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SEROLOGICAL DIAGNOSIS OF ANTI-*TOXOPLASMA GONDI IGM* AND IGG IN IRAQI WOMEN USING THE ENZYME LINKED FLUORESCENT ASSAY (ELFA)

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ABSTRACT
A serological diagnosis of anti –*Toxoplasma gondi* IgM and IgG in women (pregnant at first trimester of pregnancy and non pregnant,19-39 years old) using the Enzyme linked fluorescent assay (EIFA) .The Results revealed that all the pregnant and non pregnant women were IgM were negative except that 3 of 112 (2.7% for group one) and 7 out of 112 (6.3%) for group two, indicate that all these women were rarely infected with acute *T. gondi* whereas the pregnant and non pregnant women showed in group two IgG positive with a ratio (43.75%) and IgG negative with a ratio (56.25%) indicate that all the IgG positive women (49) (21 pregnant and 28 non pregnant) were chronically infected with *T. gondi* whereas 63 (27 pregnant and 36 non pregnant) were non infected.

KEY WORDS: anti-*Toxoplasma gondi*, IgM and IgG, ELFA.

INTRODUCTION

Toxoplasma gondi is an obligatory intra- cellular parasite and has a primary host is the filidae and scattered in nature and invade all orders of mammals and human being[1]. Toxoplasmosis is usually asymptomatic but can have severed consequences if it occurs in immune deficient subjects and fetuses[2]. Women who are seropositive before they become pregnant are essentially protected from transmitting the infection to their unborn child[3] , whereas, women who are sero negative at risk of becoming infected during gestation [4]. Transmission to the fetus occurs during the acute stage of the infection in the mother. *Toxoplasma* crosses the plasenta barrier to cause congenital infection which depend on virulence of parasite, the size of the inoculum, the immune response of patient and during gestation when the mother became infected[4,5]. Regarding the prevalence of toxoplasmosis varies depending geographical location, age and gender of population. In Europe the prevalence range from 20% to 85%, while in the united states, the prevalence is lower (12% to 41%). Prevalence in other countries (Arabic countries) can vary from 18% to 65% [6]. The diagnosis of *T. gondi* infection is depend on biological examination and specific immunoglobulin detection IgM and IgG [6]. The diagnosis of acquired infection during pregnancy is established by demonstration of a seroconversion or by significant rise in antibodies titer in sequential sera assayed concomitantly[7]. The studies of this disease are scanty in Iraqi women in addition to confusion associated with diagnosis. This study aimed to confirm a serological diagnosis for anti- *T. gondi* IgM and IgG in women using enzyme linked fluorescent assay (ELFA).

MATERIALS & METHODS

1- Specimens (112) serum samples for IgM detection were taken from women (23) pregnant at first trimester of gestation and (89) non pregnant. All the women had a history of spontaneous abortion and submitted to Gyenocology private clinics during the period between 19/4/2011 -19/ 9/2012, women ages were between 19-39 years.
2- Specimens (112) serum samples for IgM and IgG detection were taken from the women (26) pregnant at first trimester of gestation and (86) non pregnant also with a history of spontaneous abortion and the similar ages and periods of time which submitted to the same Gyenecology private clinics .
3- Toxoplasma IgM and IgG test kits : vidastoxo IgM and IgG tests is a qualitative and quantitative tests respectively for use the minividas instrument for detection IgM and IgG antibodies (Ab) against *toxoplasma gondi*in human being .

Content of toxoIgM and IgG test kits

1- Solid Phase Rsceptacle (SPR) for IgM, IgG
2- Positive control (C1) for IgM & IgG
3- Negative control (C2) for IgM & IgG
4- Standard (S1) for human IgM & IgG
5- MLE card specific sheet containing the factory master Calibration data required to calibrate the test (IgM & IgG).

***Principles of IgM and IgG tests**

1- Both had a similar principles, but in case of IgM the assay principles combine an enzyme immune assay method by immune capture with a final fluorescent detection where as in case of IgG the assay principle combine a two-step enzyme immune

assay sandwich method with a final fluorescent detection .

1- The solid phase receptacle (SPR) serves as the pipetting device for the assay. Reagent for the assay was ready to use and predispensed in the sealed reagent strip.
2- All the assay steps are performed automatically by the instrument, the reaction medium is cycled in and out of the SPR several times .
3- After a sample dilution step, the IgM are captured by the polyclonal Abs coating the inferior of the SPR, the IgM are specifically detected by inactivated toxoplasma Ag which itself revealed by an alkaline phosphatase labeled murine monoclonal antitoxoplasma Ab (anti-p30) . In case of IgG after a sample dilution step, also the sample is cycled in and out of the SPR, the antitoxoplasma IgG Ab in the specimen will bind to the toxoplasma Ag coating the inferior of the SPR. Unbound component were eliminated during washing steps. Mouse monoclonal anti-human IgG conjugated with alkaline phosphates is cycled through SPR and will attach to any human IgG bound to the SPR wall .
4- During the final detection step the substrate (4-methyl –umbelliferyl phosphate) is cycled in and out of the SPR .The conjugate enzyme catalyzes the hydrolysis of the substrate into fluorescent product (4methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of fluorescence is proportional to the concentration of antibodies present in the sample .
5- At the end: In case of IgM assay an index is automatically calculated by the instrument in relation to the S1 standard stored in memory and then printed out .

