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Original Research Article

Role of *Toxoplasma gondii* and Human Herpes Simplex Virus Type-2 in Women with Abortions and Congenital Abnormalities in Kirkuk City

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Abstract

A study was conducted during November 2013 to June 2014 to evaluate the role of Human Herpes Simplex viruses (HHSV) and toxoplasmosis among women with abortions and congenital outcomes in Kirkuk city, Iraq. A total of 232 sera were extracted from venous blood of women attending private gynecological clinics and Ibn-Alnafies private medical laboratories for detecting *Toxoplasma gondii* antibodies. Also 117 sera were extracted from women for detecting HHSV. The age group of women ranged from 15 years to 45 years and above. For both types of microorganisms, ELISA kits were used for detecting *Toxoplasma* antibodies (IgM and IgG) and HHSV type-2 (IgM and IgG). Of the 232 cases screened for *T. gondii* antibodies, 90 (38.98%) showed positive. High rates of *Toxoplasma* antibodies were recorded between the age group, 15 and 25 years, contributing 35.16% and 14.28% for *Toxoplasma* IgG and IgM respectively ($p<0.05$). No statistical difference was observed in the frequency of *Toxoplasma* occurrence (IgM and IgG), within the age groups of women ($p>0.05$). Regarding HHSV, the overall rate was 34.18% in 40 sera from total of 117 sera with a high rate of HHSV type-2 IgM (24.78%). Relationship between age group of women and frequency of HHSV type-2 was significant ($p<0.05$). Antibody crosses of HHSV type-2 were detected in 24 sera (18.46%) positive for *Toxoplasma* IgM compared to 80 sera (61.53%) positive for *Toxoplasma* IgG antibodies. The rates of *T. gondii* and HHSV antibodies were high among child bearing women in Kirkuk city. Due to antibody crosses between *Toxoplasma* and HHSV, it is wisdom to check women during pregnancy for *Toxoplasma* antibodies and HHSV-2 using ELISA technique to avoid congenital, postnatal outcomes and abortion.

Keywords

Antibody crossing

ELISA

Human Herpes Simplex virus

Kirkuk city

Toxoplasmosis

Introduction

The Human Herpes Simplex Viruses (HHSV) belonging to Herpes-viridae group produce infections in humans, especially HHSV type-1 and type-2 produce infections ranging from painful skin and genital ulcers (Rayan and Ray, 2008). Genital herpes is a recurrent lifelong inflammatory disease of the male and female genital tracts and most of people acquire genital herpes by sexual contact with an asymptomatic person infected with HHSV. Clinical manifestations involve primary genital herpes, which develops after short incubation period of 2-7 days, exerting local pain, tenderness, pruritus and dysuria (Chamberlain, 2008). In females, initial lesions are papules on a red erythromatous base that rapidly develop in to vesicles. The breakdown of vesicles develops into ulcers covered with a grayish exudate on the labia majora and minora, vaginal mucosa, cervix and perineal region (Brooks et al., 2008). The second manifestation is recurrent genital herpes which resembles but less severe and resolve rapidly. Recurrences may be hormonally triggered during menses specially after four months from first episode and recurrences of genital herpes in HHSV-2 are more frequent and sever than HHSV-1 infected patients (Georing, 2008). The virus can cross the placenta and cause stillbirth or extreme teratogenic effects. Newborns who acquire the virus during passage through the birth canal of a symptomatic mother usually develop disseminated disease, which includes vesicular lesions, pneumonia, hepatitis and infections of central nervous system (Cooke, 2008).

Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*. It is commonly transmitted to humans by accidental ingestion of oocyst stage of the parasite after cleaning an infected cat's litter box. Other routes of transmission include accidental ingestion of the parasite in contaminated soil and drinking water and consumption of infected raw meat. It can also be transmitted congenitally during pregnancy (Dubey, 2010). Generally, it is estimated that about one third of the World's population is infected with *T. gondii*. High prevalence of the infection have been reported among pregnant women and women of childbearing age from different foci in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa (Pappas et al., 2009). However, the prevalence of *T. gondii* in pregnant women in China was less than 10% (Gao et al., 2012). In Africa, overall sero-prevalence rate as high as 92.5% has been reported (Ayi et al., 2009). In Iraq, exactly in Kirkuk province, the rate was 16.54% (Salman, 2014). Most pregnant women infected with *T. gondii* are chronically infected while few acquire the infection during pregnancy (Hassan et al., 2014). Pregnant women with acute infection during pregnancy are at risk of congenitally transmitting the infection to the fetus. Congenital transmission as a result of primary infection during pregnancy is higher if the infection is acquired during the third trimester of pregnancy and is lower if the infection occurs during the first trimester. But, congenital infection occurring during the first trimester may result in high risk of tragic outcomes, which may include abortion (ANowakowska et al., 2006), than the infection at the third trimester (Dunn et al., 2006). In spite of the presence of stray cats and suitable climatic conditions favoring survival of the parasite in the study area, most of cervical or vaginal women infections due to HHSV are symptomatic. To our knowledge, there is no documented data on the prevalence of HHSV infections in Kirkuk city. Absence of documented data initiated us to undertake this study for evidence-based role of *T. gondii* and HHSV in women abortions and congenital outcomes and also to assess antibody crosses in sera of women between to microorganisms.

Materials and methods

Time and location

From 1st November 2013 to 30th June of 2013 a cross sectional study was carried out in Ibn-Nafies Private medical laboratory-Kirkuk city-Iraq.

Patients' selection and blood sampling

Three groups of women were selected for sampling who were referred by obstetricians and gynaecologists in private clinics to medical

laboratory. Prior to blood sampling a special questionnaire was completed for each patient, which contained all required information.

The women in the study were classified into three groups: **First** group included 232 women with Bad Obstetrician History (BOH), who suffered from previous abortions, congenital abnormalities and some cases with recent abortion were also included in the group. **Second** group involved 117 women with urogenital problems such as genital herpetic lesions or with vaginitis and cervicitis. **Third** group contained 100 women without previous or recent abortion, or any signs of urogenital infections.

The age group of women participated in the study were from 15 years to 46 years and above. Five ml of venous blood sample was drawn from each woman selected for the study. Sera were extracted after centrifugation and kept at -20°C till use. The serological tests were done using IgM and IgG ELISA Bio-kits reagents manufactured by Barcelona-Spain to detect anti-IgM and anti-IgG specific for *T. gondii* and HHSV-2, in accordance with the manufacturer's instructions; ELISA procedures for *Toxoplasma* and HHSV was done according to Al- Jubori (2005).

Statistical analysis

The data processing was done by using Statistical Package of Social Science (SPSS) version 16.0. Data description was presented as means with their standard errors (SE) and standard deviation (SD) that were calculated to reflect the size and precision of the estimated values. The independent sample *t*-test of significance was used for the comparison between two groups. The lowest level of significance was chosen when the probability (p) was less than or equal to 0.05($p \leq 0.05$).

Results

By examining the total 232 sera of women, the overall rate of toxoplasmosis was 38.79%, among which *Toxoplasma* IgG antibodies and *Toxoplasma* IgM antibodies accounted for 29.74% and 9.05% respectively ($p<0.05$). The relationship between *Toxoplasma* antibodies distribution and age group of women was significant ($p<0.05$), especially in the sera of women whose age group was between 15 and 25 years: *Toxoplasma* IgM-14.28% and *Toxoplasma* IgG-35.16%. Statistical analysis showed that the overall rate of toxoplasmosis with women age group was not significant, $p>0.05$ (Table 1).

Table.1 Frequency of *T. gondii* antibodies in sera of women with abortion and congenital abnormalities with reference to age group (*$p<0.05$)

Age group in years	Sera of women examined		*Toxoplasma* IgM Antibodies		*Toxoplasma* IgG antibodies		*Toxoplasma* total	
	No.	%	Positive		Positive		Positive	
			No.	%	No.	%	No.	%
15 to 25	91	39.22	13	14.28*	32	35.16*	45	29.54
26 to 35	75	32.32	5	6.66	18	24.00	27	26.02
36 to 45	48	20.68	3	6.25	11	22.91	15	27.65
46 and above	18	7.75	0	0.00	3	5.55	3	50.00
Total	**232**	**100.00**	**21**	**9.05**	**69**	**29.74**	**90**	**38.79**

Table 2. Distribution of Human Herpes virus IgM and IgG antibodies in sera of women with reference to age group (*$p<0.05$).

