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Seroprevalence of Antibodies to Cytomegalovirus, Rubella Virus and *T. gondii* among aborted women in El-Beida City

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Abstract

This work was carried out to investigate the seroprevalence of antibodies to Cytomegalovirus, Rubella virus and *T. gondii* among aborted women in the 9 consecutive months (July 2018 to March 2019) at El-Beida City. A total of 95 women was tested using ELISA method for presence of *T. gondii*, Rubella and Cytomegalovirus (CMV) antibodies (IgG and IgM) by commercial kit. Data from samples of serum found that, the sero-prevalence of *T. gondii* (IgG/ IgM) were the highest followed by Rubella (IgG/ IgM) and then CMV (IgG/ IgM). 67.74 % of total women was get two type of antibodies against two different parasites. 22.6% were sero-positive for IgG anti-*T. gondii* and anti-CMV, 18.3% sero-positive for IgG anti-*T. gondii* and anti-Rubella and 26.9% were sero- positive for IgG anti-CMV and anti-Rubella at same time.The highest prevalence was noted in age group 21-25 years followed with age groups 31-35 and 26-30. The rate of one miscarriage in abortive women was 50.6% higher than two and three miscarriages 29.9% and 11.5% respectively. Results were might give a better understanding about Rubella, *T. gondii*, and cytomegalovirus infections during pregnancy that cause the foetal loss.

Keywords: Seroprevalence, Antibodies Cytomegalovirus, Rubella Virus, *T. gondii* and El-Beida City.

INTRODUCTION

Abortion is an issue in pregnancy wastage with its concomitant social and economic [1]. Pregnancy loss has been attributed to several factors involved in human reproduction. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, infectious agents, environmental pollutants, psychogenetic factors and endometriosis are most important causes of spontaneous abortion [1]. Infections caused by *T. gondii*. gondii (T.gondii), Rubella virus and Cytomegalovirus (CMV) are major causes of pregnancy lose in human. Women affected with any of these diseases during pregnancy are at high risk for miscarriage, stillbirth, or for a child with serious birth defects and/or illness [2, 1].

Among apicomplexan, *T. gondii* is unique as it is probably the most successful parasite with its widespread distribution worldwide causing one of the most common parasitic infection, called toxoplasmosis. Remarkably, *T. gondii*'s host range is exceptionally broad since it can infect virtually all nucleated cells of all warm-blooded animals including birds, marsupials, and mammals [3].

Rubella is caused by RNA virus of paramyxovirus group. It, also called German measles, is a viral infection that can easily be passed from person to person through sneezing or coughing. Rubella is less common today because a vaccine protects against it. But pregnant women with rubella can pass the virus to a fetus, which may be a serious condition [4]. Approximately 30%–50% fetuses of women who contact with Rubella during the first 3 months of pregnancy will be adversely affected by the virus. The Rubella virus readily invades the placenta and fetus during gestation [5]. A woman in the first 2 or 3 months of pregnancy who is exposed may develop the infection and give birth to child with serious congenital defects such as deafness and blindness [6].

CMV is a member of the family Herpesviridae. It is a public health problem which can be a potential killer or lifelong silent companion. Morphologically is very similar to other herpes viruses [7]. Acute infection usually occurs in

immunosuppressed and AIDS patients, and the symptoms vary from mild to severe. The severity of infection depend on a number of factors, such as the age of host, his immune status and the stage of pregnancy at the time of acquiring the infection ("congenital") [8]. CMV infection in pregnancy is extremely serious and considered a major health issue in several parts of the world which requires precautionary measures. Congenital infections are acquired when the developing fetus becomes infected by CMV crossing the placental tissues from a mother who acquired a primary CMV infection during the pregnancy. Some of these congenitally infected infants are born with severe deformities and die shortly after birth. CMV is among the leading congenital causative agents in developed countries [4]. Furthermore, the immunity of the mother does not provide protection against congenital infection but it reduces the severity of symptoms during acute infection [4]. Fetal damage is more likely to be severe when maternal infection occurs early in pregnancy [9].

