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EPIDEMIOLOGICAL STUDIES ON TOXOPLASMOSIS IN PREGNANT WOMEN USING SEROLOGICAL METHODS IN GEZIRA STATE-CENTRAL SUDAN

دراسة وبائية لداء المقوسات في الحوامل باستعمال طرق مصلية في ولاية الجزيرة- وسط السودان

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ABSTRACT

Objective: To study the relationship between toxoplasmosis and the outcome of pregnancy (full term and abortion) and to find out the epidemiological risk factors.

Methods: This case control study was carried out at Wad Medani Teaching hospital, Gezira State, Central Sudan during the period March 2002-May 2004. The cases were 100 pregnant women who presented with abortion between the second and fourth months of gestation, and the controls were 100 full term normally delivered women.

Results: ELISA IgG test (n = 94 in each group) revealed 35.1% and 39.4% seroprevalence rates in the study and control groups respectively with no significant difference (p=0.6). When performed on IgG seropositives, ELISA IgM seroprevalence rates were 15.2% and 16.2%, in the study and control groups respectively. Statistically, there was no significant difference between them (p=0.9). When correlated to sociodemographic characteristics and risk factors, *Toxoplsma gondii* seroprevalence showed significant relationship for age, education, occupation, parity and past history of congenital malformations in both groups. There was significant difference between the two groups for ethnicity. The most important risk factor in both groups was the intake of raw meat (herbivorous viscera).

Conclusion: The findings of this study suggests that, ingestion of infected raw meat is an important risk factor for toxoplasmosis. (OR, 3.87; 95%CI, 1.59-9.44) in the study group, while in the control group (OR, 4.41; 95%CI, 1.76-11.09).

Key words: Toxoplasmosis, pregnancy, seroepidemiology, risk factors

ملخص البحث

هدف الدراسة : بحث العلاقة بين الإصابة بداء المقوسات (Toxoplasmosis) و منتوج الحمل (حمل تام وإجهاض) ومعرفة العوامل المؤهبة لحدوث المرض.

طريقة الدراسة :أجريت دراسة الحالات و الشواهد هذه بمستشفى واد مدني التعليمي بولاية الجزيرة في وسط السودان في الفترة من مارس 2002- مايو2004. تشتمل مجموعة الحالات على 100حامل أتوا للمستشفى في حالة إجهاض بين الشهرين الثاني والرابع و مجموعة الشواهد عبارة عن100 حامل أكملن فترة الحمل وأنجبن أطفال بولادة طبيعية .

النتائج :عندما أجري اختبار التحليل المناعي على (94 حامل من كل مجموعة (لقياس الضد القلوبيني جي (ELISA IgG) ، كانت نسبته في مجموعة الحالات هي 35.1%، بينما كانت 39.4% في مجموعة الشواهد. وكانت نسبة القلوبين المناعي أم (IgM) في المجموعات التي تحمل القلوبين المناعي جي (IgG) هي 15.2% و 16.2% في مجموعتي الحالات و الشواهد على التوالي. الفرق غير معتد إحصائيا بين المجموعتين في نسبة وجود الأضداد المناعية (p=0.6) IgG و IgM. (P=0.9) عند دراسة العلاقة بين نسبة انتشار القلوبين المناعي جي IgG)) لطفيل المقوسة القوندية *(Toxoplasma gondii)*وبعض العوامل الاجتماعية وجدنا علاقة معتدة إحصائيا بين انتشار المرض والعمر ، مستوى التعليم، طبيعة العمل، عدد الولادات السابقة وتاريخ ولادة أطفال يعانون من تشوهات خلقية في كل من المجموعتين. كما أن هناك فرق معتد إحصائيا بين المجموعتين في نوع الإثنية .

