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Detect the Infection with Rubella Virus and Toxoplasmosis in Pregnancy Cases Suffering from Early Abortion by Using Real Time PCR

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Abstract

Congenital infections may cause fetal death or newborns with brain malformations, little information is available about the but the impact of these diseases on the outcome of pregnancy. In this study we tested the IgG and IgM antibodies for both Toxoplasma gondii and Rubella. among 58 pregnant women with abortion. and in order to differentiate between the serological and molecular detection methods the samples were alsotested for both Toxoplasma gondii and Rubella by RT-PCR. The results showed that there is a positive relation between the infection with rubella and the infection with toxoplasma (p=0.022, Odd= 4.9) and this co-infection attribute to abortion in women. and these results of RT-PCR were significantly different than those with ELISA test.

Keyword: *Toxoplasma gondii, pregnancy, abortion, newborns, brain malformations*

Introduction

Birth defects are one of the most urgent global health problems affecting millions of births worldwide, but the causes remains unconfirmed [1]. Genetic and environmental factors have been found to cause these defects [2]. Congenital infections such as toxoplasmosis and rubella are known to play a non-negligible role in the development of brain malformations [3].

Toxoplasmosis is caused by the obligate intracellular protozoan *Toxoplasma gondii.* It is one of the most prevalent chronic infections affecting one third of the world's human population[4]. Toxoplasma gondii infections can cause to a more serious progression when accompanied with some other infection such as HIV and HBV [5].Congenital infections such as toxoplasmosis and rubella are known to play a non-negligible role in the development of brain malformations [6]. Toxoplasma gondiiseropositivity and coinfection with TORCH pathogens have been investigated in Qatar with the intention of testing the patients who are considered to be in the high risk group for TORCH pathogens[7]. Primary infections of toxoplasmosis, CMV, rubella and HSV during pregnancy can bring wide ranges of clinical symptoms dependent on the stage of pregnancy. TORCH infections during the early stages of pregnancy may result in congenital malformations, intrauterine growth restriction (IUGR) or fetal death [8].

Rubella infection during pregnancy has a devastating consequence; defined as congenital rubella syndrome (CRS). Miscarriage and stillbirth is the most important sequel of CRS [9]

Material and Method

Subjects:

This study was performed among 68 pregnant women collected from maternity unit at Al-Yarmook teaching hospital in Baghdad, Iraq, from 2018 to 2019. These samples were categorized into two groups, first group include 34 women with spontaneous abortion the other 34 women with normal delivery were enrolled as the control group.A questionnaire including demographic, epidemiological criteria and clinical symptoms was recorded in both the case and control groups by interview.

Five milliliters of venous blood samples were collected from each pregnant woman. Serum were separated from the blood samples and stored at −20 °C until use.

Serological evaluation

Specific IgG and IgM antibodies to *Toxoplasma gondii* and Rubella were measured by the Enzyme-linked immunosorbent assay, with commercial ELISA kits (ab108778 –Anti-Toxoplasma gondiiIgM) according to the manufacturer's instructions and the optical density (OD) was read at 450 nm by the spectrophotometer ELISA reader (Awareness Technology INC Stat Fax-2100). Diagnostic criteria IgG and IgM was defined the upper limit of the standard 10 U/mL (Cut-off).

Nucleic acid extraction

DNA and RNA were extracted from *Toxoplasma gondii* and Rubella, respectively using (RIBO-sorb, K2-1-Et-50-CE, Italy) according to the manufacturing procedure.

RT-PCR for detection of viruses

Both viruses *Toxoplasma gondii* and Rubella were detected by using commercial RT-PCR kits that allow the qualitative detection of viral nucleic acids in plasma, Toxoplasma gondii Real-TM (Sacae, CAT#TP1-50FRT, Italy) for *Toxoplasma gondii* and Real-TM Qual (Sacace, CAT#V24-50FRT, Italy) for Rubella.