Wide array of organisms, like TORCH agents, Chlamydia trachomatis, Niesseria gonorrhoeae and others can cause recurrent pregnancy wastage due to maternal infections during pregnancy [10]. Perinatal infections account for 2% to 3% of all congenital anomalies. TORCH, which includes Toxoplasmosis, other (syphilis, varicella-zoster, parvovirus B19), Rubella, CMV, and Herpes infections, are of the most common infections associated with unfavorable outcome of pregnancy [11]. Primary infection with any of the TORCH agents during pregnancy can lead to adverse outcomes, which are initially inapparent or asymptomatic and thus difficult to diagnose on clinical grounds [12]. The ultimate consequences of intrauterine infection can be any one of the following [11]: no significant problem, asymptomatic chronic infection, spontaneous abortion, intrauterine fetal death, stillbirth or neonatal death, fetal malformation or neonatal infection. This study was aimed to find out seroprevalence of *T. gondii*, CMV, rubella and herpes virus infections in a group of abortion women in El-Baida City, Libya.

MATERIALS AND METHODS

Ninety five abortive women who attended to different private clinics for consultation and investigations during the period between July and September 2018 were taken as a study group. Topic of prevalence antibodies for acute and chronic infection for more causes of abortion spreading that is including *T. gondii*, CMV and Rubella virus. All sera samples of 95 abortive women were analyzed by using TORCH panel Rapid Test to measure the titer of immunoglobulin G (IgG) and immunoglobulin M (IgM) against previous parasites.

The onsite TORCH panel Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of antibodies (IgG and IgM) to *T. gondii*, Rubella virus, CMV, (Herpes simplex virus1 (HSV-1) and herpes simplex virus2 (HSV-2). The onsite TORCH panel rabid test detects and differentiates IgG and IgM antibodies for all 5 TORCH infections. In general, a negative test result indicates that the individual is at risk for acquiring a primary infection. All the samples were firstly tested for the TORCH infections by using sandwich and capture ELISA based commercial kits (Biotech Lab. Ltd, UK and Omega Diagnostics, UK) and the BioTek ELx50 biochemistry analyzer (Winooski, Vermont, U.S.A) in the Serology Section of the Department of Microbiology. All the sera were assayed according to the manufacturer's instructions and the results were calculated on the basis of the cut off Activity Index (AI). The values which were 10% above the AI were considered as positive, whereas those which were 10% below the AI were taken as negative. The values which were in between these values were considered as equivocal. A 95% confidence interval was calculated for the positive cases in each of the TORCH components.

RESULTS

Total of 95 cases was tested for presence of antibodies (IgG and IgM) against *T. gondii*, Rubella, CMV and Herpes. The seroprevalence of *T. gondii* IgG was the highest followed by Rubella IgG and then CMV IgG, From total 95 sample of serum, 49.5% were found to be seropositive for *T. gondii*, IgG, 15.9 % seropositive for *T. gondii*, IgM and 34.7% were negative. 42.1% were found to be seropositive for CMV, IgG, 16.8% seropositive for CMV, IgM and 41.1% were negative. In case of Rubella, 44.2% were found to be seropositive for Rubella, IgG, 18.9 % seropositive for Rubella, IgM and 36.8% were negative, as shown in (Figure-1). Herpes antibodies were not recorded. And also compare them with those who have couple antibodies against two different parasites was 33.3%. was found to have IgG anti-*T. gondii* and anti-CMV, 27% found to have IgG anti-*T. gondii* and anti-Rubella and 39.7% found to have IgG anti-CMV and anti-Rubella at same time (Figure-2).

In term of distribution of positive cases of *T. gondii*, Rubella and CMV antibodies at different age groups showed the most prevalence was at 20-30 age group followed by 31-40 age group and then 41-50 as shown in Table-1. All subjects (95) were subdivided into three groups according to number of miscarriages (1, 2 and more than 3 miscarriages) as shown in Table-2. The rate of one miscarriage in abortive women was higher than two and three miscarriages.

