World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644

RUBELLA VIRUS AND TOXOPLASMA GONDII INFECTION WITH IMMUNE ANTIBODIES DIAGNOSIS AMONG BAD OBESTETRIC AND PRIMIGRAVIDA PREGNANT WOMEN IN BAGHDAD CITY

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Article history:

Received: May 6th 2022
Accepted: June 6th 2022
Published: July 12th 2022

Abstract:

Infection with rubella virus causes a little and mild illness, infected mother during pregnancy can result in birth abnormalities known as congenital rubella syndrome (CRS). Toxoplasma gondii is a parasitic infection that affects one-third of the world's population. Toxoplasmosis is extremely essential, particularly in pregnant women. Infection in pregnant women can be life-threatening and severely disrupt the fetus.. The goal of this study was to find out how many pregnant women had the rubella virus and antibodies and to assess the parasite antibody seroprevalence in pregnant women in relation with their age and the trimester of pregnancy in Baghdad city although to look into the link between Toxoplasmosis and Rubella virus infection in those patients. The study focused on pregnant women who visited some private hospitals in Baghdad in three different senatorial districts. From the period of time between January 2018 until January 2020. 300 pregnant women's blood serum samples were collected and tested for rubella and toxoplasma gondii IgM and IgG antibodies using an enzyme linked immunosorbent assay (ELISA) as well as confirm tested by polymerase chain reaction (RT-PCR) and gene sequencing. The risk factors for those parameters transmission in these pregnant women have been identified. The 300 patients were divided into two groups: 150 pregnant women with a bad obstetric history and 150 primigravida women who aborted at various dates (1st, 2nd, and 3rd trimesters of pregnancy) , group I had a gestational period of (69(46%), 48(32%),33(22%) and group II had a gestational period of (87(58%), 42(28%),21(14%)) respectively . There were 121 women aged 18-28 and 177 women aged 29-44 and 2 patients ≤ 44 years of age among the 300 women with a mean age of 27.9 years and a range of (18-44) years. Anti-rubella IgM levels in group I were significantly higher than in group II, according to the current data. (60%) Group I scored 90 out of 150, which was greater than group II (48%) 72 out of 150. While, IgM antibodies of anti toxoplasma among group II (40.6%)61 out of 150 was higher than group I which was (28.6%) 43 out of 150 women, IgG among group I for anti toxoplasma (63.3%)95out of 150, was higher than group II which was (56%)84 out of 150. While anti-rubella IgG in group I (67.3%) 101 out of 150 was greater than in group II (57.3%) 86 out of 150, whereas, rubella virus infection detected by ELISA in 210 (70%) and toxoplasma detected in (64.3%)193 out of 300 patients. and in order to differentiate between the serological and molecular detection methods the samples were also tested 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 and this co-infection attribute to abortion in women. and these results of RT-PCR were significantly different than those with ELISA test, There were reduced after re-testing by RT-PCR to the 156(52%)and 147(49%) out of 300. These RT-PCR results were significantly different from those of the ELISA test. Anti Rubella-Anti Toxoplasma antibodies were found in 49,57 of 150 people in group I and 41, 56 of 150 people in group II. the positive combination existence of specific anti-Rubella IgG and IgM

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644

antibodies, which were 21 (14%) in group I and 33 (22%) in group II. IgM and IgG antibodies against toxoplasma were present in 37(24.6%) and 22(14.6%) of group I and II, respectively. There is a statistically significant correlation at (p 0.01).

Keywords: Rubella virus, Toxoplasma gondii , BOH, primigravida, pregnant women.

INTRODUCTION

Birth malformations are one of the most pressing global health issues, impacting millions of babies around the world, yet the reasons are unknown [1]. These abnormalities have been linked to both genetic and environmental causes [2]. Toxoplasmosis and rubella, for example, are known to play a significant influence in the development of brain abnormalities [3].

