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Detection of *Toxoplasmagondii* Infection among Pregnant Women in Hail, Saudi Arabia

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Abstract: ***Toxoplasmosis is caused by infection with intracellular protozoa parasite T.gondii . One form of the disease, congenitally acquired toxoplasmosis, can result in sever central nervous system malformation or perinatal mortality. Other forms of the disease range from chronic, asymptomatic toxoplasma infection to ocular infection and adult disseminated cerebral toxoplasmosis. This research shows the number of cases which examined in the regional lab in Hail region, 5537 samples for women in the first few months of pregnancy has been tested for toxoplasmosis by using ELISA technique 1621 samples show positive result for TOXO-IgG which constitutes 9.8% of the total samples, which indicates that the 68% of pregnant women have an old infection. In addition, the samples were examined by TOXO-IgM to find out the number of pregnant women that have been infected by toxoplasmosis recently, the study show 17 pregnant women positive for TOXO-IgM, this means that 0.3% of pregnant women are infected by Toxoplasma parasite recently.***

Keywords: *Toxoplasma gondii*, Prevalence, IgG & IgM, Pregnant women, Hail, Saudi Arabia

1. Introduction

Toxoplasmagondii (T. gondii) is an obligate intracellular protozoan parasite causing toxoplasmosis, which is one of the mostpredominant chronic infections affecting one third of the humanaround the world(1-3).The importance of toxoplasmosis in pregnant women comes from the high prevalence of T. gondii infection and its severe consequences to the fetus and infant(4,5),and prevention from infection of the fetus and complications by antibiotic treatment has not been very effective (6). Diagnosis of T. gondii infections during pregnancy is mostly done by detecting immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies by using serological methods such as latex agglutination test, ELISA, and indirect immunofluorescence antibodies test(7-10) Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy. Primary infection with T. gondii in pregnant women occurs all over the world with frequencies between 0.1% and 1%. (11-12) Few studies have been conducted to explore the seroprevalence of T. gondii among pregnant Saudi women, with varying results. (13-16) Pregnant women with primary toxoplasmosis infection are often asymptomatic or have only mild symptoms, and approximately 70%-90% of infants born with congenital toxoplasmosis are asymptomatic at birth. Nevertheless, infection may cause spontaneous abortion, still birth, prematurity or serious fetal damage. (17,18,19)to determine the prevalence of toxoplama antibodies in pregnant women in Hail city, KSA. In 2013, 6076 pregnant women were examined for IgG and IgM antibodies using ELISA technique. The age range was 19-43 years. The overall IgG seroprevalence was 9.8% and IgM seoprevalnce was 0.6%. The IgM is indicative of low recent exposure to the parasite. In conclusion, the overall seroprevalance indicate a very low percentage in pregnant women living in Hail, KSA. This lowers the risk of contracting *T. gondii*infections which minimize the risk congenital toxoplasmosis. Most recently, in Makkah, a study reported that seroprevalence of IgG was 21.2% and that of IgM was 1.2% in a group of pregnant women with history of previous abortion.[31]This seems to be very near to the findings of the current study, perhaps due to similarity of the selected group of patients, or more similar demographic and culture factors affecting transmission of the infection in both holy Islamic cities.The varying results from those regions of the Kingdom may be due to differences in patient numbers, demographics, history and used techniques, or the results may actually point to more public awareness and implementation of preventive measures against toxoplasmosis, such as in Almadinah Almunawwarah. Epidemiological studies suggest that prevalence of T. gondii infection in pregnant women varies substantially among different countries; in Europe it varies from 9% to 63%, 63.2% in Germany (20) 19.8% in Italy (21), and 9.1% in the UK (22). In Asian countries the seroprevalence of toxoplasmosis was reported low: 3.7% in Korea and 11.2% in Vietnam (23-24) while prevalence is as high as 41.6% to 45% in Indian pregnant women (25), 66.9% in Jordan and 53.1% in Kuwait (26, 27). In the American continent, the seroprevalence of toxoplasmosis was reported to be 77.5% in Brazil (28) and 63.5% in Colombia (29).

The aim of this study to find out how many pregnant women in Hail region infected with toxoplasma through examination of serum sample in serology department in regional lab using ELISA technique for toxoplasma IgG and IgM, with trying to find out the number of abortion cases due to this parasite

All of serum samples that have been sent to regional lab from hospitals and dispensaries in hail region which collected from pregnant women to follow up in year 2016

2. Material and Methods

1-Toxoplasma IgG ELISA by United Diagnostic Industry (UDI)

The UDI EG127 Toxoplasma IgG test system is an Enzyme Linked Immunosorbent Assay kit providing material for the detection of IgG –class antibodies to Toxoplasma gondii parasite in human serum or plasma.

Principle of the Test:

The EG127 Toxoplasma IgG kit is based on the ELISA technique. In the assay, calibrators and unknown are incubated in microtitration wells coated with purified and inactivated T.gondii antigen. After incubation and washing, the wells are treated with conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti –T.gondii IgG antibodies present.

Materials:

Antigen –coated microtitration strips:
One strip holder containing 12x8 (96) microtitration wells coated with *T.gondii* antigen

Wash concentration:
One bottle containing 100ml of a phosphate buffered saline. Dilute with deionized or distilled water 1:10 prior to use

Sample diluent:
One bottle containing 100ml of BSA solution with 0.09% sodium azide as a preservative.

Toxoplama IgG calibrators:
Each 2ml of human serum calibrated according to WHO reference standards Anti –Toxoplasma IgG in a 0.01M phosphate buffer containing BSA with 0.09% sodium azide as a preservative.

2nd antibody conjugate:
One bottle containing 12ml of anti –human IgG monoclonal antibodies labeled with peroxidase in a phosphate buffer solution with 0.02% proclin.

