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SEROPREVALENCE OF TOXOPLASMA GONDII AND RUBELLA VIRUS INFECTIONS AMONG PREGNANT WOMEN ATTENDING EL-HASAHIESA MATERNITY TEACHING HOSPITAL GEZIRA STATE, MEDAN CITY, SUDAN.- JULY 2016-2017

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ABSTRACT

Background: Primary infections caused by Toxoplasma gondii can lead to serious complications in pregnant women. The aim of this study was to determine the Seroprevalence of *T. gondii* and Rubella virus among pregnant women in EI-Hasahiesa Teaching Hospital and to evaluate the use of outcomes for the prevention of congenital rubella syndrome and congenital toxoplasmosis. **Material and Methods:** Data were obtained from sera collected from eighty of pregnant women; during their visit to Maternity Hospital, between May-August 2016. Serological analysis for latent toxoplasmosis and Rubella virus levels of (IgG) and active toxoplasmosis and Rubella (IgM) was done using Immunochromatography test and Electro Chemiluminescence Immuno Assay. **Results:** The specific IgG antibodies were found to be positive in 2cases (2.5%) for toxoplasmosis and none of the examined women had IgM toxoplasma antibodies and the examined women had IgG anti Rubella antibodies(1.2%) and (0%) for IgM antibodies. The rate of infection was detected among women aged between (15 – 25)years for Rubella virus and(26-35) years for *Toxoplasma.* Rubella vaccination programs during childhood and preconceptional periods rather than antenatal screening appear to be more efficient for eliminating congenital rubella syndrome. In this study showed that, there were high percentages of negative *T. gondii* & Rubella antibodies in pregnant women and suggests that the prevention of congenital rubella syndrome can be achieved through efficient vaccination programs. **Conclusion:** The study revealed that, there were high percentages of negative *T. gondii* Rubella antibodies in pregnant women**.**

KEYWORDS: Toxoplasma gondii, Rubella, vaccination.

INTRODUCTION

Toxoplasma gondii and Rubella virus were the causative agents of a worldwide infections. In immunocompetent individuals they mild infection, but in pregnant women at certain stage of gestation they are able to cross the placenta and infect the fetus causing fetal damage andwide range of malformations in newbornssuchas hearing loss, mental retardation, developmental delay, mirocephaly, cataract (Ross and Boppana,2005; Atreya et al.. 2004; Jones, 2003).

Rubella Virus is the causative agent of the disease Rubella, which was first describe clinically in1740, also the cause of congenital rubella syndrome (CRS), when infection occurs during the first 20 weeks of pregnancy (Wesselthoeft, 1949).RV is the only member of the genus Rubivirus and belongs to the family of Togaviridae which has a single- stranded RNA of paramyxovirus group, its genome of positive polarity and icosahedral capsid. RV is only known to infect humans, responsible for the common childhood disease, known as German measles (Lee and Bowden, 2000).

Toxoplasma gondii is an obligate intracellular parasitic protozoan, capable of infecting a wide range of warm-blooded hosts, including humans. Infection with *T. gondii* has very different clinical signs in human, such as abortion and congenital eye disease (Weiss and Kim 2012.) Humans are infected with the cystic form through the gastrointestinal system via contaminated food or raw or uncooked meat. Cats are the main source of infection in humans (Eger man and Beasley, 1998).

MATERIALS AND METHODS

The study was carried out at among pregnant women attending EL-Hasahiesa Maternity Teaching Hospital in

Gezira State during March – August 2016.The population of study was all Pregnant Women Who attending Maternity Hospital during study period. The following groups of patients were excluded: a) Non pregnant women b) Pregnant women not attending the target hospital. The consent of the patient was obtained & ethical approval from ministry of health. A full explanation of the purposes, nature and procedures of the study was conveyed to them. The potential participants were clearly assured that their participation in this study is voluntary and that they could withdraw at any stage and that any data obtained would be treated confidentially and for the purpose of the research only. The data was collected by using a questionnaire. A questionnaire was designed to include socio-demographic data, clinical data and laboratory results. The sample size was determined using prevalence rate from different countries, therefore the sample was 320 according to this equation n=Z2p (1-p)/d2, where n: required sample size, Z: confidence level at 0.95% =1.96, P: expected prevalence, d :1margin of irro at 0.05, n= (1.96)*(-) (1-0.37)/ (0.05)2 =320 approximately However, the number was restricted to 160 due to high cost of investigations. The data obtained from the questionnaire and the result of laboratory analysis were analyzed using SPSS (statistical package for social sciences) computer program (version16.0).

1. Blood sampling and processing

Three ml blood sample was collected by vein puncture under aseptic condition, transferred into sterile plain container and allowed to clot. The clotted blood sample was centrifuged (3000 rpm, 5 min), and the serum (the supernatant) was transferred into Eppendorf tube using automatic peppite and stored at -20°C until required for use.