Age group in years	Sera of women examined		HHSV-2 IgM antibodies		HHSV-2 IgG antibodies		HHSV total	
	No.	%	Positive		Positive		Positive	
			No.	%	No.	%	No.	%
15 to 25	35	38.68	14	40 *	3	8.57	17	48.57 *
26 to 35	45	27.98	8	17.17	2	4.44	10	22.22
36 to 45	22	26.53	5	22.72	5	22.72*	10	45.45
46 and above	15	6.81	2	13.33	1	6.66	3	20.00
Total	**117**	**100.00**	**29**	**24.78**	**11**	**9.40**	**40**	**34.18**

In order to assess the antibodies cross reaction between *T. gondii* and HHSV, 40 sera positive for HHSV were tested by using ELISA kit for *Toxoplasma* antibodies IgM and IgG, and 130 sera positive for toxoplasmosis (50 for IgM and 80 for IgG) were tested for HHSV type-2 as shown in Table 3. High frequencies of 31.03% and 18.18% *Toxoplasma* IgG were detected in sera of women with HHSV type-2 IgM antibodies and HHSV type-2 IgG respectively; whereas HHSV type-2 IgM and HHSV type-2 IgG was detected in 6.89% and 9.09% of cases respectively ($p<0.05$).

Table.3 Cross reaction of *T. gondii* IgM and IgG antibodies with Human Herpes Simplex Virus type-2 (HHSV-2)

	T. gondii		*Toxoplasma* IgM		*Toxoplasma* IgG	
			No. positive	% positive	No. positive	% positive
HHSV-type 2	**No. positive**	**% positive**	21	9.05	69	29.79
HHSV-2-IgM	29	24.78	2	6.89	9	31.03
HHSV-2-IgG	11	9.40	1	9.09	2	18.18
Total	**40**	**34.18**	**24**	**18.46**	**80**	**61.53 ***

Total *Toxoplasma* IgM+HHSV-2 IgM =50; Total *Toxoplasma* IgG+HHSV-2 IgG = 80. Total of *Toxoplasma* and HHSV-2=130 * $p<0.05$.

Table 4 is showing the frequency of *T. gondii* and Human Herpes Simplex virus type-2 (HHSV-2) among different age group of women in control group, where two positive *Toxoplasma* IgM antibodies were recorded one each in sera of women aged between 15 and 25 years and 46 years and above respectively. Among age group of 26 to 35 years, sera positive for *Toxoplasma* IgG antibodies was 7 which was highest among age groups. Regarding sera positive for HHSV type-2, only four sera revealed positive for HHSV type-2 IgG antibodies ($p>0.05$) (Table 4).

Table.4 Frequency of *T. gondii* and Human Herpes Simplex virus type-2 (HHSV-2) among different age groups of women in control group

Age group in years	No. Exami-ned	*T. gondii*						HHSV type-2					
		IgM		IgG		Total		IgM		IgG		Total	
		No.	%+ve	No.	% +ve	No.	%+ve	No.	%+ve	No.	%+ve	No.	% +ve
15 to 25	31	1	3.03	3	9.09	4	12.12	0	-	2	6.06	2	6.06
26 to 35	34	0	0.00	7	20.5	7	20.50	0	-	1	2.94	1	2.94
36 to 45	23	0	0.00	3	13.04	3	13.04	0	-	1	4.34	1	2.94
46 and above	12	1	8.33	2	16.66	3	24.99	0	-	0	0.0	0	0.0
Total	**100**	**2**		**15**		**17**		**0**	-	**4**		**4**	

