

Investigation of Actual Rate of Primary and Mixed Infections with some TORCH Agents among Iraqi Gravidas with History of Recurrent Abortions

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Abstract: *Viral and bacterial infections in pregnant women have long been known to play a critical role in pregnancy wastage and adverse fetal consequences in terms of recurrent and spontaneous abortions especially at the first trimester of gestation with the increasing risk of in-utero transmission. Aim of the study: The correlate between the incidence of Rubella, CMV, HSV and Ct primary infection among first trimester gravidas with recurrent abortion, and estimating the rate of mixed infection with these agents. Materials and methods: A cross-sectional study was carried out at the private laboratories of Wasit province/Iraq between September 2015-August 2016 serum level of Rubella, Cytomegalovirus (CMV), Herpes simplex virus 1 and 2 (HSV) and Chlamydia trachomatis (Ct) antibodies were evaluated using enzyme-linked immunosorbent assay (ELISA) method and IgG avidity. Results: 82 serum samples were collected from gravidas in their first trimester, the study group included 67 (81.7%) gravidas suffering from previous history of recurrent abortions. High rate 31(46.27%) of study group were suffering from 1time abortion. the lowest rate 7 (10.45%) were for gravidas with ≥ 4 times abortion. The control group included 15(18.3%) gravidas with no history of abortion and healthy pregnancy. The rate of primary infection among group 1 arranged as follow high rate of Ct infection (16.42%) followed by CMV, HSV and Rubella primary infection (14.92%, 5.97% and 4.48% respectively). Conclusion: Early and accurate diagnosis of TORCH agents in first trimester will aid in management of infection especially Ct which infect all ages recurrently. Using of IgG avidity test will decrease false positive results and aid in management of Rubella and CMV and lower the risk of in-utero transmission of infection.*

Keywords: recurrent abortion, Rubella, CMV, HSV, Chlamydia, IgG avidity test

Abbreviations: *Chlamydia trachomatis*= (Ct), Cytomegalovirus= (CMV), Enzyme-linked immunosorbent assay= (ELISA), Herpes simplex virus 1 and 2= (HSV), immunoglobulin M= (IgM), immunoglobulin G= (IgG)

1. Introduction

Viral and bacterial infections in pregnant women have long been known to be associated with bad obstetric outcomes (1). Pregnancy induces a transient immunosuppression, which is thought to increase the vulnerability of pregnant women to infections with different bad outcomes known as Bad Obstetric History (BOH) which includes women with previous adverse fetal consequences in terms of two or more successive spontaneous abortions (recurrent abortions), early neonatal deaths, stillbirths, intrauterine fetal deaths, intrauterine growth retardations and congenital anomalies (2,3).

Infections with organisms that are able to be passed from mother to child and to produce congenital defects have been described with the acronym TORCH (also known as STORCH or TORCHES), these are a medical acronym for a set of perinatal infections including (*Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV), Herpes simplex virus (HSV), and others agents like *Chlamydia trachomatis*, *Treponema pallidum*, *Neisseria gonorrhoea*, HIV, etc. (4, 5).

Rubella is a single-stranded RNA virus infecting humans who are the only natural host, the human host receptor is unknown and the virus can infect any organ of the developing fetus. Primary Rubella infection can lead to Congenital Rubella Syndrome and fetal damage (6). Human

CMV and Herpes simplex virus (HSV) both are DNA viruses that belong to the beta herpes viruses. CMV is the largest of this group and can cause lytic and productive infections. Like other herpes viruses, it can be latent and reactivate. CMV infection in pregnancy can be primary (initial acquisition in pregnancy) or recurrent. Vertical transmission can occur transplacentally and it is mainly associated with congenital infection (7,8). Infection with HSV in the neonate is commonly acquired at birth through contact with the mother's infected birth canal. Primary infection of HSV enters a latent state in the nerve ganglia and may emerge later to cause recurrent active infection. Latency in nervous tissue is mainly caused by HSV-1 (3). *Chlamydia trachomatis* (Ct), is one of the major causes of BOH, this pathogen usually causes only asymptomatic or mild infection in mother, but can cause much more serious consequences in fetus and it has been connected to miscarriage (9).