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الخلاصة : وجدنا أن أهم العوامل التي تساعد على انتشار الإصابة بهذا المرض في المجموعتين هي أكل اللحوم النيئة (الأحشاء الداخلية للضأن والأبقار) وكانت في مجموعة الحالات (OR 3.87; 95%CI, 1.59-9.44) بينما كانت في مجموعة الشواهد (OR, 4.41; 95%CI, 1.76-11.09)

INTRODUCTION

Toxoplasmosis is a worldwide distributed zoonotic disease caused by *Toxoplasma gondii* parasite. Infection in humans most commonly occurs via the ingestion of raw or undercooked meat that contains *T.gondii* cysts (1,2), or by ingestion of oocyst via eating or drinking food contaminated with infected cat's faeces (3).Toxoplasmosis could be transmitted transplacentally to the fetus in utero or at the time of vaginal delivery (4). There is an inverse relationship between the severity of *T.gondii* infection in the fetus and the gestational age at the time of seroconversion (5).Diagnosis of toxoplasmosis is established by direct detection of the parasite or by serological tests (6).The frequency of *T.gondii* infection among human population varies depending on the geographical area (7). In Gabon seroprevalence has shown that, 71.2% are seropositive for IgG antitoxoplasma antibodies (8). Seroprevalence rates in Egypt among full term and aborted women were 58.1% and 44.7% respectively (9).An overall prevalence rate of 41.7% was reported among the residents of Gezira-Sudan aged 10 years and above (10). Among pregnant women in Khartoum-Sudan, seroprevalence rates of 34.1% and 27.5% were reported by Elnahas (11) and Satti (12), respectively. No studies were done among pregnant women in Gezira State- Central Sudan, so this study was done to evaluate the relationship between toxoplasmosis and the outcome of pregnancy in Gezira State, and to identify some risk factors.

METHODS

The sample collection was carried out during the period March - July 2002 in the Obstetric and Gynaecology teaching hospital, which was a 192 beds hospital, (152 beds in the obstetrics ward and 40 beds in the gynaecology ward). The hospital was run by 6 units, there were 15 consultants, 10 registrars, and 30-40 house officers. It has 2 theatres, 2 antenatal and 2 postnatal care units. The hospital was located in Wad medani city which lies about 186 Kilometers South to Khartoum, the Capital of Sudan. It was considered as the main referal hospital for the Gezira State, 26075 km² in area and 3.5 millions of populations. The cases were 94 pregnant women who presented to the hospital with abortion. The controls were 94 full term women gave birth to alive healthy babies. All women were between 15-40 years, not diabetic, nor hypertensive. All the study population was clinically evaluated by an obstetrician using pre-designed forms for the history and general condition. Urine samples were collected for sugar and acetone tests, blood samples were collected in EDTA for detection of blood group, Rhesus factor, and haemoglobin estimation. 5mls of venous blood samples were collected, allowed to clot at room temperature and centrifuged to separate the sera for ELISA test. Highly lipaemic sera samples were excluded. *T.gondii* IgG and IgM antibodies were detected using ELISA IgG and IgM kits produced by Human Gesellschaft für Biochemica und Diagnostica mbH- Germany. The tests were performed at the Serology Laboratory/ King Saud University / Riyadh/ KSA. Results were analysed using chi-squire test.

RESULTS

Seroprevalence and basic sociodemographic characteristics

As shown in Table 1, the prevalence of IgG antibodies to *T. gondii* gradually increased by age, the correlation between age and IgG seroprevalence rate was statistically significant in both study and control groups. In both groups no significant difference was found between urban and rural populations. The prevalence rate was inversely decreased as the level of education increased in both study and control groups with a significant relationship between them. (p value = 0.01 and 0.02) in study and control groups,

respectively . In the study group, *T. gondii* sero-prevalence rate among Arab races was 41.2%, whereas it was only 16% among Nigroid races, with a statistically significant correlation between them. (p- value = 0.02). In the control group, the prevalence rate in Arab and Nigroid races was 42.9% and 31.6%, respectively, with no statistically significant difference between them. (p- value = 0.37). Further analysis of the results revealed that the IgG sero-prevalence rate was affected with the occupation of the respondents among the study and control subjects; the higher prevalence rates were observed among housewives, whereas the lower ones were obtained by employee. Socio-economic level had no correlation with sero-prevalence rate in both study and control groups.