2. Laboratory analysis

(2.1) Immunochromatography test (ICT)

Torch Toxo / Rubella/CMV/HSV 1/2 IgM and IgG Antibodies Combo Rapid Test Device (Serum/Plasma).

(A) Principle of the test

The Torch IgM Antibodies Combo Rapid Test Device (Serum/Plasma) is a qualitative, lateral flow immunoassay for the detection of IgM antibodies to Toxoplasma, Rubella, CMV and Herpes Simplex Virus 1/2 in serum or plasma specimens. In this test, antigens of Toxo, Rub, CMV and HSV 1/2 are coated in the test line regions of each section in the test. During testing, the serum or plasma specimen reacts with Goat anti-human IgM coated particles in the test strip. The mixture then migrates upward on the membrane by capillary action and reacts with the Toxo, Rub, CMV and HSV 1/2 specific antigens on the membrane in the test line regions of the respective sections. The presence of a colored line in the test line region of a particular section indicates a positive result for the corresponding infection. Toxo, Rub, CMV, HSV 1/2, while its absence indicates a negative result for that infection. To serve as a procedural control, a colored line will always appear in the respective control line regions of all the four strips indicating that proper volume of specimen has been added and membrane wicking has occurred.

(B) Materials used

Test device, disposable droppers, and buffer and serum samples.

(C) Procedure

Allow the test device and specimen to equilibrate to room temperature (15-30°C) prior to testing then place the test device on a clean and level surface. Hold the dropper vertically and transfer 1 full drop of serum or plasma (approximately 10 µL) and 2 drops of buffer (approximately 80 µL) to each specimen well of the test device respectively, and then start the timer. Avoid trapping air bubbles in the specimen well. Wait for the colored line(s) to appear. The results should be read at 15 minutes and should not interpret the results after 20 minutes.

(2.2) The Electro Chemiluminescence Immuno Assay

"ECLIA" is intended for use on Elecsys and Cobas Immunoassay Analyzers.

Intended use Immunoassay for the in vitro qualitative determination of IgM antibodies to cytomegalovirus in human serum and plasma. Results obtained with this assay are used as an aid in the diagnosis of recent CMV infections.

(A) Test principle

µ-Capture test principle (total duration of assay is 18 minutes). First incubation: 10 µL of sample are automatically prediluted 1:20 with Diluents Universal, Biotinylated monoclonal anti-h- IgM- specific antibodies are added Second incubation T.gondii and Rubella-specific recombinant antigen labeled with a ruthenium complex and streptavidin-coated micro particles are added. Anti-T. gondii and Rubella IgM antibodies present in the sample react with the ruthenium-labeled T.gondii and Rubellaspecific recombinant antigen. The complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration. The analyzer automatically calculates the cutoff based on the measurement of T.gondii and Rubella IgM calibrator one (Cal1) and T.gondii and Rubella IgM calibrator two. (Cal2). The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index

(signal sample/cutoff). Results obtained with the Elecsys T.gondii and Rubella IgM assay can be interpreted as follows: Non-reactive < 0.7 COI, Indeterminate ≥ 0.7 - < 1.0 COI, Reactive ≥ 1.0 COI.

RESULTS

Eighty blood samples were collected from pregnant women who were met the study randomly and personal interviews by using a specifically designed questionnaire for collecting the required data.The collected data were then analyzed interpreted in the following sequence. The age range was 20-38 years with a mean of 28.5 years for the study group. The age groups of the study population were 25 (31.2%) aged (15-25) years, 43(53.8%) aged between (26-35) years, 12(15.0%).aged between 36-45 years(Figure I). All individuals live in Gezira state at the time of study for at least a year**,** 53(66%) from rural area, 27(34%) from urban area(Figure 2). Parity in the study population, 11(13.8%) primigravida, 20(25.0%0 Para1. 15(118.8%) Para,. 18(22.5%) Para 3-5(6.5%) Para4 and 11(13.8%) %) is multiparous (Figure 4-5). Duration of gestation in the study population, 9(11.2%) in first trimester, 20(25.0%) in second trimester and 51(63.8%) in third trimester. (Tables 2). The specific IgG antibodies were found to be positive in 2cases (2.5%) for toxoplasmosis and none of the examined women had IgM toxoplasma antibodies (Table 3,4,5) and the examined women had IgG anti Rubella antibodies(1.2%) and (0%) for IgM antibodies (Table 6,7,8). The rate of infection was detected among women aged between (15 – 25)years for Rubella virus and(26-35) years for *Toxoplasma*. The seropositivity of the IgG antibody for *T.gondii* and rubella were 2(2.5%) and 1(1.2%), respectively. The seropositivity of the IgM antibody for *T.gondii* and rubella were 0(100.0%) and 0(100.0%) respectively.