TMB –substrate:
One bottle containing 12ml of tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer with pH 3.8

Stopping solution:
One bottle containing 15ml of 0.3M H_2SO_4

1-Toxoplasma IgM ELISA by United Diagnostic Industry (UDI)

The UDI EM127 Toxoplasma IgM ELISA is an Enzyme Linked Immunosorbent Assay kit providing materialfor detection of IgM –class antibodies to T.gondii in human serum or plasma

Principle of the Test:

The toxoplasma IgM assay is based on the principle of the capture of these immunoglobulins and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified an inactivated *Toxoplasmagondii* antigen

MATERIALS:

Antibody –coated microtitration strips:
One strip holder containing 12x8 (96) microtitration wells coated with anti –human IgM antibodies.

Wash concentration:
One bottle containing 100ml of a phosphate buffered saline. Dilute with deionized or distilled water 1:10 prior to use

Sample diluent:
One bottle containing 100ml of BSA solution with 0.09% sodium azide as a preservative.

Toxoplasma IgM controls:
Three vials, negative, cut off and positive each 2ml of human serum in a 0.01M phosphate buffer with BSA containing 0.09% sodium azide as a preservative.

Toxoplasma –HRP –Conjugate:
One bottle containing 12ml of purified toxoplasma antigen conjugated with peroxidase in a phosphate buffer solution with 0.02% proclin.

TMB –substrate:
One bottle containing 12ml of tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer with pH 3.8

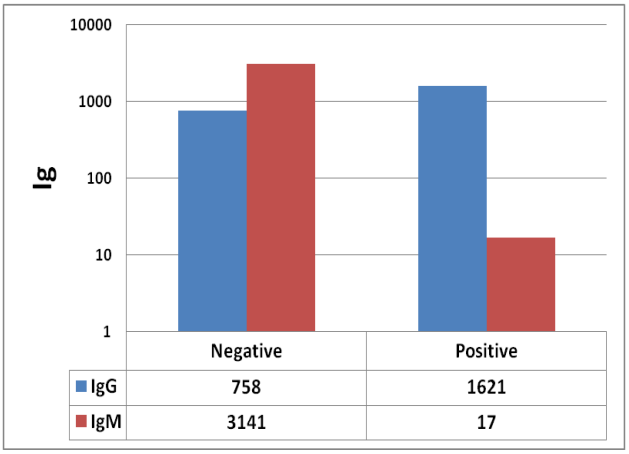
Stopping solution:
One bottle containing 15ml of 0.3M H_2SO_4[4]

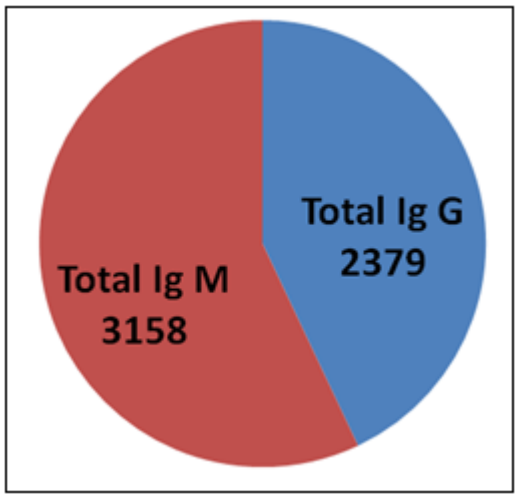
3. Results

In the present results cases which examined in the regional lab in Hail region, 5537 samples for women in the first few months of pregnancy has been tested for toxoplasmosis by using ELISA technique 1621 samples show positive result for TOXO-IgG which constitutes 9.8% of the total samples, which indicates that the 68% of pregnant women have an old infection.

In addition, the samples were examined by TOXO-IgM to find out the number of pregnant women that have been infected by toxoplasmosis recently, the study show 17 pregnant women positive for TOXO-IgM, this means that 0.3% of pregnant women are infected by Toxoplasma parasite recently.Table.1 and Figure. 1

TOXOPLASMOSIS STATISTIC IN 1437H		
HAIL REGIONAL LAB		
	NEGATIVE	POSITIVE
IgG	758	1621
IgM	3141	17
TOTAL IgG	2379	
TOTAL IgM	3158	
Total Patients Number	5537	





4. Discussion

In the present study, a rapid, inexpensive serodiagnostic test (one step *Toxoplasma*-IgM/IgG test, InTec) was used. This test was simple to perform in only 15 min without special equipment or experienced personnel. (30-33)

In our study, IgG seroprevalence rate among the study subjects in Hail was 29% and IgM seroprevalence was 0.3% among pregnant women. Compared to rates previously reported from other KSA cities, this rate is found to be higher than those of Almadinah Almunawwarah 21.3%,but it islower than the rates reported from Abha (34) in which IgG seroprevalence was 31.6%,and Makkah; 35.6% and 29.4%, in 2002 and 2006 among pregnant women.(14 and 35). However, some of them develop manifestations at childhood and adolescence. The seroprevalence of T. gondii in Hail province is 9.8% which is lowerthan the other cities with the results of the pervious cross sectional studies carried out in other regions in Saudi Arabia and neighboring Gulf countries, showing that the prevalence of T. gondii ranges between 25 and 36% (36-40). Low prevalence rates of 10% were reported in the United Kingdom (22), Korea (23), and Norway (41)and as high as 77.5% in Brazil (28) and 63.5% in Colombia (29). Higher prevalence rates were also reported in some neighboring Arab countries like Jordan 66.9% and Kuwait 53.1% (26,27). The difference in prevalence rate between different countries can be explained by variation of geographical and climatic conditions between different areas as the success of oocysts sporulation better in hotter and wetter areas (42).

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