Original Research Article

Correlation between *Toxoplasma gondii* and Anti-Mullerian Hormone Levels in Sera of Women In Kirkuk City Using ELISA Method

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A B S T R A C T

Keywords

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Toxoplasmosis is one causative agent of women abortion and congenital outcome. While anti-Mullerian hormone (AMH) is an essential hormone that indicates ova reserve in female and have had vital role in women infertility. This study was planned to determine *Toxoplasma* incidence among women with abortions and to assess correlation between toxoplasmosis and AMH levels among women in Kirkuk city-Iraq. To achieve that, a total of 441 sera were tested using AMH Elisa kit, while 224 sera were chosen for detecting toxoplasmosis using *Toxoplasma* Elisa kit. Results: women AMH levels were distributes in to 205(49.88 %) for low AMH level, followed by 120(30.41%) and 81(19.71 %) for satisfactory AMH level and ovary potential level respectively, P<0.05. Low AMH was more frequent among women aging from 36 to 45 years, while ovarian potential AMH was among women aging from 15 to 25 years. The overall sero-prevalence of *T. gondii* in the study area was 29.46 % with frequent of 25.78 % of *Toxoplasma* IgG compare to 2.67 % for *Toxoplasma*IgM ,P<0.05.Relationship between *Toxoplasma* distribution and women ages was not significant P>0.05. The all rate of toxoplasmosis according to AMH levels was 16.05 % with high dominance of *Toxoplasma*IgG antibodies rate 14.59 %   compare to 1.45 % for *Toxoplasma*IgM ,P<0.05 . Women sera of low AMH reveal 11.70 % of *Toxoplasma* antibodies compare to 12.34 % of toxoplasmosis with high level of AMH P>0.05.Conclusion:Rate of toxoplasmosis among women in kirkuk city is high. AMH levels among women in Kirkuk city are variable and the relationship between toxoplasmosis and women AMH level is significant P<0.05.

Introduction

Toxoplasmosis is a parasitic infectious disease caused by a protozoan Toxoplasma gondii*(T. gondii)*, which is transmitted to humans through the infection of food or water contaminated with cat faces or eating undercooked meat of the infected sheep, goat, cow, or pig and other avian species

(Sadeq *et al.,*2012) Identification of positive titer of immunoglobulin G (IgG) and immunoglobulin M (IgM) during pregnancy in women with previous negative titers of anti-*Toxoplamsa*IgG antibodies suggests a proliferative disease condition dangerous to the fetus(Taher, 2012) and (Noori,2013). If a

pregnant women contract toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital *Toxoplasma* infection stimulates humoral immune response as antibody production, which includes IgM and IgG, in addition to cell mediated immunity (Darcy and Santro,1994) .Toxoplasmosis is one of the classical conditions known to have a profound adverse effect on human reproductive functions (Akarsu *et al.,* 2011). Experimental evidence has established that, mice undergo acquired hypo-gonadotrophic hypo-gonadism secondary to hypothalamic dysfunction after chronic infection with *T. gondii* (Stahl, 1994) and (Antonios *et al.,* 2011). Furthermore, it has been recently reported that children with congenital toxoplasmosis have a high frequency of precocious puberty (Setian *et al.,* 2002) Although previous reports suggest that *Toxoplasma* infection may cause transient hypo-gonadotrophic hypo-gonadism, no detailed analysis has been conducted in humans (Eslamirad *et al.,* 2013) In women during toxoplasmosis, the proliferative tachyzoites may invade female reproductive organ specially the oviducts, subsequently evoke tissue cysts formation in it, that may produce tubule dysfunctions, the latter action can leads to hormonal abnormalities which give rises into secondary infertility sequels (Salman, 2014).