In Iraq and worldwide, many studies have been conducted to evaluate the role of TORCH agents among women with abortion (2, 10, 11, and 12). Large number of these studies found that Rubella and CMV formed the highest rate among the other TORCH agents those studies depended on the serum IgM level for detection of primary infection (13, 14, 15 and 16). Our study was conducted to correlate between the actual incidence of Rubella, CMV, HSV and Ct primary infection among first trimester gravidas with

recurrent abortion, and estimate the rate of mixed infection with these agents.

2. Materials and Methods

Patients

A cross-sectional study was carried out at the private laboratories of Wasit province/Iraq started at September 2015 to August 2016. Some viral and bacterial TORCH agents (Rubella, CMV, HSV and Ct) primary infection rates were determined using ELISA method. A total of 82 serum samples were collected from gravidas in their first trimester of gestation living at Wasit province /Iraq. Serum samples were divided into 2-groups as following:

- 1) Group1: the study group included 67(81.71%) serum samples collected from gravidas with a history of recurrent abortion including those with one to more than four times of previous abortion times.
- 2) Group2: the control group 15 serum samples (18.29 %) collected from healthy gravidas with no history of abortion and matched to group1 for age, parity, and gestational age.

All samples were collected from women who attended the general hospital and private clinics, in Wasit province. Using special questionnaire the history of the participants were collected including the name, age, weight, residence area (urban and rural), education, number of children, obstetric history, number of abortions and time of gestation. All tests procedures and results were explained to the participants and their agreement has been taken to perform the tests.

Blood collection and serological tests

5 ml of whole blood were collected from each participating gravidas (group1 and2) by venipuncture and preserved in plain tubes, Sera were separated and stored in small screw capped vials at -20C° until serological analysis. Samples were screened for the presence of IgM and IgG antibodies against Rubella and CMV using ELISA kits from (Demeditec diagnostic GmbH) the test were performed according to manufactures instructions with 1:40 dilution of serum. Anti-HSV IgM was tested using microwell ELISA from DIAGNOSTIC AUTOMATION, INC, USA. Ct IgM was tested using (De medi tec diagnostic GmbH) ELISA kit. Optical density (OD) was read at 450 nm on an ELISA reader, all samples with OD reading above cut off value were considered to have a significant antibody titer to the mentioned organisms. IgG avidity test was performed for the anti-CMV IgM and anti-Rubella IgM positive cases. Results were interpreted for each test as shown in table 1 (13, 17).

Statistical Analysis

Data were obtained from special questionnaires, the results of the laboratory analysis were entered into Microsoft Excel, The χ^2 test at a 95% confidence interval and a significance level of 0.05 was used. $P < 0.05$ was considered significant.

3. Results

Sociodemographic and reproductive characteristics

A total of eighty twogravidas were divided into 2-groups and evaluated during their first trimester of pregnancy for their immune status for TORCH agents. Group1 included 67 gravidas (81.7%) high rate of this group included educated women living in rural and urban area (46.27% and 19.4%) respectively in comparison to a lower rates (19.4% and 14.93%) respectively of non-educated gravidas living in rural and urban areas (figure 1).

Group 2 included 15 gravidas (18.3%) who were having normal pregnancy with no previous abortion; high rates (53.33% and 13.33%) of this group were educated women living in rural and urban areas respectively. The non-educated gravidas recorded the lowest rates (26.67% and 6.67%) among group1 respectively.

The age of the gravidas varied between 17 and 44 years old. The total rate of gravidas with one time abortion recorded the highest rate 46.27% while the lowest was the rate 1.45% recorded by gravidas with ≥ 4 times abortion. Number of abortion times decreased gradually with the increase of gravidas age, in which one time abortion recorded 60%-47.62% among age groups 17-23 and 24-30 years respectively while 4 times recorded the lowest rates (0% and 9.52%) among those two groups (table 2). The results recorded a non-significant difference $P > 0.05$.