Rubella virus is a common human pathogen that causes rubella, little red, 3-day measles, or German measles, an acute and contagious sickness[4]. This virus has only one reservoir: humans. The virus has a 2–3 week incubation period. The transmission path is in postnatal situations, it is airborne, and during pregnancy, it is transplacental[5,6]. This virus's illness is very prevalent. It is characterized by a maculopapular rash that appears in children low-grade fever, lymphadenopathy, and malaise are all symptoms[7]. In adults, it can cause joint problems, headaches, and conjunctivitis[8]. Rubella infection is regarded to be relatively benign, and it is usually moderate and self-limiting in the absence of pregnancy .However, it has a terrible effect on a pregnant woman. On the fetus as it develops this is a huge public health concern around the world[9,10]. There is currently no specific treatment available[11]. because of the virus Its burden, on the other hand, can be significant. The use of a live attenuated rubella vaccine helps to reduce the risk of infection[12,13].Rubella and congenital rubella syndro me (CRS) are controlled by a high degree of communit y immunity[14]. The World Health Organization (WHO) recommended that rubella vaccine be introduced in each country by the year 2000. and various activities are underway in various WHO areas. As a result, the load has decreased, albeit primarily in developed countries. Rubella vaccination, on the other hand, is still unavailable in many impoverished nations. It's also not covered by their immunization regimens[15,16].

Toxoplasmosis is a zoonotic illness caused by the intracellular protozoan Toxoplasma gondii, which infects humans and warm-blooded animals as an intermediate host [17]. While many members of the cat family, including cats, are the terminal hosts of the parasite, sexual reproduction and the creation of Oocysts are the main sources of infection and disease dissemination [18]. Humans become infected by congenital transmission of the parasite from the infected mother to the fetus via the placenta, resulting in miscarriage and abortion, particularly if the infection occurs during the first trimester of pregnancy However, if the infection occurs during the third trimester of pregnancy, the infection may not cause clinical signs in the baby in the early stages, but as the child grows older, the condition worsens and can develop to chorioretinitis or ocular toxoplasmosis [19]. The severity of the sickness is determined by a number of factors, including the host's immunity, parasite strains, parasite size, and the infection causes the body to develop an immune response known as cellular immunity, which is the main line of defense against the parasite and disorientation. Immunity is represented by immunoglobulins or antibodies that kill parasites outside the cell [20]. Single infections in immunocompromised patients are usually asymptomatic or accompanied by influenza-like symptoms, but infection in immunocompromised patients is extremely dangerous and can result in an increase in the severity of symptoms as well as mortality [21]. Serological method as enzyme-linked immunosorbent assay are used to diagnose toxoplasmosis and rubella virus infection (ELISA), [22]. Genetically for the parasite as well as the virus illness detection by polymerase chain reaction (PCR) is important for the definitive diagnosis of toxoplasmosis and rubella infection during pregnancy[23].

MATERIALS AND METHODS

Collection of samples

Samples were taken from 300 pregnant women ranging in age from 16 to 44 years old, all of whom underwent an abortion. The samples were gathered from various private laboratories in Baghdad. Ten milliliters of venous blood were taken at random from participants .between January 2018 and January 2020, for each woman blood to separate serum from blood, samples were centrifuged at 4000 rpm for 5 minutes.

Serological testing

The Enzyme-linked immunosorbent test (ELISA) was used to assess specific IgG and IgM antibodies to Toxoplasma gondii and Rubella virus with commercial ELISA kits (Human. Germany). The optical density (OD) was measured at 450 nm using a spectrophotometer ELISA reader, as directed by the manufacturer.

The preparation of samples

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Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644

The samples were made by diluting 10 µL of patient serum in the test tube with 1 mL of sample diluent and gently mixing for five minutes. After covering with adhesive strips, incubated for 30 minutes. After that, it was washed four times with washing solution at room temperature. After that, adding 100µL of conjugate to each well. Then, covering and incubating for 30 minutes at room temperature. Wash the wells five times with washing solution before adding 100L of substrate and incubating for 30 minutes. When we finally add 50 µL of stop solution measuring the absorbance during 30 minutes only at 450 nm.

PCR technique

Real-TM, Sacace's (Italy) kit was used to test T. gondii, and Rubella virus, samples . According to the manufacturer's manual guide , the real-time instrument utilized was the Applied Biosystem Fast Real time (7500) PCR system, and the amplification program was the real-time instrument. Using Toxoplasma gondii Real-TM (RT-PCR kit), a commercial product that enables the qualitative identification of viral nucleic acids in plasma, Toxoplasma gondii and Rubella viruses were both identified.

