortions) is known to be significantly higher than those without it [5]. A recent study from India reports a statistical difference between IgG antibody levels against *T.gondii* in habitual abortions as compared to sporadic abortions or normal pregnancies [6]. Another study from Egypt revealed that there was a significant IgG and IgM *T.gondii* antibody level difference between women with no history of abortion, women with one or two abortions, women with more than three abortions and the control group [7].

In Gaza, studies of this nature have not been carried out. However, a screening study of productive-age women was done in 1990 as a first effort to explore the epidemiological pattern of toxoplasmosis in a specific group of the community. The study concluded that toxoplasmosis is endemic in the area, and is one of the multiple aetiologies of threatened abortion in the investigated group [8]. Our objectives were:
1-To determine the prevalence of Toxoplasmosis among women with abortion in Gaza.
2-To detect levels of *T.gondii* specific IgG, IgM antibodies among women with regard to the trimester of abortion and infection with toxoplasmosis.
3-To look for any relationship between animal rearing and the presence of Toxoplasmosis.
4-To identify any abnormalities that could be related to infection with Toxoplasmosis.

Materials and Methods:

Subjects: A hospital-based study was implemented to detect, describe and analyze toxoplasmosis among women who have had an abortion.
Study place: The study was carried out in Antenatal clinic in Al-Shifa Hospital in Gaza strip.
Sample size: The size of the sample for this study was determined by using the statistical calculator of the EPI-INFO program. Assuming that the prevalence of toxoplasmosis was 10% with a confidence level 95%. It was estimated that 312 women with abortion would be required for the study.
Eligibility criteria: Women who had abortion without any obvious cause admitted to the antenatal clinic.
Sampling process: A random sample was used to collect 312 through six

months, 14 blood samples from the subjects was collected weekly. All subjects admitted in antenatal clinic at the time of data collection of the study and diagnosed as abortion with no obvious cause will be taken as participants in the study.

Data collection: Quantitative investigation of each blood sample for the levels of the specific IgG and IgM of *T.gondii* was done.

Methods: Blood was collected into plain sterile containers. Detection of antibodies' levels was done using Enzyme Linked ImmunoSorbent Assay ELISA techniques according to manufacturer recommendations (DiaMed EuroGen, Belgium)

Questionnaire: All women were interviewed and information was annotated on adedicated form including medical history, previous times of pregnancy, previous abortion, if any, and contact with animals.

Ethical considerations and procedures: An official letter of approval to conduct the study was obtained from the Helsinki Committee (Ethical Committee in Gaza Strip) dated in 3-6-2009. Also from ethical point of view, all subjects were informed about the objectives of the research before collecting blood samples.

Data analysis: Data were entered into the computer and analyzed using SPSS, version 12. Frequency, cross tabulation, chi-square and the existing association was investigated. $P<0.05$ was considered significant.

Results:

The present study included 312 subjects with abortion who attended Antenatal clinic at Al-Shifa Hospital to investigate the causes of the abortion. The prevalence of Toxoplasma IgG was found to be 17.9% and Toxoplasma IgM was found to be 12.8%.

The age ranged from 16 to 45 years old; where 216 came from the Gaza city (69.2%) and the others reside in the near village 96 (30.8%). Most of the participants (59.0%) had secondary education level. Animal breading was reported for 33.3% of the aborted women. The age group 23-28 years old were at great risk of toxoplasma infection (25.5%) than other groups but no statistical difference was found as indicated in table 2. Table 3. show that 5.8% of the women with abortion had a history of congenital malformation that ranged between a hydrocephalus and abnormal.

While the stillbirth recorded 41.7%. In the present study 25.6% of the women had >5 deliveries while 42.9% had 2-3 abortion. It was found that 4 women with abortion 1.2% had the fifth abortion, 4 women with abortion 1.2% had the sixth abortion and 2 0.6% had the seventh abortion as indicated in table 4.

Table 5. Shows that 10 cases (35.7%) of the aborted women for the fourth abortion occurred in the first trimester. Two cases of women with abortion had fifth and sixth abortions separately. Occurring of the fourth abortion was found high among women with toxoplasmosis in the first trimester, and this was statistically significant compared to the second and third trimester (χ^2=7.025, P=0.03). But 71.4% of the fourth abortion occurred in the second trimester with a statistical significance (χ^2=35.893, P=0.001). Similar prevalence for occurring the second abortion in both second and third trimester (χ^2=26.873, P=0.001).