In case of IgG assay results are automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out results and interpretation:

Index (IgM)	interpretation	Titer IgG (Iu/ ml)	interpretation
< 0.55	Negative	< 4	Negative
0.55-< 0.65	Equivocal	4≥ -< 8	Equivocal
≥ 0.65	Positive	≥ 8	Positive

*Minividastoxo IgM and IgG Assay, biomerieux, SA, France

*Procedure for IgM and IgG test Assay:

Both IgM and IgG test Assay are similar with slight variation.

1- All the reagent strips, SPR are come to room temperature.
2- Use one IgM strip and one SPR for each sample ,control and standard to be tested for IgM ,also one IgG strip and one SPR for each sample, control, standard to be tested for IgG.
3- Type or select either toxo IgM or toxo IgG to enter the test code, the standard must be identified by S1 and tested in duplicate. If positive or negative control is to be tested, it should be identified by C1,C2 respectively .
4- Mix the standard, control and sample using a vortex – type mixer.
5- Pipette 100ml of standard ,sample or control into the sample well .
6- Insert the SPR and strip into the instrument.
7- Initiate the assay as directed in the operator's manual ,all the assays steps are performed automatically by the instrument .The assay will be completed for IgM and IgG within 40 minutes .

*Minividastoxo IgM and IgG assay, Biomerieux SA, France.

RESULTS & DISCUSSION

First group ,all the serum samples of women (table 1) showed negative IgM (*i.e.* the index ratio were < 0.05) except 3 of 112 (2.7%) and indicate that there is rare acute infection with *Toxoplasma gondi* in these group of women (23 pregnant & 89non pregnant), similar result were obtained from the second group of women (26 pregnant and 86 non pregnant) who serum samples give negative IgM level (*i.e.* index ration were < 0.05 and only one serum sample give equivocal IgM level except 7 of 112 (6.3%). The negative IgM in these two group of women revealed that these women were rare from acute *T. gondi* infection[1] although the majority of toxoplasma infection were subclinical and asymptomatic associated with low or absence level of IgM (negative)[8] and therefore , other serum samples must be taken from the same groups of women at different periods of time to confirm the diagnosis of acute *T. gondi* infection, since IgM level were persist with variable durations time and even it persist with high level in certain cases of chronic infection with T. gondi [8] .

Regarding the second group of women who their serum samples take for IgM and IgG .The IgG positive level (*i.e.*> 8 iu / ml) were present in(49) out of (112) serum samples of women with the ratio (43.75%), whereas (63) out of (112) were negative IgG level (<4 iu / ml) (*i.e.* 56.25 %) indicate that these women (27 pregnant and 36 non pregnant) were free from *T. gondi* infection ,where as all the women in these group with positive level of IgG (21pregnant at first trimester and 28 non pregnant) were chronically infected with *T. gondi* for a long time because the IgM positive level was evident in acute form of toxoplasmosis and disappear gradually during gestation where as positive IgG level increase gradually during the gestation period and persist with high level for a long time even in some women for months and year depending on immune state ,health condition, age and gender [5]. The prevalence rate of IgG positive level (43.75%) in women in this study were close to other studies in Iraq[9] 22.95%[10] 18.5%, these results were close to

other studies in Arabic countries in Saudia Arabia a several prevalence rates were reported in women for *T. gondi* infection (positive IgG)[11,[12,13] (13.6%, 35.6% and 29.4 % respectively. In Jordan [14] 37%, whereas in Lebanon 46%, Egypt 23.3%, Syria 65% and Ethiopia 77% [15]. Also the prevalence rate of toxoplasmosis in women were varied in other countries in Europe were arranged between 20% - 85% in United States of America the range of toxoplasmosis were low (12%-41%)[16, 5]. This variation in the prevalence rates of this disease in different parts of the world were related to immune response, age and gender population , period of gestation, virulence of parasites, dose of inoculum and geographic area of distribution [9, 5].

CONCLUSION

1- This study revealed that the vidastoxo IgM and vidastoxo IgG assay (ELFA) were considered to be dependable and standard test for diagnosis of toxoplasmosis in individuals .
2- Although most of serum samples of women showed negative IgM level were indicated that there is rare acute infection with *T. gondi* to confirm these result, must be a sequential serum samples were taken from these women at different periods of time to see if a significant rises in IgM level or not .
3- Regarding the IgG positive women which showed chronic infection with *T. gondi*, although the vidastoxo IgG assay (ELFA) is a confirmatory and standard test, also we preferred to polymerase chain reaction test in addition to IgG avidity test .

TABLE 1: Seronegative and seropositive of anti *Toxoplasma gondi* (IgM and IgG) in relation to pregnancy and non pregnancy.

Group One IgM	Group two IgM and IgG
IgM negative all women (112) 23 pregnant, 89 non pregnant except 3 out of 112 (2.7%)	IgM negative all women (112) 26 pregnant, 86 non pregnant except 7 out of 112 (6.3%) IgG negative 56.25% (63 out of 112) 27 pregnant and 36 non pregnant IgG positive 43.75% (49 out of 112) 21 pregnant and 28 non pregnant

*All pregnant women were at first trimester of pregnancy *Women ages were between 19-39 years.

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