RESULTS

This study was created to look into the frequency of Rubella virus and *Toxoplasma gondii* infection in pregnant women, in relation with their age as well as their trimester of pregnancy. n 300 pregnant women, the levels of IgG and IgM were assessed. The method used to detect antibodies is ELISA. The participants in the study had an average age of 27.9 years. ranging in age from 18 to 44. With 177 women who are 29 to 44 years old and 121 women who are 18 to 28, whereas, 2 ≤ 44 years. figure1 shows that. It was discovered that rubella virus and toxoplasmosis substantially correlated with the age and the gestational age of pregnancy (p 0.01). The results are shown in Table 1. Two groups were created from the 300 patients: 150 pregnant women in their first, second, and third trimesters who had a bad obstetric history (69(46%), 48(32%),33(22%)). While the remaining 150 primigravida women had abortions in the first 87(58%) second 42(28%),and third 21(14%) trimesters of their pregnancies, respectively. Figure 2 , the table 2 both display a strong significant association, as demonstrated. According to the most recent data, Table 3 demonstrates that group I had significantly greater seroprevalence of newly acquired Anti-Rubella IgM levels than group II (60%) Group I received 90 out of 150. which, as indicated in figure 3, was higher than group II (48%) 72 out of 150. Additionally, group II had a greater level of IgM in anti-toxoplasma (40.6%)61 out of 150 was higher than group I which was (28.6%) 43 out of 150 women. Figure 4 demonstrates high significance. While anti-Rubella IgG levels in group I were higher (67.3%) 101 than in group II (57.3%) 86 among the 150 women, there was no statistically significant difference between the two, as shown in figure 3, group II anti-Toxoplasma antibody IgG (63.3%)95out of 150 was more than group I score (56%)84 out of 150**.**Figure 4 illustrates that there is also no significant assosiation . Anti Rubella-Anti Toxoplasma antibodies were found in 49,57 of 150 people in group I and 41, 56 of 150 people in group II. Figure 4 illustrates the positive combination existence of specific anti-Rubella IgG and IgM antibodies, which were 21 (14%) in group I and 33 (22%) in group II. IgM and IgG antibodies against toxoplasma were present in 37(24.6%) and 22(14.6%) of group I and II, respectively. There is a statistically significant correlation at (p 0.01). figure 3. Since RT-PCR relies on molecular detection of the active viruses, it is claimed to be more sensitive than serological methods for virus detection. Table 4 is a summary of the findings

Table 1: Pregnancy age distribution in relation to women in groups I and II of the BOH and primigravida

Age	Number tested	Group I(BOH)	Group II (primigravida)	P value
18 - 28	121	54(44.6 %)	106(87.6%)	P= 0.017 *
29 - 44	177	94(53%)	44(24.8%)	
≤ 44	2	2(100%)	0 (0%)	

***significant at (p<0.01)**

Table 2: Prevalence of pregnancy by abortion time for BOH women in group I and primigravida women in group II.

Trimester of	Group I(BOH)	Group II (primigravida)	P value

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Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644

pregnancy			
First trimester	69(46%)	87(58%)	**P= 0.0124 ***
second trimester	48(32%)	42(28%)	
third trimester	33(22%)	21(14%)	

***significant at (p<0.01)**

Table (3): IgM and IgG frequency distribution of Rubella virus and Toxoplasmosis infections utilizing ELISA tests in (BOH) and (primigravida)

Factors	Group I(BOH)	Group II (primigravid)	P value
Rubella Positive (IgM)	**90(60%)**	**72(48%)**	**0.0423 ***
Positive(IgG)	101 (67.3%)	86 (57.3%)	**0.847 NS**
positive (IgM,IgG)	21 (14%)	33 (22%)	**0.0029 ***
Toxoplasma Positive (IgM)	43(28.6 %)	61(40.6%)	**0.0342 ***
Positive(IgG)	84 (56%)	95 (63.3%)	**0.965 NS**
positive (IgM,IgG)	37(24.6 %)	22(14.6%)	**0.0013 ***

***significant at (p<0.01) NS- no significant**

Table 4: Abortion distribution in relation between ELISA and RT-PCR.

Group	Case		p-value
	RT-PCR	ELISA	
Rubella	156(52%)	**210(70%)**	**0.003***
Toxoplasma	147(49%)	193(64.3%)	**0.009***