Table 1. Some demographic characters for the subjects (n=312)

Variable	No.	%
Age (year)		
16-22	**46**	**14.7**
22-28	**94**	**30.1**
28-34	**92**	**29.5**
35-45	**80**	**25.6**
Residence		
City	**216**	**69.2**
Village	**96**	**30.8**
Education		
Primary level	**84**	**26.9**
Secondary level	**184**	**59.0**
University level	**44**	**14.1**
Animal breading		
Yes	**110**	**33.3**
No	**202**	**64.7**
Type of animal breaded		
Cat	**34**	**10.9**
Chicken	**46**	**14.7**
Others	**30**	**9.6**
No breeding	**202**	**64.7**

Table 2. Distribution of IgG and IgM antibodies for the examined subjects due to age groups (n=312

Age (year)	No. examined	Positive for IgG (%)	Positive for IgM (%)
16-22	**46**	**6 (13)**	**4 (8.7)**
22-28	**94**	**24 (25.5)**	**2 (2.1)**
28-34	**92**	**12 (13)**	**-**
35-45	**80**	**14 (17.5)**	**-**
		(χ^2=5.936, P>0.05	(χ^2=17.364, P=0.008)

Table 3. The clinical profile of the woman with abortion (n=312)

Variable	No.	%
History of congenital malformation (number)		
Yes	**18**	**5.8**
No	**254**	**94.2**
Type of congenital malformation		
Hydrocephalus	**8**	**2.6**
Abnormal	**10**	**3.2**
Normal	**294**	**94.2**
Death of embryo inside the uterus (Stillbirth)		
Yes	**130**	**41.7**
No	**182**	**58.3**
The number of deliveries		
<2 delivery	**122**	**39.1**
2-5 delivery	**110**	**35.3**
> 5 delivery	**80**	**25.6**
The number of abortion		
1 time	**110**	**35.3**
2-3 times	**134**	**42.9**
> 3 times	**68**	**21.8**

Table 4. Distribution of abortion according pregnancy trimester (n=312)

Trimester	First abortion		Second abortion		Third abortion		Fourth abortion	
	No.	%	No.	%	No.	%	No.	%
First trimester	**232**	**(74.4)**	**154**	**(44.4)**	**102**	**(32.7)**	**28**	**(9.0)**
Second trimester	**70**	**(22.4)**	**34**	**(10.9)**	**14**	**(4.5)**	**8**	**(2.6)**
Third trimester	**10**	**(3.2)**	**14**	**(4.5)**	**6**	**(1.9)**	**-**	
Without abortion	**-**		**110**	**(35.3)**	**190**	**(60.9)**	**276**	**(88.5)**

Table 5. Distribution of abortion with 56 women who IgG positive with pregnancy trimester (n=56)

Trimester	First abortion		Second abortion		Third abortion		Fourth abortion	
	No.	%	No.	%	No.	%	No.	%
First trimester	**44**	**(19)**	**28**	**(18.2)**	**24**	**(23.5)**	**10**	**(35.7)**
Second trimester	**10**	**(14.3)**	**14**	**(41.2)**	**10**	**(71.4)**	**2**	**(25)**
Third trimester	**2**	**(10)**	**6**	**(42.9)**	**-**		**-**	
Do not	**-**		**8**	**(7.3)**	**22**	**(11.6)**	**44**	**(15.9)**
Without abortion	**-**		**-**		**-**		**-**	
	(χ^2=0.829, P=0.661)		**(χ^2=26.873, P=0.001)**		**(χ^2=35.893, P=0.001)**		**(χ^2=7.025, P=0.03)**	

Seroprevalence of Toxoplasmosis according to life style rural (village) or urban (city), education, and animal breading.

The seroprevalence for IgG between the village and the city is represented in Table 6. Subjects came from the village had high prevalence than who reside in the city with no significant difference (P>0.05).

Both subjects of primary and secondary education level had the same prevalence for Toxoplasma, while who had university degree was found to be the lower. There was no statistical difference (P>0.05) in the seroprevalence of IgG between subjects who breading animals and who did not. High association was found between the infection by Toxopasma and breading of cats 23.5%.

Table 6. Distribution of IgG with some demographic characters for the subjects with abortion (n=312)

Variable	No.	%	(χ^2, P-value)
Residence			
City (n=216)	**36**	**16.7**	**(0.783,**
Village (n=96)	**20**	**20.8**	**0.232)**
Education for each woman with abortion			
Primary level (n=84)	**16**	**19.0**	
Secondary level (n=184)	**36**	**19.6**	**(2.740, 0.254)**
University level (n=44)	**4**	**9.1**	
Animal breading			
Yes (n=110)	**18**	**16.4**	**(0.290, 0.354)**
No (n=202)	**38**	**18.8**	
Type of animal breaded			
Cat (n=34)	**8**	**23.5**	
Chicken (n=46)	**4**	**8.7**	**(3.581, 0.310)**
Others (n=30)	**6**	**20**	
No breeding (n=202)	**38**	**18.8**	

Seroprevalence of Toxoplasmosis according to the clinical profile of the study subjects **with abortion:**

Table 7 illustrates the seroprevalence of Toxoplasma was high among women with history of congenital malformation (22.2%) compared to women without. While the high prevalence of toxoplasmosis was clear among women with no stillbirth. Also, the seroprevalence IgG was high among women with deliveries between 2-3 deliveries (21.8%). A statistical significant difference was found between the presence of IgG and the number of abortion times for women had >3 abortions (P=0.001).

Discussion:

To our knowledge, there have been few researches on *T.gondii* conducted in Palestine. The seroprevalence of *T.gondii* in pregnant women, on worldwide scale, varies from 7% to 52.3% and in women with abnormal pregnancies and abortions the seroprevalence varies from 17.5% to 53.3 % [6].