Statistical Analysis

All data were analyzed by SPSS version 11.5 (SPSS, Chicago, IL, USA) using Fisher exact test. The odd ratio (OR) and *P*-value were also calculated

Results

The difference in age between case and control groups wasn't statistically significant, As shown in Table 1. Also the contact with cats showed no statistically significant differences

Table (1): The difference in age between case and control

Risk factors	Variables	Case (n = 34)	control (n = 34)	P-value
Age	<20	2	1	0.5
	20–40	29	30	
	>41	4	3	
Contact to cat	yes	9	7	0.6
	no	25	27	

*Toxoplasmagondii*IgGantibodywasdetectedin19(55.8%)ofcaseand11(32.3%)ofcontrolgroup(OR=1.23,P=0.5). Rubella IgG antibody was detected in 75.3% versus 86.7% in case and control groups(OR=0.46,P=0.05). **Table (2):** The comparison results *Toxoplasma gondii*IgG antibody and Rubella

Infection type	Case	Control	OR	p-value
Toxoplasma gondii	19 (55.8%)	11 (32.3%)	1.23	0.53
Rubella	25 (75.3%)	29 (86.7%)	1.4	0.32
Toxoplasma gondii+ Rubella	16 (47.0%)	2 (5.8%)	4.6	0.001

The detection of both viruses were also done by using RT-PCR and the resulted curves showed in figure-1 and the resulted data were compared to data obtained by using ELISA detection method. The comparison results summarized in table-2.

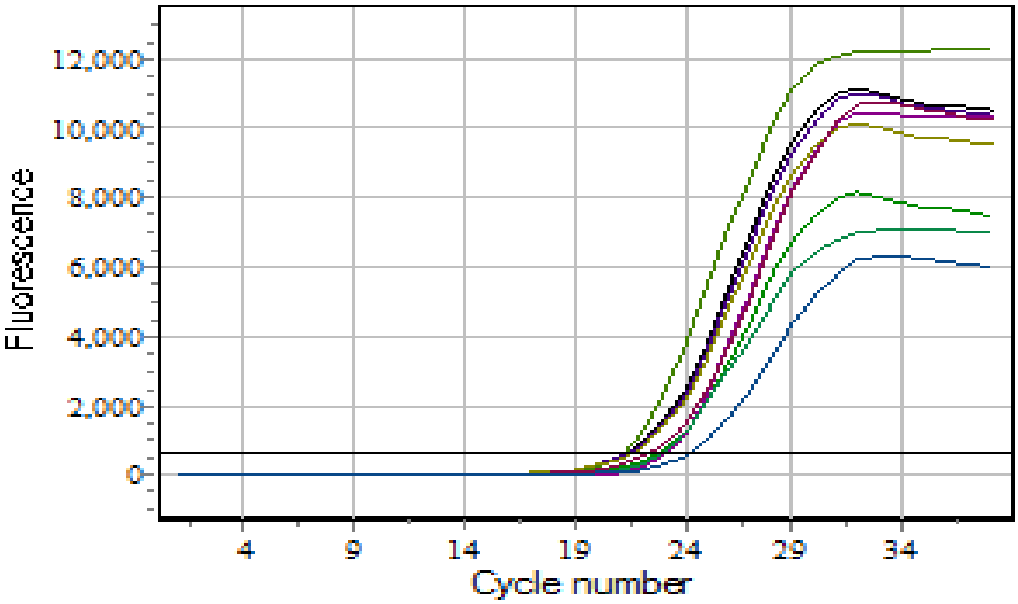

Figure 1:- resulted RT-PCR curves of Rubella and Toxoplasma detection.

The detection by using RT-PCR reported to be more sensitive than serological method as this method based on molecular detection of the active viruses. The results were summarized in table-3. 25 case subjects (with continues abortion) infected with rubella detected by ELISA were minimized to 20 sample after re-tested by RT-PCR and 29 control subjects minimized to 3 only this comparison were highly significant. While the infection with toxoplasma were recorded in 19 case subjects by ELISA minimized to 14 by RT-PCR and 11 control subjects to only 5 only this were also highly significant. The statistical test were done by ANOVA two way .