According to the clinical presentations taken from women in the study, the analysis of data revealed significant relationship between toxoplasmosis and abortion especially among women with double abortions. The results recorded showed a high positive rate of 36.76% and 7.24% recorded for *Toxoplasma* IgG and IgM antibodies respectively. HHSV type-2 IgM showed 33.3% and 19.4% which were recorded in women with tetra and penta abortions respectively. Stillbirths were recorded in women positive for *Toxoplasma* antibodies, and the rates were 66.66% and 33.34% for *Toxoplasma* IgM and IgG antibodies respectively ($p<0.05$) (Table 5). Neonatal herpes and complications were recorded only in sera of women positive for HHSV type-2 IgM, the positive rate was 100%. While ocular complaints were distributed in sera of women positive for *Toxoplasma* IgM and HHSV type-2 IgM antibodies, and the rates were 66.66% and 33.34% ($p<0.05$). Sera of women positive for *Toxoplasma* IgM of 58.33% and 41.67% for HHSV type-2 IgM antibodies were recorded in women whom they had premature babies (Table 5).

Table.5 Distribution of *T. gondii* and Human Herpes Simplex virus type-2 (HHSV) with reference to selective clinical presentations

Clinical presentation	Number of cases	*Toxoplasma gondii*				HHSV-type-2				All total	
		IgM		IgG		IgM		IgG			
		+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Abortions:											
Single abortion	72	3	4.16	19	26.38	7	9.72	3	4.16	27	37.5
Double abortions	69	5	7.24	25	36.76	7	10.14	4	5.79	36	52.17
Triple abortions	48	4	8.33	12	25.00	5	10.41	0	0.00	21	43.75
Tetra abortions	31	3	9.67	11	35.48	6	19.35	3	9.67	23	74.19
Penta abortions	12	6	50.00	23	3.33	4	33.33	0	0.00	12	100.00
Stillbirths	6	4	66.66	2	33.34	0	0.00	0	0.00	6	100.00
Neonatal herpes	5	0	0.00	0	0.00	5	100.00	0	0.00	5	100.00
Ocular complains	9	6	66.66	0	0.00	3	33.34	0	0.00	9	100.00
Premature	12	7	58.33	0	0.00	5	41.67	0	0.00	12	100.00
Total	**264**	**38**	**14.39**	**71**	**26.89**	**42**	**15.90**	**10**	**3.78**	**161**	**60.98**

Total number examined for *Toxoplasma* =232; Total number examined for HHSV type-2=117;

Discussion

This study showed an overall rate of 38.79% sero-prevalence of anti-*T. gondii* antibody among women in Kirkuk Province. This finding was higher than the prevalence among women in the same province reported by Salman (2014) and Al-Jubori (2005). Similarly, the IgG sero-prevalence of *T. gondii* obtained in this study was higher than those reported from Palestine (Nijem and Al-Amleh, 2009), Saudi Arabia (Mohammad et al., 2010), Brazil (Vaz et al., 2010), Sudan (Elnahas et al., 2003), Morocco (El Mansouri et al. 2010) and China (Gao et al., 2012). In contrast, lower sero-prevalence of *T. gondii* reported in the present study differs from the study by Kasim (2013) and Tewfik (2013) in Kirkuk, whom they found the following rates respectively 91.6% and 48.9%. Also the reported rate in the study was lower than that recorded in Ethiopia (Zemene et al., 2012), whom they recorded 83.6% (Acharya et al., 2014). The variances in the results may be attributed to differences in the number of the specimens, type of methods, source and type of *Toxoplasma* kits or due to large number of specimens included in the present study. This wide variability could be attributed to differences in climatic conditions and personal hygienic practices, feeding habits, socio-economic and literacy status of the study category.

Regarding HHSV-2 sero-prevalence studies show variation in infection by geographic location. Some of the highest prevalence of HSV-2 among women with abortions has been found in Nepal-84.4% (Acharya et al., 2014), in Babylon 82.7% (Al-Marzoqi et al., 2012) and Diala-Iraq-73.9% (Jasim et al., 2011). Lower prevalence has been found in India (Haider et al., 2011) and in Baghdad (Tuma et al., 2013) whom they record 16.8% and 8.26 % of HHSV-2 among women with BOH respectively. HHSV-2 prevalence has also been found to vary by individual-level characteristics, including gender, age, sexual activity level, marital status, socio-economic status, education and race/ethnicity (Xu et al., 2006). However, these characteristics are insufficient to explain differences within and between countries, regions and population sub-groups, suggesting the need to identify ecologic factors which may help to explain the differences (Smith and Robinson, 2002).