Anti-Mullerian hormone (AMH)also known Mullerian Inhibiting Substance (MIS), it is a glycoprotein that belongs to the transforming growth factor-B (TGF-B)—a member of the superfamily of growth and differentiation factors. It has been studied for its regulatory role in male sex differentiation (Ibrahiem, 2012). AMH produced by sertoli cell of the fetal testis, includes regression of mullerian ducts, the anlagen of female reproductive tract and is a substance produced by granulosa cells in ovarian follicles. It is first made in primary follicles that advance from the primordial follicle stage. At these stages follicles are microscopic and cannot be seen by ultrasound (Josso *et al.,* 1993). AMH production is highest in parental and small antral stages (less than 4mm diameter) of development. Production decreases and then stops as follicles grow. There is almost no AMH made in follicles over 8mm. Therefore, the levels are fairly constant and the AMH test can be done on any day of a woman's cycle (Pellat, 2007) Anti-Müllerian hormone seems to be the best endocrine marker for assessing the age-related decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan (Guyton and Hill, 2006). The most established role for AMH measurements is before in vitro fertilization is initiated, because AMH can be predictive of the ovarian response, namely poor and hyper-responses (Grynnerup *et al.,* 2012).

The clinical implications of infection due to Toxoplasma in pregnant patients are manifold. Such patients mayhave spontaneous abortions, stillbirth, intrauterine growthretardation, preterm deliveries or fetal anomalies. In addition to the risk of gestational complicationsand congenital infections, it has been suggested that toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women (Flegr, 2013). The role of toxoplasmosis in habitual abortions is well established, however, only a few studies had been done for its association with infertility. This baseline study is probably the first study from North Iraq (Kirkuk Province) to evaluate the role of *T. gondii*in infertility. The present study assessments were tested role of T.*gondii*in two distinct groups: (i) in women with Bad Obstetrician History BOH; and (ii) in women with primary and secondary infertility.

Materials and Methods

1. Time and location: From 1st October 2013 to 31th of March 2014, a cross sectional study was carried on in Ibn-Nafies Private medical laboratory-Kirkuk city-Iraq.

2-Patients selection and blood sampling: Two groups of women were selected by obstetricians in private clinics, they referred to laboratory. Prior to blood sampling a special questionnaire was completed for each patient, that contains all required informations , then they classified either they are infertile or they are fertile, but they suffering from Bad Obstetrician History(BOH),such as abortion, congenital abnormalities . All women participate the study their age were from 15 years to 46 years over. For women with infertility a total of 441 of venous blood samples were drawn and the same volumes of venous blood samples were drawn from 224 women with BOH. Sera were extracted after centrifugation and kept at -20 ^{0} C till to use. Inclusion criteria involve:patients agreement participation in the study, while infertile women have five basic investigations(Ovulatory assessment ,Hysteo-salphengeo-graphy, laparoscopy, post coital test and seminal fluid analysis).Any patient has abnormal investigation apart from ovulatory assessment was excluded from the study.

3. Procedures: For detecting toxoplasmosis, direct toxoplasma agglutination test was performed for detecting positive cases, and then confirmed by Elisa using separate kits of Elisa-Toxo-IgM kit and IgG Kit manufactured by Bio-kit company-Spain. All Elisa kits were purchased from local scientific shops. For the second group of women anti-Mullerian hormone assay was performed using Elisa kit imported from USA. Prior to assessment all sera and the contents were brought to room temperature and the procedure assay included the following steps: 100µl of diluent buffer was transferred in to microplates and then after 20 µl serial standards, positive and negative controls and for each serum has been added except the blank