Seroprevalence and risk factors

As shown in Table 2 there is no significant relationship between toxoplasmosis and cat contact, eating undercooked meat, drinking raw milk, and eating inadequately cooked vegetables and fruits in both study and control groups. Significant association between sero-prevalence rate and eating raw herbivorous viscera (marrara) and raw liver was found in both study and control groups (OR was 0.002 and 0.001), respectively.

Seroprevalence and past obstetrical history

As shown in Table 3 women with past history of abortion did not show significantly different seroprevalence rate than others with no such history in both study and control groups. There was a significant correlation between *T.gondii* seroprevalence and parity in both study and control groups. A higher seroprevalence rate was observed in grandmultipara compared to primigravidae. Women with previous history of congenitally malformed babies showed seroprevalence rates significantly higher than others with no such history.

When ELISA IgG was performed, 33 out of the total 94 patients in study group, were sero-positive by ELISA IgG test with 35.1% prevalence rate compared to 39.4% sero-prevalence rate in control group (37 sero-positive out of 94). There was no statistical significant difference in prevalence rate between the two groups (p- value = 0.55).

Positive samples by ELISA IgG test were further subjected to ELISA IgM test. The IgM sero-prevalence rate in study group was 15.2% compared to 16.3% in the control group. Statistically there was no significant difference between them (p- value = 0.90).

DISCUSSION

There was no correlation found between *T.gondii* antibodies seropositivity and abortion in this study. This result supported by Adnan (13), who found no correlation between *T. gondii* antibodies seropositivity and abortion in a study performed in Khartoum-Sudan. Satti (12), and Abd Elraoaf (14), also found that abortion was not correlated to *T.gondii* seroprevalene in Khartoum. Another study carried out in Khartoum reported prevalence rates of 35.4% and 34% among women with past history of abortion and others with no past history, with no significant difference between them (11). In Egypt no association was found between the prevalence of *T.gondii* antibodies and repeated abortion (15).

In the present study, the prevalence rates of 35.1% and 39.4% in the study and control groups, respectively, were almost nearer to the prevalence rates reported from Khartoum-Sudan among pregnant women by Elnahas (11), (34.1%), and Adnan (13), (30.1%), using ELISA test. Other studies done among Sudanese pregnant women in Khartoum State (12,14), have shown prevalence rates of 27.45% and 20%,

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respectively. Compared to these rates ours were higher; this discrepancy could be due to variation in hygienic conditions and the type of tests used.

The increase in seroprevalence by age in the present study agrees with the finding of Abd Elhameed (10), who found that, *T.gondii* seropositivity increased by age in Gezira State- Central Sudan. The results correspond well to others obtained among Nigerian women of childbearing age (16). Similar correlations of increased *T.gondii* prevalence rate by age were detected in Eastern England among pregnant women (17), USA, (18), and Brazil (19). The increased prevalence by age may be explained by more exposure to the causative agent of infection, or frequent handling of meat.

The lack of association between seroprevalence and residence in both groups could be explained by the stability of some epidemiological factors between the various habitats. This finding agrees with that obtained by Abd Elhameed (10) who found no association between *T.gondii* seropevalence and residence among both sexes in Gezira State-Central Sudan. In Khartoum Elnahas (11) also found no difference in seroprevalence according to residence. Similar results were found in Eastern England among 13,000 pregnant women (17), Denmark (20) and in Sweden (21).