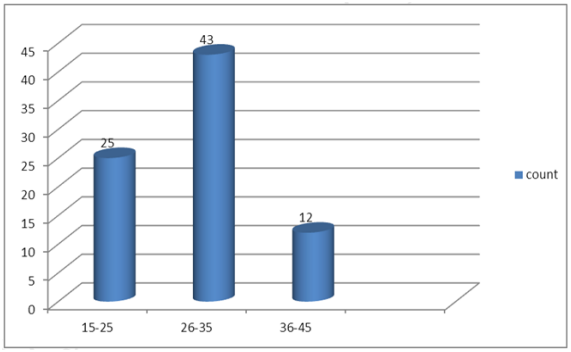

Figure 1: Distribution of study population according to the age groups.

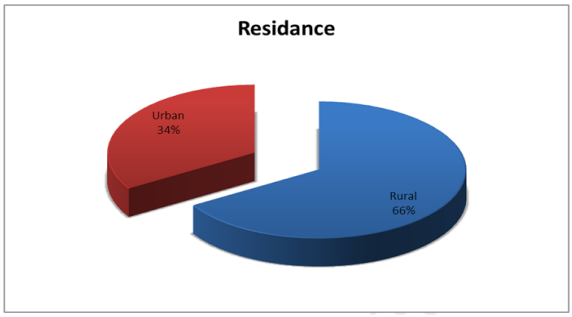

Figure 2: Distribution of the study population according residence.

Parity

Table 1: Distribution of samples according to parity.

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid primigravida	11	13.8	13.8	13.8
para1	20	25.0	25.0	38.8
para2	15	18.8	18.8	57.5
para3	18	22.5	22.5	80.0
para4	5	6.2	6.2	86.2
Multiparous	11	13.8	13.8	100.0
Total	80	100.0	100.0	

Table 2: Distribution of samples according to duration of gestation.

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid first trim ester	9	11.2	11.2	11.2
second trimester	20	25.0	25.0	36.2
third trimester	51	63.8	63.8	100.0
Total	80	100.0	100.0	

Table 3: Result of Toxoplasma gondii.

Result	Prevalence of Toxo- IgG		Prevalence of Toxo- IgM	
	NO	%	NO	%
Positive	2	2.5%	0	0
Negative	78	97.5 %	80	100%
Total	80	100%	80	100%

Table 4: Seroprevalence data together with personal and demographic variables.
Toxoplasma ICT IgG

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Positive	2	2.5	2.5	2.5
Negative	78	97.5%	97.5%	100.0
Total	80	100.0	100.0	

Toxoplasma ICT IgM

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Negative	80	100.0	100.0	100.0

Table 5: Seroprevalence data together with personal and demographic variables.

		Positive	Negative	Total
Age in group per years	15 – 25	2	23	25
	26 – 35	0	43	43
	36 – 45	0	12	12
Residence	Rural	2	51	53
	Urban	0	27	27
Level of education	Illiterate	0	8	8
	Primary	2	5	7
	Intermediate	1	20	21
	High education	0	44	44
Parity	Primigravida	1	10	11
	Para 1	1	19	20
	Para 2	0	15	15
	Para 3	1	18	18
	Para 4	0	11	11
	Multiparous	1	12	13
Occupation	House wife	1	73	74
	Employee	1	9	9
Duration of gestation	First trimester	0	15	16
	Second trimester	0	20	20
	Third trimester	2	49	51

Table 6: Result of Rubella virus.

Result	Prevalence of RV IgG		Prevalence of RV IgM	
	NO	%	NO	%
Positive	1	1.2%	0	0
Negative	79	98.8%	80	100%
Total	80	100%	80	100%

Table 7: Rub. ICT.IgG.

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid yes	1	1.2	1.2	1.2
no	79	98.8	98.8	100.0
Total	80	100.0	100.0	

Table 8: Rub. ICT.IgM.

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid No	80	100.0	100.0	100.0

Rub.ICT.IgG * premature. Labor P=.87 (no significant)

DISCUSSION

Maternal rubella and toxoplasmosis infection pose risks to the fetus and the newborn, particularly when acquired in early gestation. When susceptible women are exposed to these agents, congenital defects, affecting almost all of the organ systems of the fetus, known as CRS and CT occurs. These infections cause fetal and neonatal mortality and an important contributor to early and later childhood morbidity.(Seth P, *et al;*1985) (Binnicker MJ *et al;* 2010).

Rubella virus is a Rubivirus genus of the Togaviridae family. It is a single-stranded RNA virus enveloped by an icosahedral symmetrical capsule and causes infections only in humans. Clinically, it manifests with mild measles-like symptoms. During the prodromal period, fever and flu-like symptoms accompanied by a maculopapular rash are common. The transplacental transmission of the virus during early gestation results in congenital defects. Embryonic cells infected with the rubella virus display chromosomal fragmentations and an inhibition of mitosis (Gladwin and Trattler, 2004).