Serological test results

Primary infection with Ct recorded the highest rate (16.42%) among group1 followed by the rates (14.92%, 5.97% and 4.48%) of CMV, HSV and Rubella respectively.

Among group2 gravidas, Rubella and HSV primary infections recorded the highest rate (13.33%) for both, followed by the rate of CMV and Ct which also recorded the same rate 6.67% (table3). Serology tests results of group1 and group2 showed a non-significant difference $P > 0.05$.

As shown in table 4 primary infection with Ct recorded high rates (38.46%, 25%, 50% and 66.67%) among gravidas with 1, 2, 3 and ≥ 4 time's abortions respectively. Followed by the rate of primary infection with CMV (38.46%, 37.5%, 25% and 33.33%) respectively. Rubella did not show any positivity among gravidas with 3-4 times abortion and recorded 15.38% and 12.5% among 1 and 2 times abortions respectively. HSV recorded a low rate among gravidas with 1 and ≥ 4 times' abortion (7.7% and 0% respectively) and gave equal rates 25% among gravidas with 2 and 3 times abortion as shown in table4. The results were non-significant and $P > 0.05$.

Comparison between number of mixed infections among group 1 and 2 have been shown in table 5 the number of gravidas of group 1 written between brackets. high number of mixed primary infections recorded by Rubella, HSV and CMV respectively among group1 and 2 gravidas in comparison to a lower number recorded by Ct (the total cases were 11 only 2 were mixed with CMV and HSV).

4. Discussion

Viral and bacterial infections have long been connected to abortion (2, 3, and 14). Earlier studies placed the incidence of infection and abortion between 5.4 and 8.2% of all different causes of abortion (18). There were previous studies conducted to predict the causal agents and rate of some prenatal and perinatal infections which were designated as TORCH agents (10, 19, and 20). These agents infect and affect the fetus at various stages of gestation either once, as is usual in Rubella virus infection, or recurrently, as seen notably with HSV, CMV and *Ct* infection (21). Primary infection with TORCH agents is initially asymptomatic and thus difficult to diagnose on clinical grounds in pregnant women so most physician request a serological test for diagnosis (2, 3, and 22). Detection of agent specific IgM antibodies in a single serum sample has been taken as a reliable indicator of recent infection and hence, this method was employed in many studies (1, 2 and 3) as well as our study.

The current study tried to correlate between the incidence of Rubella, CMV, HSV and *Ct* primary infection among first trimester gravidas with recurrent abortion, also, tried to predict the actual rate of mixed infection with these agents among group 1 and 2 gravidas. Eighty two gravidas in their first trimester of gestation were included in the recent study they were divided into two groups, there were a non-significant difference between rate of infection among all gravidas despite their different levels of education and area of residency. Our results disagree with a study conducted in turkey which found that rural gravidas recorded infection rate higher than those living in an urban area (22). The differences in immunization rates from one geographical area to another and from age group to another results in a considerable variation in the prevalence of infection among women of child bearing age in different geographical areas globally (23). The rate of abortions were seen maximally between the age groups of 17-30 years in our study and this is might be due to the fact that marriage in Iraq is most common in this age group. Our results agree with the results of Mohammad and Salman, 2014 in which they found that infection increases in young ages and decrease in older women (10) and disagree with the results of Al-Marzoqi and colleagues who mentioned that the main age of infection was from (25-40 years) (2). As such as, age, education level, residency and many other factors do not seem to affect the risk of infection (19).

Results in table 3 showed that, *Ct* infection rate was the highest rate (16.42%) followed by CMV, HSV and Rubella infection rate (14.92%, 5.97%, and 4.48%). Our results went against many other studies conducted in Iraq, for example Al-Marzoqi and colleagues depended on ELISA for titration of serum IgG and IgM from aborted women and their results revealed that CMV recorded the highest rate 55.3% followed by Rubella 45.5%, *Ct* 36.3% and Herpes 5.8% (2) these results are very high comparing to our results except for HSV infection rate. Similarly Kishore and colleagues recorded prevalence rate about 15% for rubella, 30% for CMV and 3.3% for HSV (21). This difference could be attributed to the use of IgG avidity testing to confirm Rubella and CMV primary infections among our