Table 7. The clinical profile of the woman with abortion (n=312)

Variable	Positive for IgG	Negative for IgG	(χ^2, P-value)
-History of congenital malformation (number)			
Yes	**4 (22.2)**	**14 (77.8)**	**(0.237, 0.409)**
No	**52 (17.7**	**242 (82.3)**	
- Type of congenital malformation			
Hydrocephalus	**2 (25.0)**	**6 (75.0)**	**(11.177, 0.01)**
Abnormal	**52 (17.7)**	**242 (82.3)**	
Normal	**2 (25.0)**	**6 (75.0)**	
-Death of embryo inside the uterus (Stillbirth)			
Yes	**20 (15.4)**	**110 (84.6)**	**(0.995, 0.199)**
No	**36 (19.8)**	**146 (80.2)**	
-The number of deliveries			
<2 delivery	**22 (18.0)**	**100 (82.0)**	**(2.732, 0.266)**
2-3 delivery	**24 (21.8)**	**86 (78.2)**	
> 3 delivery	**10 (12.5)**	**70 (87.5)**	
-The number of abortion			
1 time	**8 (7.3)**	**102 (92.7)**	**(18.286,**
2-3 times	**26 (19.4)**	**108 (80.6)**	**0.001)**
> 3 times	**22 (32.4)**	**46 (67.6)**	

In the present study the participants were women with abortion admitted to Antenatal clinic in Al-Shifa Hospital seeking miscarriage. The overall prevalence of IgG seropositivity was 17.9% for IgG and 12.8% for IgM.
Tabbara and Saleh [9] reported a prevalence of 21.8% for IgG among selected groups in Bahrin. In Turkey (60.4%) for IgG and (3%) for IgM [10]. In Saudi Arabia 25% for IgG and 5% for IgM [11].

Health status in Palestine Ministry of Health (MOH) annual report in 2003 reported 72 cases of toxoplasmosis in Gaza governorates, especially in Gaza city. According to the Ministry of Health no cases were reported in any other city in Gaza governorates or in West Bank [12].

A considerable number of IgG seropositive samples for *T.gondii*, especially among women with abortion was detected. Linking this observation to: The information that the total number of

recorded abortions in MOH hospitals in Gaza strip during 2004 was 1233 out of 13151 delivery cases i.e. abortion rate of 9.3 % [13].

Toxoplasmosis can be transmitted to humans by three principle routes. First, humans can eat raw or inadequately cooked infected meat or eat uncooked foods that have come in contact with contaminated meat. Second, humans can inadvertently ingest oocysts that cats have passed in their faeces, either in a cat litter box or outdoors in soil (e.g., soil from gardening or unwashed fruits or vegetables). Third, a woman can transmit the infection to her unborn foetus [14]. In our study 23.5% of aborted women with toxpoplasmosis were found to rear cats.

The organism in humans produces either congenital or postnatal toxoplasmosis. Congenital toxoplasmosis develops only when non immune mothers are infected during pregnancy and is usually of great severity; postnatal toxoplasmosis is usually much less severe [5]. Congenital infection may cause abortion or result in live-born infants with evidence of disease [16].

In our study we used ELISA technique and this may need using more diagnostic methods where diagnosis of toxoplasmosis requires the isolation of the organism or serological tests for the antibody formation. Cultures have proved to be very difficult, and most laboratories are not equipped to do it. Tissue cultures are positive in about 40% of cases [17].
Serologic tests form the backbone of diagnosis. These may include the titration of serum for IgG, IgM and IgA immunoglobulins. IgA immunoglobulin is useful in the diagnosis of recent foetal and newborn infections. IgG immunoglobulin is very useful for screening purposes and follow up of active infection [18]. IgM immunoglobulins are specific; however, their presence is not dependable for making decisions on therapeutic abortion since they persist for several months after initial infection [19].
Other laboratory techniques include indirect haemagglutination agglutination test (IHA), enzyme linked immunosorbent assay (ELISA), Immunofluorescence antibody (IFA), immunoblot technique and polymerase chain reaction (PCR) [8].
Our results indicated that women from village were found more risky to toxoplasma infection (20.8%) than who reside in the city (16.7%) but no significant difference was found. Khalil et al., (20) reported that women in El Masoudia village are more exposed four times to toxoplasmosis infection than women in El-Nuba village. While Ertug et al., (21) stated that no statistical meaningful difference was observed between urban and rural areas for toxoplasmosis seroprevalence in Aydin province, Turkey.
Antibodies were found more prevalent among persons who had cat or cats in the household of farmers in Finland (22). This was similar to our results where women who bread cats in their houses were more infected with toxoplasmsis. Other studies were found not associated with toxoplasma infection like; educational level, touching cats, handling raw meat and farming (23). In the current study 47.7% of the women were educated but no association between the infection by Toxoplasma infection and education level was found.
Because our study involved a specific group of women (aborted women), we could not generalize the prevalence into all Palestinian women. Both IgG and IgM tests are recommended as routine tests among pregnant women in Gaza.

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