T. gondii is an obligatory intracellular protozoan. Many organs are susceptible to infection with *T. gondii*. Humans are infected with the cystic form through the gastrointestinal system via contaminated food or the consumption of raw or undercooked meat. Cats are the main source of infection in humans. (Egerman and Beazley, 1998). Symptomatic infants may have a rash, chorioretinitis, blindness, con-vulsions, mental retardation, mirocephaly or encephalitis (Egerman and Beazley, 1998).

Current study aimed to determine the prevalence of T.gondii and Rubella virus antibodies among pregnant women in Gezira State. The diagnosis made by immuno chromatographic test. Total of 80 samples were collected randomly from pregnant women attending EL-Hasahiesa Maternity Teaching Hospital, the women categorized into three aged group;(15-25) years represent 31.2%, (26-35) years represent 53% and (36-45) years 15.0%.

Seropositivity of Toxoplasma IgG antibodies by Immunochromatography test(ICT) was2.5% of 80 women.This indicate low prevalence rate of T.gondii IgG among population and this agree with Ayi *et.al*;2009 which considered that the IgG has a low prevalence among population. This lower incidence may be due to the dry climates contain lower incidence of than temperate and moist climate (Jeffrey et al, 2001).

And disagreed with Musa et al; 2014; Khalil *et al*; 2013;AbdelHameed,1999;Amir et al; 2003in Sudan; Tagreed,2011 in Baghdad, Iraq and Sood *et al*;2004 in Ahmadabad. The IgG Seropositivity reported in age group (26-35) 31.2%; and the seroprevalence of IgG in rural area equal to that in urban that means the risk factor such as contact with cat, eating raw meat, eating unwashed vegetables are the same in two areas.

Seropositivity of Rubella IgG antibodies by Immunochromatography test(ICT) was 1.2% of 80 women. This indicate low prevalence rate of Rubella virus IgG among population. The IgG Seropositivity reported in age group (15-25) 53%; and the seroprevalence of IgG in urban area, this infection may be due no vaccination or failed. The participant had an intermediate education, and multiparous parity.

The surveillance system for congenital toxoplasmosis varies in different European countries, and principally, it depends on the prevalence in that region. We do not have a routine national screening program for *T. gondii* and rubella infections in Sudan. Because the cost-effectiveness of screening is not yet clear, these tests are not a routine element of nationwide antenatal care. Worldwide, there is considerable variation in the prevalence of rubella antibodies among women of childbearing age. Seroprevalence is higher in European women (93.2%) than in women of African (86.7%) and Asian origin (78.4%) (Lever *et al.*, 1987). In our study, we detected a low rate of susceptibility (0.6%) to rubella infection among pregnant women. In Sudan, the mumps-measles-rubella (MMR) vaccine has been part of the national vaccination program. There may be cases in which the vaccine fails or the protective level of antigens declines in a few years. For this reason, some researchers recommend screening for rubella antibodies among women of childbearing age (Uyar *et al.*, 2008). It seems that the prevention of congenital rubella syndrome may be possible only through efficient vaccination. The World Health Organization (WHO) reported various strategies for the prevention of congenital rubella syndrome. These strategies include providing direct protection to women and schoolgirls (a selective vaccination strategy), vaccinating boys and girls to provide indirect protection by reducing the transmission of rubella virus infections (a universal vaccination strategy) and a combination of these two approaches (a com-bined strategy) (Robertson *et al.*, 1997). Worldwide, there is great diversity in the seroprevalence of *T. gondii* reported. The epidemiology of toxoplasmosis is documented in many countries. The seroprevalence of toxoplasmosis is related to factors including socioeconomic status, age, dietary habits, and rural or urban settlement. In a recent study with sera from pregnant women living in northeastern China, the two main risk factors related to *T. gondii* infection were the care of pet animals and the consumption of raw meat (Liu *et al.*, 2009). In the first trimester, the risk of congenital infection with T.gondii is 10-15%, and fetal effects can be disastrous. During the second and third trimesters, the probability of fetal infection increases up to 68%, but the fetal effects are milder (Remington etal., 2006). Ocular lesions, which may cause blindness, are the most common long-term sequelae of toxoplasmosis. It has been demonstrated in several studies that chorioretinitis and cerebral lesions are more common in cases with lack of antenatal screening and treatment

(Wallon et al., 2004). When serocon-version occurs in a pregnant individual, spiramycin is pre-scribed.

CONCLUSIONS

The study revealed that, there were high percentages of negative *T. gondii* Rubella antibodies in pregnant women.

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