The inverse relationship between *T. gondii* seroprevalence and level of education may be explained by the fact that more appropriate hygiene measures are expected to be maintained by educated women regarding eating habits and food preparation. The results disagree with those of Elnahas (11) who found no association between *T.gondii* seropositivity and educational level among pregnant women in Khartoum-Sudan. Our results were supported by the findings of Jones etal., (18) who found an inverse relationship between *T.gondii* seroprevalence and educational level in USA. Similar findings were also detected in Brazil (19).

The significant correlation between *T.gondii* seroprevalence and ethnicity in study group was similar to that of Elnahas (11) among pregnant women in Khartoum. Compared to some studies, there was a significant correlation between *T.gondii* seroprevalence and ethnicity in Taiwan (22), Malysia (23) and West London (24). The lack of correlation in control group is similar to that reported by Satti (12), and Abd Elraoaf (14), who studied different populations in Khartoum, their results revealed no association between *T. gondii* seroprevalence and ethnicity. These results agree with those of Julvez (25), who found no correlation between *T.gondii* seroprevalence and ethnicity in Niamy, Niger. No correlation was detected in Brazil in a cross sectional study of 1,261 pregnant women (19). The difference between the study and control group in the present study is difficult to explain. One possibility is the tribal composition in each group. For example 7.4% of the control group is from Southern Sudanese tribes with 0% in the study group. The tribes from central Sudan constituted 47.9% and 34% of the study and control group, respectively. It is well known that tribes differ in their eating and preparing habits of food.

The significant reltionship between seroprevalence and occupation may be attributed to the frequent handling of raw meat by housewives during food preparation. The results agree with others reported in Khartoum (12). These results were also supported by Spalding (26) who found a significant increase in seropositivity among housewives in Brazil.

Socioeconomic level in the study and control groups was found to be not correlated to increased or decreased *T. gondii* antibodies seroprevalence rate. Similar findings were reported in Khartoum (11,12,14). The results are not different from those of Ahmed (27) and Almalki (28) who found no significant association between *T. gondii* antibodies seroprevalence and socioeconomic status in KSA/ Riyadh. Also among Indian volunteers, *T.gondii* seroprevalence was found not to be correlated to socioeconomic level (29). The results may be explained by that, hygiene measures are similar in between low and high socioeconomic levels.

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No significant role was played by cats in the prevalence *of T.gondii* in this study. Although there are many stray cats in Sudan, they are not popular pets among our population. The lack of association between the prevalence of *T.gondii* and contact with cats could be explained by the fact that, infection in man is acquired via the oocysts shedded in cat's faeces, which represent the main cause of environmental contamination, so contact with cats is not the main problem. Other explanation was that most cats in Sudanese households do not come in close contact with people, but they live in courtyards and outside houses. Similar findings were reported by Abd- Elhameed (10) who found no correlation between *T.gondii* seroprevalence and cat contact in Gezira State-Central Sudan. Elnahas (11) also found no relationship between *T.gondii* seroprevalence and contact with cats among pregnant women in Khartoum, Similar findings were obtained in UAE (30), Malaysia (23), Brazil (26), and USA (18).

Women who eat undercooked meat among the present subjects were very few. They represented 0.05% (5 out of 94); accordingly, the number is too small to draw reasonable conclusions. A similar situation was found in the control group. The result may be explained by the fact that the vast majority of people in our community properly cook their meat. Elnahas (11) found no association between *T.gondii* seroprevalence and eating undercooked meat among pregnant women in Khartoum-Sudan. Similar findings were obtained from Saudi Arabia (27), Malaysia (23), and Brazil(31).

In both study and control groups women who used to eat marrara (raw herbivorous viscera) were around 4 times susceptible to *T.gondii* infection than others who did not. The strong association between seroprevalence and eating marrara could be attributed to that marrara is very popular in Sudan, and most of our community like eating (sheep marrara) so our subjects could probably get the infection from sheep, as they are considered a frequent host for *T.gondii* (32). Highly significant relationship was detected in Khartoum (11). In many studies, the association between acute *T.gondii* infection and raw or undercooked meat was a consistent finding (1,33,26).