Results and Discussion

From assessments of 411 sera of women with infertility, low level of anti-Mullerian hormone was detected in 205 sera with a rate of 49.88 %, followed by satisfactory AMH level 30.41 % in sera of 125 women, while ovarian potential rate was 19.71 % in sera of 81 women, P<0.05.According to women age high rate of low AMH 85.32 % was recorded among women aging from 36 to 45 years, controversy to low rate 19.49 % among women aging from 15 to 25 years. Satisfactory AMH level 47.16 %was highly distributed among women aging from 15 to 25 years compare to 7.33 % in sera of women ageing from 36 to 45 years. The same relationship was obtained in relation AMH distribution and ovary potential, via which ovary potential (High level of AMH) was recorded among women age from15 to 25 years, the rate was 33.33 % compare to 7.33 % and absence of ovary potential among women aging from 36 to 45 years and among women aging 46 and over respectively, Table 1 and Table 2 showing frequency of *Toxoplasma gondii*IgM and IgG antibodies among 224 sera from women with different levels of AMH .The overall rate of *Toxoplasma* was 29.46 %, this rate was divided into 26.78 % of *Toxoplasma*IgG antibodies and 2.67 % for *Toxoplasma*IgM,P<0.05. According to women age, *Toxo* positive rate in 224 sera from women with different levels of AMH; *Toxoplasma*IgM was highly frequent among women aging from 36 to 45 years the rate was 4.25 % .While *Toxoplasma*IgG rate 50 % was exert in sera of women aging from 46 years and over. Correlation between

Toxoplasma distribution and women anti-Mullerial levels was exerting in Table 3,via which high rates of *Toxoplasma*IgM antibody 3.2 % and *Toxoplsam*IgG 22.4 % were recorded among women with satisfactory AMH level, P <0.05. Regarding to *Toxoplasma*IgM antibody, this type of antibodies was not recorded in sera of women with low level of AMH.

From the results in Table 1,it is obvious that the rate of low AMH is higher than satisfactory AMH ,this finding can reflects that women has had baby or child conceiving problem rather than toxoplasmosis. The more suitable cause to this may be tubule obstruction because the rate 50.43 %of low AMH among women aging from 26 to 35 years is high and this is also pointing to rate of infertility among this age group. While high rates of low AMH among women aging from 36 and 0ver 46 years and low rate 7.3 % of satisfactory AMH levels can be considered normal hormonal condition, because it has been found that serum AMH level decreases gradually with aging, and becomes undetectable after menopause (Shine, 2008). Low AMH level in the present study is agree with that recorded by (Barad *et al.,* 2009) and with that recorded in Kirkuk province in 2012 by Ibrahim, (2012). Considering high level of AMH among women except women over 46 years, this finding can be attributed to abuse of ovulatory inductive drugs by women .On the other hand the abuse may be by the obstetrician advising such drugs in condition when the result of follicle stimulating hormone (FSH) is not normal, or an error in administration of ovulatory inductive drug prior to In-Vitro fertilization (Pellat *et al.,* 2007) and (Kamil, 2011) Also a patient with elevated estrogen level for instance may lead to false assumption of normal FSH and ovarian reserve (Fanchin *et al.,* 2003) Serum AMH level fluctuated very little during menstrual cycle and therefore