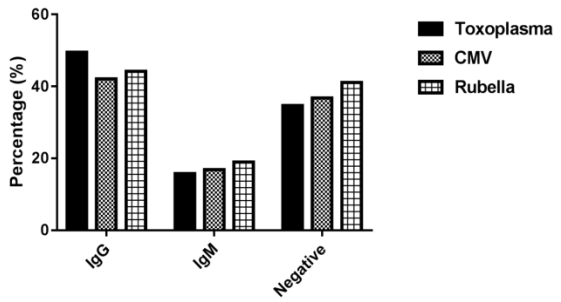

Fig-1: Percentage incidence of *T. gondii*, Rubella and CMV antibodies in miscarriage women.

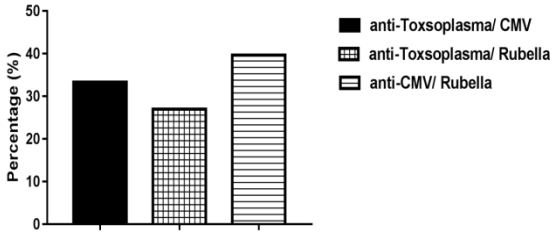

Fig-2: Percentage levels of antibodies (IgG and IgM) against couple parasites at same time (*T. gondii*, CMV and Rubella) in miscarriage women

Table-1: Distribution between prevalence of *T. gondii*, Rubella and CMV antibodies at different age groups

	No of cases		
	20-30	**31-40**	**41-50**
All subjects	42	32	21
Positive- *T. gondii*	20	12	8
Positive CMV	10	10	6
Positive Rubella	12	10	7
Positive for two or more	30	19	15

Table-2: Number of miscarriage in all 95 subjects

Number of miscarriages	**No of cases (%)**
One	46 (48.4)
Two	28 (29.5)
Three	12 (12.6)
More than three	9 (9.4)

DISCUSSION

Rubella, *T. gondii* and CMV agents are known to cause infections in utero and are often responsible for pregnancy lose, but there are considerable geographical variations in the prevalence of these agents among the women of child bearing age and are responsible for pregnancy wastage [5]. Topic of prevalence antibodies for acute and chronic infection for more causes of abortion spreading that is including *T. gondii*, CMV and Rubella virus [13]. Therefore, a total of 95 cases were tested for presence of *T. gondii*, Rubella and CMV antibodies (IgG and IgM). In current study was found that the prevalence of *T. gondii* (IgG/ IgM) were the highest followed by Rubella (IgG/ IgM) and then CMV (IgG/ IgM). These results were in concordance with the findings of a comparable study from India [14]. Previous study in Turkey, also reported overall anti-*T. gondii*, anti-Rubella, and anti-CMV IgM and IgG antibody positivity rates were 1.1%, 0.5% and 2.6%, and 37.6%, 86.5% and 100%, respectively [15]. Another study in El-Beida City also reported that prevalence of IgG against Rubella infection (98.59 %), IgG CMV (96.36%), IgG *T. gondii* (39.39%), anti-Rubella IgM and anti-CMV IgM antibodies were (0%) and (2.8%) respectively [16].

Most of the previous studies in Libya have concentrated on the prevalence of *T. gondii* infection among pregnant women. These studies appear to be in accordance with the high prevalence rates of *T. gondii* infection which reported by most of the previous studies in Arab countries for example in Saudi Arabia [16, 17], in Tunisia [19, 20]. In Libya, many studies done to detect the prevalence of *T. gondii* antibodies and show high prevalence rates in their studies [21-26]. However, the main reasons for this pattern of high prevalence rate of *T. gondii* infection in Libya needs more studies particularly in Northeast of Libya.