patients. A positive IgM result for Rubella and CMV may not always indicate a primary acute infection, as IgM has a tendency to persist, even at high levels, after primary infection. False-positive IgM results may occur due to rheumatoid factor and antinuclear antibodies. Hence, IgG avidity testing is recommended to differentiate between primary infection, IgM persistence and reactivation (24). Persons develop IgG antibodies by 30 days post-infection, pregnant women that had IgG antibodies are immune (21). Virus-specific IgG antibodies of low avidity are produced during the first months after onset of infection, and subsequently, a maturation process occurs by which antibodies of higher avidity are generated. Hence high IgM levels and a low avidity index are highly suggestive of a recent (less than 3 months) primary infection (25). IgM antibody is present 4-8 weeks following the primary infection and can persist for years. False positive results can occur due to detection of high IgM levels with a high avidity index (26). The measurement of IgG avidity for Rubella and CMV has proved to be a highly useful procedure, especially in combination with conventional serological assays. A low avidity index may also be seen in a proportion of infected persons for months. Hence it is important to perform IgM testing initially to avoid misinterpretation of results (13).

Accuracy in diagnosis of congenital infections with Rubella and CMV is very important because they are a significant cause of neonatal mortality and infant morbidity especially when they are acquired during the first trimester of the pregnancy (27). So, our results agree with the results of Idris *et al* and Kamel *et al* (13, 28) in the importance to verify between the primary and recurrent infection with an IgG avidity testing especially with CMV infection because women with past infection with rubella are unlikely to have reinfection/recurrence whereas reinfection/recurrences with CMV are common (13).

For *Ct* infection serological diagnosis play an important role in diagnosis if compared to other methods. Isolation and culturing of *Ct* is technically difficult, due to its strict intracellular life cycle, infection sometimes could be localized at deeper sites not amenable to sampling. Even with molecular approaches, detecting *Ct* can be difficult because of Polymerase Chain Reaction inhibitors or low number of copies often present in the lesions (29). In our study *Ct* IgM positive rate was the highest 16.42% among all group 1 gravidas and it formed 38.46-66.67% among gravidas with 1 time abortion and 4 ≤ times abortion i.e. increased with the increasing number of abortions, these findings are consistent with previous reports stating that a significantly increased incidence of *Ct* infection was observed among women with abortions. Early pregnancy loss could be induced by persistent, asymptomatic Chlamydial infection spreading to the fetal tissue (18). Although, the results showed a non-significant difference $P > 0.05$ which might be due to the low number of gravidas. However, our results were similar to Al-Husseinei and colleagues results who showed that the prevalence of Chlamydial infections in 273 females were (14.6%) among aborted women (20). On the other hand, our results disagree with the results of Kishore *et al.*, who stated that the anti-*Ct* IgM positivity was 46.5% versus 13.8% in control group (30).

As shown in table 5 mixed infection between TORCH agents was high among gravidas group 1 and 2. Mixed infections recorded high number between Rubella and CMV and a lower degree with Ct and HSV. Our results agree with the results of Aftab and colleagues (3).

In conclusion, viruses and bacteria cause clinically significant intrauterine fetal infections leading to abortion and other bad obstetric outcomes. Since the abrogation of congenital rubella infections by vaccination, Ct, CMV, HSV and other TORCH agents are the most important causes of intrauterine fetal infections. Although vaccination of women with child bearing age is important but still there are some degree of immunization failure due to many factors. Screening of immune status to all TORCH agents for each gravidas especially in first trimester is very important and aid in management of infection and avoiding fetal loss, also, mixed infection with more than two agents could be an expectable result. Using of IgG avidity and determining primary and recurrent infections with Rubella and CMV will save time and money as well as decrease the risk for in-utero transmission.

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6. Conflict of Interest

No conflict of interest was declared by the authors.

