

SEROPREVALENCE OF RUBELLA, CYTOMEGALOVIRUS, HERPES, AND TOXOPLASMA GONDII IN RECURRENT ABORTED WOMEN IN BAGHDAD

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ABSTRACT

Recurrent Abortion (RA) in the general populations is common occurrence and often changes among different communities.

This study was to determination the prevalence of *Rubella*, *Cytomegalovirus*, *herpes*, and *Toxoplasma Gondii* in Baghdad. Blood samples were collected from 210 women, 180 women with recurrent abortion (three or more abortions) in the first trimester and 30 women with a healthy pregnancy. Based on the clinical examination, and diagnostic laboratory findings of the rapid lateral immune chromatography assay (RLICA), and Enzyme-linked Immunosorbent assay (ELISA) test for TORCH examination measured serum levels of immunoglobulin (IgM and IgG) for *Toxoplasmosis*, *cytomegalovirus*, *herpes*, and *rubella* in aborted women. Depending on the result of ELISA test the patients were included women with recurrent abortion with sero-negative for TORCH test was 50 (27.8%), women with recurrent aborted with sero-positive for anti-*toxoplasma* antibodies was 44 (24.5%), women with recurrent abortions suffering from infected with different causes such as *cytomegalovirus*, *Rubella* or *Herpes* ratio were 86 (47.7%) and women with a healthy pregnancy (normal third delivery or more and with no previously recognized miscarriage) was 30 (14.3%). Also the results pointed out that the seropositivity rate for CMV IgG, *T. gondii* IgG, *Rubella* IgG, and *HSV* IgG were higher in recurrent aborted women compared with CMV IgM, *T. gondii* IgM, *Rubella* IgM, and *HSV* IgM also in recurrent aborted women with highly significant difference ($P < 0.05$).

The results indicated that the efficacy of ELISA test was higher than rapid test in detecting of TORCH infections.

Aim of this Study: Determination the prevalence of *Rubella*, *Cytomegalovirus*, *herpes*, and *Toxoplasma Gondii gondii* in Baghdad and to detect the efficacy of ELISA test in detecting of TORCH infections.

INTRODUCTION

Recurrent abortion (RA) is the most common complication of early pregnancy, refers to one of the most frequent reproductive events is recurrent spontaneous abortion (RSA). It is defined as three or more repeated pregnancy losses before the fetus has reached a viable gestational age (Williams and Wilkins, 2012). Reasonably accepted etiologic causes include, genetics, immunologic factors, placental abnormality, endocrine disorder, nutritional, environmental factors and infection with microorganisms like *Toxoplasma gondii*, *Cytomegalovirus*, *syphilis*, *rubella*, *herpes* and maternal disease (such as diabetes mellitus, thyroid disease) (Tang and Quenby, 2010; Klaewklad, *et al.*, 2017).

Toxoplasmosis caused abortions usually to occur during the first half of gestation and effects on liver and spleen functions (Abdul-Hadi *et al.*, 2016; Ermawati and Wibisono 2017; Jain 2017). When the congenital toxoplasmosis occurs early in, it may lead to severe damage or abortion. Embryo acts as an allograft to the mother's body, it is remaining normally in the mother's womb during the entire gestational period in the case of normal successful pregnancy (Malarvizhi *et al.*, 2012).

MATERIALS AND METHODS

Patients and control: The current study included 210 women in Baghdad during the first-trimester. The ages of these women were ranged between 20-35 years. These women were referred to Obstetrics

and Gynecology Department of AL-Yarmook Teaching Hospital, Baghdad Teaching Hospital and Fatima AL-Zahraa Hospital. Excluded criteria patients were without infection with diabetes mellitus, hypertension, fever, thyroid disease, drinking alcohol, smoking, any chronic disease, and if taking any drugs during the period from March to December 2016.

Samples collection: The 5 ml of venous blood was taken at the time of miscarriage by using sterile disposable syringes, the blood placed in a plain tube with gel clot and left to stand for one hour at room temperature for clot formation, for serum collection, the tube centrifuged for 10 minutes at 3000 rpm. Then the serum aspirated by using a Pasteur pipette, dispensed into sterile eppendorf tube, and stored at -20 C° until used.

TORCH rapid Test for IgM and IgG: Practical work was done according to the instructions of manufacturers (Ecotest, China).

Enzyme Linked Immunosorbent Assay for the detection of IgG or IgM antibodies in human serum: The practical work was done according to the instructions of manufacturers (Diagnostic Automation, INC, USA).

RESULTS

Table 1 shows the distribution of studied groups according-to TORCH results by lateral flow chromatographic immunoassay and TORCH of ELISA test as following divided into group1: recurrent abortion with seronegative for TORCH test the percentage was 50 (27.8%), group2: recurrent abortion with seropositive for anti-*Toxoplasma* antibody was 44 (24.5%), group 3: recurrent abortion with seropositive for one or more causes of TORCH test was 86 (47.7%).