***significant at (p<0.01)**

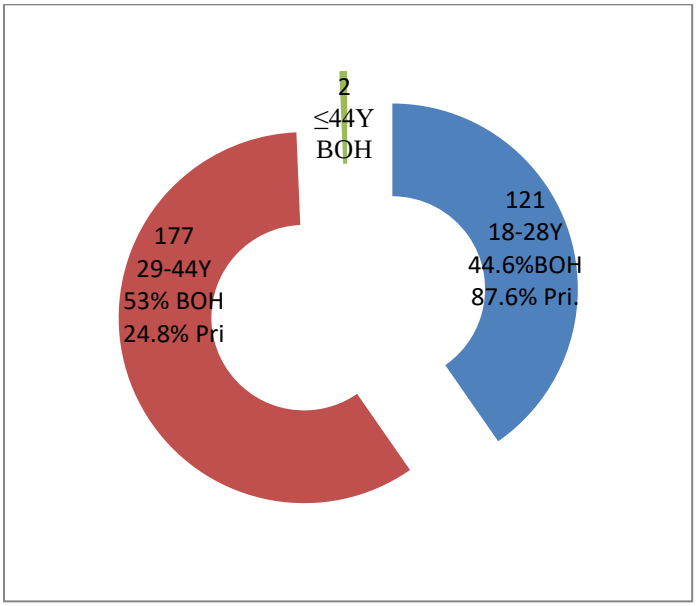

Figure 1: Pregnancy age distribution with respect to (group I) BOH and (group II) primigravida women.

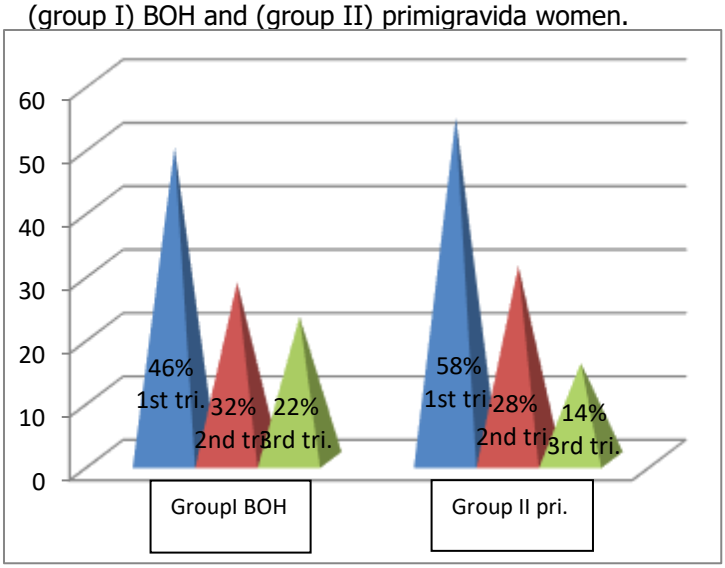

Figure (2): Distribution of pregnancies by abortion time (group I) for BOH women and (group II) for primigravida women

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Volume-12, July 2022
ISSN: 2749-3644

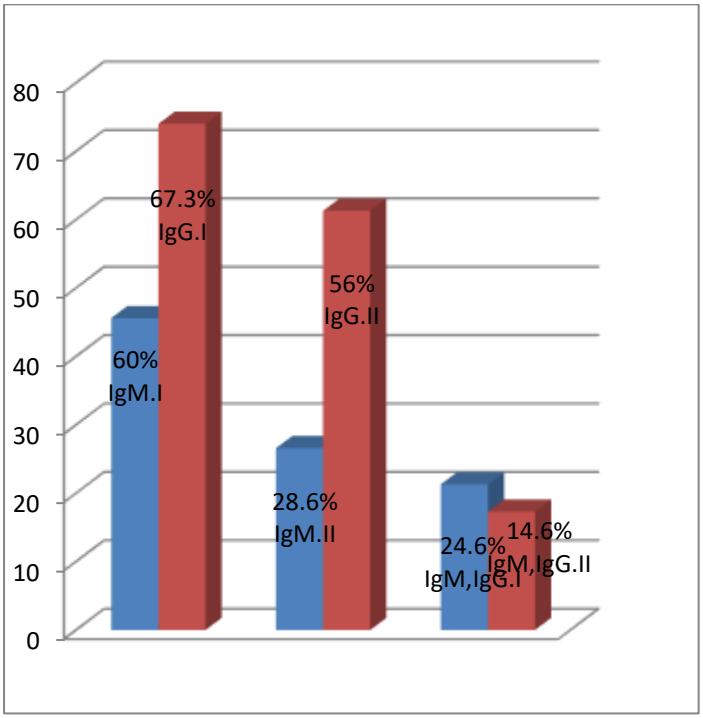

Figure (3):Seroprevalence of Rubella IgM and IgG in groups I and II positive results .