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Table 1: Interpretation of Rubella and CMV Immunoglobulin tests

IgG	IgM	IgG avidity	Interpretation
Non-reactive	Non-reactive	Not applicable	Infection unlikely
Reactive	Non-reactive	High avidity	Past infection; low risk for in-utero transmission
Reactive	Reactive	Low avidity	Primary infection; high risk for in-utero transmission
Reactive	Reactive	High avidity	Non-primary infection; low risk of in utero-transmission

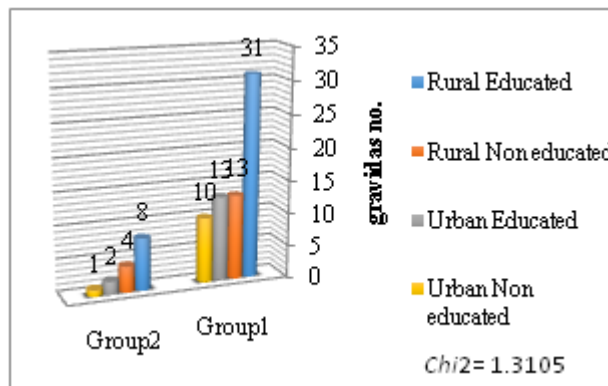


Figure 1: Description of social information for Group 1 and Group 2 gravidas

Table 2: Age group range and number of abortion times among Group1

Abortion times	1 time No.(%)	2 times No.(%)	3 times No.(%)	≥4times No. (%)	Total (%)
Age groups					
17-23	12(60)	5(25)	3(15)	0(0)	20(100)
24-30	10(47.62)	4(9.52)	5(23.81)	2(9.52)	21(100)
31-37	7(36.84)	5(26.32)	3(15.79)	4(21.05)	19(100)
38-44	2(28.57)	2(28.57)	2(28.57)	1(14.29)	7(100)
Total (% out of 67)	31(46.27)	16(23.88)	13(19.4)	7(10.45)	67(100)
Statistics	Chi ² =7.08			P=0.6286	

NO=number, %=percentage

Table 3: Distribution and percentage of IgG, IgM immunoglobulin and avidity testing for TORCH agents in serum samples among Group1 and Group2

Test type	Rubella (%)		CMV (%)		HSV (%)		Ct(%)	
	*G1	**G2	G1	G2	G1	G2	G1	G2
IgG test	14(20.9)	4(26.67)	20(29.85)	3(20)	-	-	-	-
IgM test	5(7.46)	2(13.33)	11(16.42)	1(6.67)	4(5.97)	2(13.33)	11(16.42)	1(6.67)
Avidity test	3(4.48)	2(13.33)	10(14.92)	1(6.67)	-	-	-	-
Primary infection=	3(4.48)	2(13.33)	10(14.92)	1(6.67)	4(5.97)	2(13.33)	11(16.42)	1(6.67)

*G1=Group1 rates are out of 67, **G2=Group2 rates are out of 15, P value >0.05

Table 4: Correlation of primary infection rate and number of abortion times among group1 gravidas:

Antibody Tests	Abortion times					Total
	1 time No. (%)	2 times No.(%)	3 times No.(%)	≥4times No. (%)		
<i>Rubella</i>	2(15.38)	1(12.5)	0(0)	0(0)		3(10.71)
<i>CMV</i>	5(38.46)	3(37.5)	1(25)	1(33.33)		10(35.7)
<i>HSV</i>	1(7.7)	2(25)	1(25)	0(0)		4(14.29)
<i>Ct</i>	5(38.46)	2(25)	2(50)	2(66.67)		11(39.29)
Total (out of46)	13 (100)	8(100)	4(100)	3(100)		28(100)
Statistics	Chi²=4.1			P=0.9045		

Table 5: comparison between number of mixed primary infections with TORCH agents among group1 and group2 gravidas

Versus	<i>Rubella</i>	<i>CMV</i>	<i>HSV</i>	<i>Ct</i>
<i>Rubella</i>	3*(4)**	3(3)	0(1)	0(0)
<i>CMV</i>	3(3)	10(3)	3(1)	1(0)
<i>HSV</i>	0(1)	3(1)	4(2)	1(1)
<i>Ct</i>	0(0)	1(0)	1(1)	11(1)

*Number of group1 gravidas, ** Number of group2 gravidas