Table 1: Distribution of the studied group's according-to TORCH by rapid test and by ELISA test

Study groups	TORCH by rapid test		TORCH by ELISA test	
	No	(%)	No	(%)
Recurrent abortion with seronegative for TORCH test.	76	(42.2%)	50	(27.8%)
Recurrent abortion with seropositive for anti- <i>Toxoplasma</i> antibody.	34	(18.9%)	44	(24.5%)
Recurrent abortion with seropositive for one or more causes of TORCH test	70	(38.9%)	86	(47.7%)
Pregnant normal without any abortion	30	(14.3%)	30	(14.3%)
Total	180	(100%)	180	(100%)

Comparison between rapid lateral immunochromatography assay method and ELISA test method in detecting TORCH test in the serum sample of women. The efficacy of ELISA test was higher than RLICA in detecting TORCH infections. ELISA was superior in achieving a comparatively high sensitivity and specificity. The rapid test required little technical expertise, lesser time and could be done without elaborate equipment, unlike the ELISA. Therefore, groups of study divided depending on the result of the ELISA test.

Seroprevalence of IgM and IgG among study groups: Table 2 Illustrates seroprevalence of IgM

and IgG antibody for pathological causes among study groups according-to ELISA test. The seropositive Antibodies of IgM and IgG for *Toxoplasma gondii* were 10 (5.6%), 30 (16.7%) followed by *Cytomegalovirus* were 17 (9.4%), 28 (15.6%), *Rubella* were 9 (5%), 15 (8.3%), HSV were 1 (0.6%), 4 (2.2%), while for healthy pregnant were 0(0%), 0(0%) respectively. The seroprevalence of (IgM and IgG) antibody for *Toxoplasma gondii* was 4(1.9%), *Cytomegalovirus* was 5(2.8%), *Rubella* was 6(3.3%), and *Herpes Simplex Virus* was 1 (0.6%) and Healthy pregnant was 0 (0%).

Table 2: Seroprevalence of IgM and IgG among study groups according-to TORCH of ELISA test.

Pathogens	IgM (%)	IgG (%)	IgM & IgG (%)	Total
<i>Toxoplasma gondii</i>	10 (5.6%)	30 (16.7%)	4(2.3%)	44
<i>Cytomegalovirus</i>	17 (9.4%)	28 (15.6%)	5(2.8%)	50
<i>Rubella</i>	9 (5%)	15 (8.3%)	6(3.3%)	30
<i>Herpes Simplex Virus</i>	1 (0.6%)	4 (2.2%)	1(0.6%)	6
Healthy pregnant	0 (0%)	0 (0%)	0 (0%)	0
Total	37 (20.6%)	77(42.8%)	16(9%)	130

DISCUSSION

Seroprevalence of IgM and IgG among the study groups: The rate of IgM antibody refers to acute infection, while IgG antibodies may increase in the 2-4 weeks after infection and gradually rises for many weeks then remain in low level for the rest time, while repeated exposure to the infection may cause high antibody rate for longer time (kumar *et al.*, 2004).

Toxoplasma, *Herpes*, *Rubella* and *Cytomegalovirus* are known to cause infection in uterus. Often responsible for abortion, stillbirth, premature delivery and congenital malformation, detection and treatment of such infections can prevent morbidity and mortality of the infants born to such mothers (Agrawal *et al.*, 2016).

The seroprevalence of *T. gondii* infections ranges between 7.7 and 76.7 % in different countries like in United Kingdom (7.7- 9.1%); Norway (10.9%); India, (45%); Brazil (50-76%) and Nigeria (75.4%) (Gulden *et al.*, 2009).

Turbadkar *et al.* (2003) revealed that in pregnant women who have history of abortion in India the percentage of IgG for *T. gondii* antibody was (42.10%) and IgM was (10.5%). In addition, study was done in India the rate of Toxoplasmosis in pregnant women for IgG was (45%) and only seven women were found to have IgM in rate (3.3%) (Singh and Pandit, 2004). Nash *et al.*, (2005) reported that in United Kingdom the average of *Toxoplasma* in pregnant women for IgG was (9.1%), also obviously, the rate expanded with age from (15-19) years the rate was (5.6%) and from (40-44) years the rate was (16.7%).

The National Health and Nutrition Examination Survey (NHANES) found that the prevalence of Toxoplasmosis has declined in the past decade (Jones *et al.*, 2007). Also, Acharya *et al.*, (2014) showed that in Nepal the women with spontaneous abortions had IgG for *T. gondii* in rate (77.9%). Another Study was done in Egypt on pregnant women with threatened abortion found that the prevalence of *T. gondii* for IgM was (18.3%), IgG was (38.3%), IgM and IgG were (5.8%) (Kamal *et al.*, 2015).

A study done in Mexico on pregnant women infected with *T. gondii* recorded that IgG and IgM antibodies level in rate (6.1%), (9.5%) respectively (Alvarado-Esquivel *et al.*, 2006). Result was recorded by Hadi *et al.* (2016) in Iraq showed that in aborted women the percentage rate for IgG was (35.4 %) and for IgM was (3.2 %). Also, other studies in Iraq, the women with spontaneous abortions

had IgG and IgM for *T. gondii* in rate (31.5%), (7.6%) respectively (Al-kalaby *et al.*, 2016). The percentage of IgG and IgM for *T. gondii* in aborted

women its (22.8%) and (35.1%) respectively (Abbas and Al-Hamairy, 2016).