There were few women in the study group, (4 out of 94) and none of our control group subjects who used to drink raw milk. No association was found between *T.gondii* seroprevalence and consuming raw milk; that may be due to the small number of our subjects used to drink raw milk. In addition to that, generally in Sudan, milk is taken after being boiled.

As very few women in our study consumed inadequately washed vegetables or fruits, we could not find any association between it and *T.gondii* prevalence. Other probability was the shyness of pregnant women to state a positive answer at the time of completing the questionnaire.

We did not find any correlation between *T. gondii* seroprevalence and past history of abortion in both groups. The role of *T.gondii* as a cause of repeated abortion was discussed before. Some studies found no association between *T.gondii* seroprevalence and previous history of abortion (34,15)

The increased seroprevalence by parity may partially be explained by the habit of eating raw or partially cooked liver by pregnant women in Sudan during pregnancy to elevate their haemoglobin levels or may be due to their carelessness when preparing food due to the large number of children. Prolonged exposure by age as a factor is also entertained. These findings agree with those obtained from Sudan (11), Malaysia (23), Amman (34) and Norway (35)

Twice higher *T.gondii* seroprevalence was shown by women with past history of congenitally malformed babies than others with no past history of malformations. These results are in agreement with those obtained from Egypt (36), Nigeria (37) and India (38). In China Sun et al. (39) suggested that the congenital malformations among newborn babies may be associated with congenital *T.gondii* infection.

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Further study of the same cases and controls using molecular techniques will follow.

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Table(1).Association between *T.gondii* seroprevalence and basic sociodemographic characteristics

		Seroprevalence%		Level of significance	
		Study group (n=94)	Control group (n=94)	Study group.	Control group.
Age	20-24	20	22.2	*P* = 0.03	*P* = 0.004
	35-39	68.8	76.5		
Residence	Urban	34.6	32.6	*P = 0.91*	*P = 0.19*
	Rural	35.7	45.8		
Level of	Illiterate	60	57.7	*P* = 0.01	*P* = 0.02
education	University	17.9	20.8		
Race	Arab	41.2	42.9	*P* = 0.02	*P = 0.37*
	Nigroid	16	31.6		
Occupation	House wives	58.3	61.9	*P* = 0.003	*P* = 0.0001
of respondent	Employee	9.1	7.7		
Socio- economic level	High level	26.9	39.1	*P = 0.59*	*P = 0.98*
	Low level	38.3	40.5		

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Table 2. Association between seroprevalence status and risk factors.

		Seroprevalence %		Level of significance	
		Study gr n=94.	Controlgroup. n=94	Study group.	Control group.
History of cat	Yes	36.8	32.8	*P = 0.59*	*P = 0.08*
contact	No	30.8	51.5		
Eating under Cooked meat	Yes	40	16.7	*P = 0.81*	*P = 0.09*
	No	34.8	42.7		
Eating marrara and raw liver	Yes	52.5	63.3	*P* = 0.002	*P* = 0.001
	No	22.2	28.1	*OR* = 3.87	*OR* = 4.41
Drinking raw milk	Yes	25	0	*P = 0.67*	
	No	35.6	39.4		
Eating inadequately washed vegetables and fruits.	Yes	0	0	*P = 0.30*	*P = 0.16*
	No	34.8	40.7		

Table 3. Association between seroprevalence status and past obstetrical history

		Seroprevalence%		Level of significance	
		Study group. n=94	Control group. n=94	Study group.	Control group.
Previous	Yes	44.4	41.7	*P = 0.14*	*P = 0.79*
history of abortion	No	29.3	38.6		
Parity	Primigravidae	15.6	27.3	*P* = 0.003	*P* = 0.034
	Grand multipara	56.3	59.3		
Previous history of having babies with	Yes	63.6	75	*P* = 0.035	*P* = 0.031
Congenital malformations	No	31.3	36		