The results obtained in the present study showed that the overall rate of 34.18% for HHSV infections (IgM-24.78% and IgG-9.40%) is critical for women in third trimester especially IgM, because it means acute infection with probability of transporting the infection to fetus during passage through birth canal at the time of delivery (post-delivery outcomes such as pneumonia, conjunctivitis). HHSV-IgG mostly indicate protection with probability of sero-conversion from latency in some cases (Salman, 2007). In addition to high records of HHSV-2 IgM positive in sera of 14 young aged women from a total of 40 is highlighting the exposure to degree of sexually transmitted diseases (STD) in women community in this province, and the predisposing factors

might be attributed to sexual activities, hormonal changes, use of contraception or bad care in ignoring contraceptive methods by men. Furthermore it might be the fact that most of HHSV-2 infections were asymptomatic. Co-existence of microorganisms in some cases becomes life threatening of immnuo-compromised patients and even among some conditions such as malnutrition, poverty, pregnancy, so antibody crosses finding in this study was very important among women with *Toxoplasma* IgM and HHSV-2 IgM antibodies (6.89% of *Toxoplasma* IgM positive from 24.78% HHSV-2 IgM), and this finding is close to that recorded by Jasim et al. (2011). Also this finding reflects the degree of injury to women health in childbearing or conceived women because the immune system tolerate or neutralize pathogens is other success in causing damage to fetus (Salman, 2014).

According to clinical presentations, the highest infection was reported in women who had repeated bad pregnancy with spontaneous abortion may due to primary, recurrent infection or reactivation from chronic toxoplasmosis. Primary or recurrent HSV infection in pregnancy and its serious consequences for the fetus and neonate have attracted much interest. Specifically, primary HSV infection during the second or third trimester can be related to pre-term labor, fetal abnormalities and pregnancy loss, whereas recurrent HSV infection constitutes a much lower risk for the embryo, fetus and neonate (Kapranos and Kotronias, 2009). In contrast, the association of HSV with first trimester pregnancy loss, despite the initial epidemiological observations has not been fully elucidated and remains controversial (Tuma et al., 2013). It is recognized that primary CMV infections occurring at an early gestational age are more likely to cause fetal damage than recurrent infections (Sarawathy et al., 2011). In cases of spontaneous abortion (SAs) that HSV is a causative agent, there is a risk for later SAs in spite of the fact that HSV infection is not primary and this may occur even in the absence of clinical signs and symptoms (Bujko et al., 1988).

The second woman clinical presentation was premature labour (12%) with no significance between *Toxoplasma* IgM and HHSV-2 IgM and this finding was in agreement with 12.43% recorded by Al-Marzoqi et al., 2012). The reason to this might be due to *Toxoplasma* proliferation inside the placenta and to adverse effects of herpes colonization in genitalia of women. Considering stillbirths, only 6 cases were record for *T. gondii*, reflecting severity of toxoplasmosis during gestational periods when there is high parasitemia and weak immune response against primary infections (Pappas et al., 2009). The high ocular involvement due to toxoplasmosis in the present study than HHSV-2 infection might be due to the gain of *Toxoplasma gondii* infection in third trimester, and high optical nerve infection due to parasites adverse actions while the effect of HHSV on trigeminal ganglia revealing neonatal herpes.

Conclusion

Although the two attributions of viral and protozoan infections were moderately prevalent in the present study, the abortion and congenital anomalies were highly associated with infectious agents. The present study demonstrates that infectious agents linked with abortion and congenital anomalies in women with BOH. It is recommended that all antenatal cases should be screened for these agents. In addition, carrying out further studies such as DNA detection has to be conducted to allow assessment of the serological diagnosis more accurate.

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