In this study the comparison between subjects who have couple antibodies against two different parasites was 67.74%. The highest prevalence was found in subjects who have IgG anti-*T. gondii* and anti-CMV, followed by found IgG anti-*T. gondii* and anti-Rubella then in subjects who have IgG anti-CMV and anti-Rubella at same time. These results revealed to another study repeated that the prevalence anti-Rubella IgG and anti-CMV IgG antibodies were found in high percentage [16]. The detection of IgG indicated that the pregnant women had previously been infected with Rubella, *T. gondii* and/or CMV viruses. After the infection, IgG remains in the body for life and protects considerably against the next infections. There are many studies about the association between CMV infection and pregnancy loss; these studies showed that CMV can result in abortion or stillbirth [27, 28].

In term of age, the cases per age groups were corrected according to demographic data, this study found that age group from 21 to 40 years were highest prevalence for presences of infection and this is pointing to rate of fertility among this age group. This result was not agree with that of Al-Harthi et al., [29] who reported the highest rate of sero-conversion among pregnant women aged 35–45 years. However, it is consistent with the age-group of pregnant women (15–35 years) that Jackson and Hutchison [30] reported to have the highest rate of sero-conversion, and also agrees with the data collected from Libya [31, 32]. Relation between an increases in the prevalence of chronic infection with middle age, indicate that exposure to (oocyst-contaminated) soil, which tends to be greatest during childhood, may not be the principal mechanism by which girls and women acquire *T. gondii* [33]. This high rate of sero-prevalence in 21-35 age group may also belong to higher contact with cats or

infected things and vegetables than other age groups [34]. This finding relatively also was analogous with study in Iran [35] and in Iraq [36].

Result from number of miscarriages was found that over all subjects the rate of one miscarriage in abortive women was higher than two and three miscarriages. The current study agreed with studies where they found the highest rate of infection among women who have suffered single abortion [37, 36]. On other hand the current study did not agree with [38]. The reason for the high incidence among aborted women may return to the type of acute injury or reactivate chronic injury due to decrease immunity of pregnant mother's body as the time of the injury during pregnancy has an important role to determine the fate of the fetus [37, 36].

The serological tests are useful for the diagnosis of Rubella, *T. gondii* and CMV agents through the detection of antibodies in the serum samples and determine the stage of infection that were in the acute phase or chronic [39].

The presence of the Rubella IgG antibodies were found in around 42 % of the pregnant women, which was in concordance with the findings of a comparable study in India [14]. Sero-epidemiological studies have shown that risk of the congenital Rubella infection following a maternal infection ranged from 5% to 50% in various studies, with an increasing severity when it was acquired in the first trimester of the pregnancy [10, 1]. Moreover, there is a considerable variation in the prevalence of the Rubella specific IgG antibodies among the women of the child bearing ages, with studies suggesting a prevalence of a 39% Rubella immunity, thus leaving about 61% of the women susceptible to the Rubella infection [40].

CMV infection in pregnancy has a higher incidence of symptomatic congenital infection and foetal loss [12]. Seroprevalance of CMV IgG observed in current study is agree with some other studies in different other countries in same regions include, Egypt [41], Nigeria [42], Iran [43] and Saudi Arabia [44]. Though more women who are in the child bearing ages are already seropositive, reinfection with a new strain of CMV can cause infections even in the presence of detectable IgG levels [45, 40]. This study showed a seropositive rate for the CMV specific IgM, thus indicating a higher prevalence in and around Varanasi. Therefore, it is necessary that behavioural and educational interventions for prevention are given and that an antenatal screening is routinely done.

It has already been emphasized that knowing the epidemiology of the TORCH infections is an important aspect in the development of strategies for the prevention of congenital infections. Hence, it should be recommended that all the antenatal cases should be routinely screened for the TORCH complex to avoid adverse foetal outcomes. Moreover, similar studies as this one, which document the seroprevalence of the TORCH infections, should be done to create a baseline data in the country. In such a context, the development of a vaccine strategy against these infections, especially in the developing countries, should be considered.

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