The role of some infections in recurrent abortion has been intensely investigated during the past decades by viruses, especially *cytomegalovirus* (CMV) have been more consideration since they can produce chronic/recurrent intrauterine infections. CMV causes both primary and recurrent infection (reactivation or reinfection), viral strain variation may contribute to reinfection, and low maternal IgG avidity may unexpectedly promote transmission of virus across placenta and cause production of toxic metabolites, fetal loss, placental disorder, and chronic endometrial infection (Nigro and Adler, 2011; Nigro *et al.*, 2011).

Uyar *et al.*, (2008) in Turkey reported that the pregnant women infected with CMV were IgG and IgM in rate (97.3%) and (1.0%) respectively. Another study done in Sudan revealed that the prevalence of CMV IgG in pregnant women was (97.5%), while IgM was (6%) (Khairi *et al.*, 2013). Sherkat *et al.* (2014) recorded that in Iran CMV was significantly higher in recurrent pregnant loss (RPL) than the women without history of abortion and the titer of CMV IgG was (90.6%). Study in Iraq on women with history of abortion found that IgM and IgG antibodies to *Cytomegalovirus* in rate were (7.2%), (96.6%) respectively (Aljumaili and Alsamarai, 2013). Another study in India, also on women with history of abortion the percentage of infection with CMV was (14.6%) (Faldu *et al.*, 2015). Al-Baiati *et al.* (2014) showed that in aborted women the percentages of CMV for both IgG and IgM were (85%), (10%) respectively. Also Moreover, other study in Iraq found that 90 pregnant women with an average age of 23 years had CMV IgG in rate (98.9%), while CMV IgM was (1.1%) (Altayeb *et al.*, 2016).

Rubella is caused by RNA virus of paramyxovirus group. The pregnant women if contact with *Rubella* during the first 20 weeks of pregnancy will be harmfully affected by the virus. It readily attacks the placenta and fetus then lead to miscarriage or stillbirth (Kolawole *et al.*, 2014). Other study in Turkey the seropositive of the pregnant women for *Rubella* IgG, IgM were (96.1%) and (0.2%) respectively (Tamer *et al.*, 2009). Another study was done in Iraq in aborted women with *Rubella* antibodies was positive for IgG in rate of thirty-four percent (Abdul-Karim *et al.*, 2009). Sadik *et al.*, (2012) observed in India the seropositive of IgG for *Rubella* in pregnant women was (29.06%).

Acharya *et al.*, (2014) showed that in Nepal the women with spontaneous abortions had *Rubella* IgG in rate (11.7%). Khudhair and Ahmed (2015) recorded that the women in Iraq who had a one abortion the titer of *Rubella* IgG was (40%). Other report in Iraq revealed that in pregnant women with previous abortion the rate of *rubella* IgG was (57.8%) and *rubella* IgM was (26.6%), while the rate of *rubella* IgM in pregnant women without a history of abortion was (3.8%) (Mohammed, 2015). Lamichhane *et al.*, (2016) found that in spontaneous miscarriage the percentage rate for IgG was (43.68%) and for IgM was (7.76%).

Also, pregnant women infected with *Herpes simplex virus* (HSV) can result neonatal, premature labor and abortion (Kimberlin, 2011). Biswas *et al.*, (2011) reported that the pregnant woman in India the rate of IgG antibodies for HSV was (8.7%). Also, some studies in Iraq like Al-Marzoqi *et al.*, (2012) recorded that in pregnant women the positive for HSV IgG antibodies was (28.9%), and for IgM antibodies was (2.2%), Hasan *et al.*, (2013) found that in pregnant women the rate of *Herpes simplex virus* of IgG and IgM antibody were (2.19%), (2.19%) respectively. another study done in Nepal on the women with spontaneous abortions found that the HSV IgG antibody in rate (36.4%) (Acharya *et al.*, 2014).

While in Nigeria, the titer of HSV IgM in pregnant women was (2.8%) (Okonko *et al.*, 2015). Other study in India showed that in aborted women the titer of HSV IgM was (30.10%) (Tiwari *et al.*, 2016).

Differences between studies results can be due to the several laboratories utilizing different techniques used in these aspects, type of test that had been used and the number or types of sample tested for infection and it is well-known that epidemiology of infection is different among different populations. In Furthermore, patients' characteristics have been different among studies.

Conclusion:

In the current study indicated that the efficacy of ELISA test was higher than rapid test in detecting of TORCH infections.

Depending on the result of ELISA test the patients were included women with recurrent abortion with sero-negative for TORCH test was 50 (27.8%), women with recurrent aborted with seropositive for anti-*toxoplasma* antibodies was 44 (24.5%), women with recurrent abortions suffering from infected with different causes such as *cytomegalovirus*, *Rubella* or *Herpes* ratio were 86 (47.7%) and women with a healthy pregnancy (normal third delivery or more and with no previously recognized miscarriage) was 30 (14.3%).

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