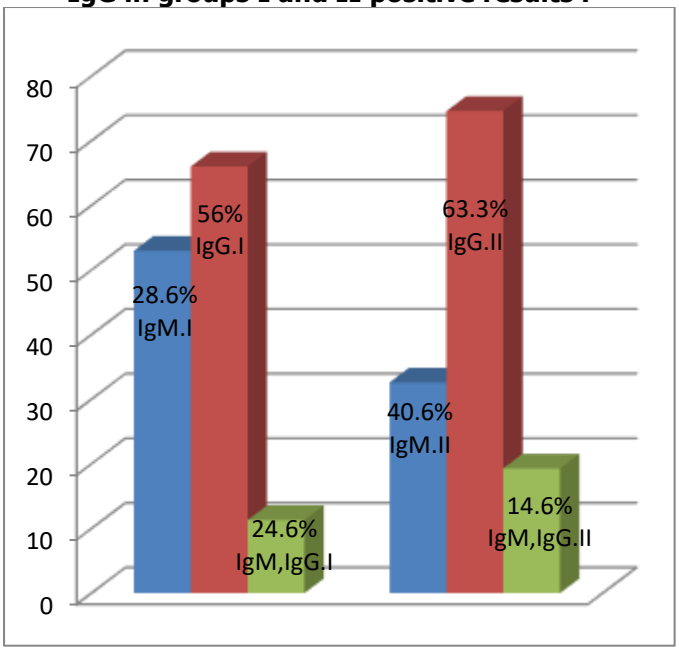

Figure (4): Seroprevalence of Toxoplasma gondii, IgM, and IgG in group I and II positive results

DISCUSSION

Toxoplasmosis is a zoonotic illness that affects one third of the world's population and is caused by the protozoan parasite toxoplasma gondii, which can infect all species of mammals and birds worldwide. It can also produce a number of clinical symptoms in both humans and animals[24,25]. viral contamination Togaviridae family member rubella is a disease that affects people all over the world[26]. Early in pregnancy, rubella can be fatal and impact many organs, leading to various birth abnormalities.[27] As other herpesviruses can establish latency and reactivate under conditions of immunocompromised people like HIV patients, HCMV infection often goes unreported in healthy people[28]. Results of this study showed that 300 aborted women were separated into two groups: BOH and primigravid. Of these, more over half of pregnancies ended in the first trimester. 69(46%),87(58%)respectively. This study's findings were in agreement with [29, 30,37] who reported that the first trimester of pregnancy had the highest prevalence, but they were at odds with [31], who discovered that the second trimester accounted for more than half of all pregnancies (51.2%), and [32], who also found that the second trimester had the highest prevalence of pregnant women. It was hypothesized that because most pregnant women visit doctors in their second and third trimesters of pregnancy, the first trimester saw the highest incidence of abortions. Our research involved patients with a mean age of 27.9 years who were between the ages of 18 and 44. They were compared to participants in other studies[33,37] whose participants had mean ages of 28.6 and 29.6 years who were between the ages of 15 and 42. There was opposition to this[34]. The current results for positive toxoplasma are shown in table 2, and they concur with [35], who reported that IgM for group I was 18% and group II was 17.5%. They also concur with [35], who reported that IgG for primigravid pregnant women was 45 %, but they disagree with BOH group, which was 26 %. also concurred with [36], who reported that 46% of people had positive IgG. The percentage of positive IgM in groups I and II was 43 (28.6%) and 61 (40.6%), respectively. IgM antibodies are a sign of an acute infection since they seldom occur in chronic illnesses and are not typically associated with acquired immunity. While the IgG response for groups I and II was 84 (56%) and 95 (63.3%) respectively, our results were in agreement with [38]. who found IgG/ was 96.1 percent in primigravid and 76 percent with BOH. According to the combination of positive precense of our results, which were reported in [39], 39.6% of individuals tested positive for both IgG and IgM antibodies. Serological findings discovered by Thakra[40]. found that 12 out of 57 individuals had IgM and IgG antibodies to rubella, and that only 50% of the results from PCR testing on these patients were

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644

positive. And Nolan [41] concurred with this outcome. Who approved that only 7 of the 18 cases of Rubella detected by ELISA and PCR were positive.

CONCLUSION

It could be concluded that, because of the high prevalence of rubella infection as well as toxoplasmosis among pregnant women, as well as the high percentage of infection among patients with a high level of disease ignorance, every pregnant woman still necessary to immunize seronegative against rubella before they get pregnant. And should be tested for rubella serological status as well as molecular diagnosis with RT-PCR to confirm the test.

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Available Online at: https://www.scholarexpress.net
Volume-12, July 2022
ISSN: 2749-3644