can be takes at any time during menstrual cycle (Marca *et al.,* 2006)).Regarding toxoplasmosis, this study showed an overall 29.46% sero-prevalence of anti-*T. gondii* antibody among women in Kirkuk city. This finding was lower than those recorded by (Othman, 2004) and (Al-Jubori, 2005) in the same province ,whom they record 36.67 % and 33.53 % respectively . It was also lower thanthoserecorded in Baghdad, Faloja ,Erbil, Mosul and Tikrit by (Ismaiel, 2012), (Abul-Mohaymen *et al.,* 2009), (Al-Doski, 2000), (AlWattari, 2005) and (Ahmed, 2008), whom they record the following rates 32%,32.50 % ,46.9%,48.7% and 55.36 % respectively. Also *Toxoplasma* rate was lower than those recorded in Tunisia 58.45 % by (Bouratbine *et al.,* 2001), 57.52 % in Eygptby (El-Tantawi *et al.,* 2014) and with 77% in Turkey by (Poryaz *et al.,*1995) . While the overall rate of toxoplasmosis was higher than those recorded in England and India by Panigrahi *et al.,* (1978) and Broadbennt *et al.,* 1981) whom they recorded the following rates 24.19% and 27.3 % respectively. The variances in *Toxoplasma* rates are factorial: First ,might be due differences in study size, because most of compared studies above used small size of specimen compare to total 635 samples in the present study. Second, type of laboratory methods; such as Elisa that applied in the study which is more specific and reliable than other methods. Third; may be attributed to some technical pitfalls or serum quality such as; usage of hyper-lipidemic ,hemolysed, icteric or incomplete thawed serum , granulated antigen in some studies (Salman, 2007). Similarly, the IgGsero-prevalence of *T. gondii* obtained in this study was higher than those reported from Palestine by (Nijem and Al-Amlih, 2007), Saudi Arabia by (Mohammad et al., 2010), Sudan (El-Nahas *et al.,*2003) and in Brazil by (Vaz *et al.,*2010). 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 subjects. The observed difference in the rates of infection could bedue to variation in age distribution and antibody profiles of the study populations. The results of *Toxoplasma* antibodies rate 16.05% according to levels of AMH is vital when it was subtracted from overall 29.46 % ,because this finding is highlighting the degree of adverse effect of toxoplasmosis on women health in Kirkuk community , in addition to high rates of toxoplasmosis among women with ovary potential 12.34 % and 11.77 % among women with low AMH. The more important finding is 3.2 % of women with satisfactory AMH level have had *Toxoplasma*IgM antibodies, which indicate that they facing outcomes of toxoplasmosis in next pregnancy. Also *Toxoplasma*IgG rate 14.59 % is crucial for the next pregnancy especially when the case is latent toxoplasmosis, which mostly gives raise to sero-conversion. **Conclusion:** Watching of toxoplasmosis is essential for child bearing women and women with abnormal AMH. Relationship between toxoplasmosis and AMH among women with infertility is significant.

Table.1 Distribution of anti-Mullerian hormone according to women ages

Age/years	Number examined	Percentages %	Low AMH		Satisfactory		Ovary potential	
			No. exam	%	No. exam	%	No. exam	%
15 to 25	159	38.68	31	19.49	75 *	47.16	53 *	33.33
26 to 35	115	27.98	58	50.43	37	32.17	20	17.39
36 to 45	109	26.53	93	85.32*	8	7.33	8	7.33
46 and over	28	6.81	23	82.14	5	17.85	0	0.00
Total	411	100.00	205	49.88	125	30.41	81	19.71

*P<0.05

Table.2 Frequency of *Toxoplasma gondii*IgM and IgG antibodies in relation to women ages

Age /years	Number examined	Percentages %	*Toxoplasma*IgM antibodies		*Toxoplasma*IgG antibodies		*Toxoplasma* total	
			Number positive	Percentages %	Number positive	Percentages %	No. positive	% +ve
15 to 25	88	39.28	3	3.40	23	26.13	26	29.54
26 to 35	73	32.58	1	1.36	18	24.65	19	26.02
36 to 45	47	20.98	2	4.25*	11	23.40	13	27.65
46 and over	16	7.24	0	0.00	8	50.00*	8	50.00
Total	224	100.00	6	2.67	60	26.78	66	29.46

*P<0.05

Table.3 Distribution of *Toxoplasma gondii*antibodies(Toxoplasma IgM, IgG) according to women anti-mullerian hormonal levels

Anti-Mullerian Hormone levels	*Toxoplasma*IgM antibodies			*Toxoplasma*IgG antibodies		*Toxoplasma* total	
	No.exam	No.+ve	% positive	Number positive	Percentages %	Number positive	Percentages %
Low	205	0.00	0.00	24	11.70 *	24	11.70
Satisfactory	125	4	3.2	28	22.4	32	25.6 **
Ovary potential	81	2	2.46	8	9.87	10	12.34
Total	411	6	1.45	60	14.59	66	16.05

*,** P<0.05

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