Table (3): The correlation betweenRubella and toxoplasmos

Groups	**Case**		**Control**		**p-value**
	RT-PCR	**IgM**	**RT-PCR**	**IgM**	
Rubella	20	25	3	29	0.001
Toxoplasma	14	19	5	11	0.008

The correlation betweenRubella and toxoplasmosis summarized in table--3 showed that 14 of the patients with Toxoplasmosis were also infected with Rubella and only 3 cases were infected with Rubella without the infection with toxoplasma. While the 20 subjects infected with rubella without toxoplasmosis infection and 21. These data showed significantly (p=0.022) high odd ratio= 4.9 (C.I.= 1.26 to 19

Table (4): Risk for congenital rubella syndrome increases when infection occurs in early stages of pregnancy

		toxoplasmosis				
		infected	non-infected	p-value	odd	
rubella	Infected	14	6	0.022	4.9	1.26 to 19.13
	non-infected	3	21			

An estimated 5% of couples attempting to have a baby experience recurrent pregnancy loss. Recurrent pregnancy loss is often defined as 3 or more consecutive miscarriages. There are many reasons a woman might miscarry, but in the past very few diagnoses were given or made known to the mother. Recent studies have shown that approximately 10

to 15% of all first time pregnancies end in miscarriages. The studies also suggested that a similar miscarriage rate could be expected for future pregnancies. [10]. Jaslow CR et al (2010) [11] and Rai R, Regan L.(2006) [12] found there is significant relationship between history of miscarriage in the family and the studied group this is may be estimated to be due to the life style in the family and their health status these studies agreed with the current study.

Risk for congenital rubella syndrome increases when infection occurs in early stages of pregnancy. Specifically, the percentage of infants with congenital malformation exceeds 50% in cases of infection during the first trimester of pregnancy while the relative percentage is significantly reduced after the 20th gestational week [13],[14],[15],[16],[17] . However, maternal viremia is not a proof of vertical transmission to the embryo, and fetal infection does not necessarily correspond to fetal damage. Namaei et al. reported that none of the infants of gravidas that received a measles-rubella vaccine at the interval between 3 months before and 3 months after conception appeared to experience viral consequences. This may be the reason why authors support detailed information provided to the mother in order to prevent unnecessary voluntary interruption of pregnancies [14,18].

Prenatal diagnostic exploration should be recommended in case of positive PCR in order to detect the affected embryos, having more precise information concerning the fetal health status before the decision of pregnancy's termination [19,20,21,22] .Studies have demonstrated an association between viral load in amniotic fluid and the risk of a symptomatic infant, whereas further investigation with a noninvasive diagnostic procedure might postulate a method to evaluate potential fetal affection [22,23,24,25].

Serological tests results by ELISA were not specific and may give false positive results that could be explain by chances of infection with other microorganism and that's also confirmed by the results asfew of the results obtained by ELISA were confirmed by PCR[26]. In addition, The results obtained in Al-Najaf indicates that the percentage of Rubella IgG was 49.12%and that revealed less than the result recorded by Lenochove in Turkey [27].

Serological results obtained bybyThikra[28]. revealed 12 out of 57 patients were IgM and IgG for Rubella and after test them by PCR, only (50%) of results were positive. And this result agreed with Nolan [29]. whose approved that out of 18 positive cases of Rubella by ELISA only 7 were positive by PCR. Our results revealed seropositive IgGfor Rubella and Cytomegalovirus percentagewere49.12 % and 70.2% respectively. After PCR test has done the results percentage has declined to 10.53 % in Rubella virus, and this lead to real incidence of infection of this microorganism and reflect the false positivity of the routine ELISA test [30]. In addition, Toxoplasma gondii and Rubella both are considerednon-active infections in human being with continuous mini foci that may lead to immune response state. Antigenic diversity in viruses may add another explanation to give false positive results in ELISA[31].

A small number of countries in Asia have a Rubella-containing vaccine in their national immunization programs. At the moment, control of Rubella through vaccination has been achieved only in Japan, Taiwan and Singapore. Rubella therefore remains poorly controlled in many Asian countries. Data from 2009 shows that, in the Southeast Asian continent, the vaccination coverage rate is only (4%) [32], while in Iraq, the vaccination coverage was 67.6% and 88% as demonstrated in retrospective and prospective study respectively [33]. Various vaccine strategies have been used the world over to mitigate Rubella infections. As inoculation is now well received, vaccination programs strive to immunize all young people before the onset of puberty using a two-stage Rubella vaccination. In the event of a negative result, there is a chance of being immunized during the early stages of pregnancy which lead to specific IgM being detected [34].

Conclusion

After representing these results we conclude that co-infection with both Rubella and Toxoplasma infection might increase the risk of miscarriageand we showed the sensitivity of molecular test and specially the RT-PCR is significantly sensitive than the serological test.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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