

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article

doi: 10.12980/jclm.3.2015j5-164

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Serologic survey of cytomegalovirus and rubella virus in Tabriz, Iran

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ARTICLE INFO

Article history:

Received 28 Aug 2015

Received in revised form 19 Sep 2015

Accepted 9 Oct 2015

Available online 4 Dec 2015

Keywords:

Rubella

Cytomegalovirus

Tabriz

Chemiluminescence

Seroprevalence

ABSTRACT

Objective: To determine the prevalence of rubella and cytomegalovirus (CMV) among women in Tabriz City, Iran in 2013.**Methods:** This study was carried out in numerous laboratories in city of Tabriz. In 2013, 26618 women aged 18–35 years were enrolled in this study. Serum samples were analyzed with chemiluminescence apparatus and SPSS software was used to conduct the statistical analysis.**Results:** A total of 26618 individuals were examined (10598 cases for anti-CMV and 16020 cases for anti-rubella). About 98.9% and 1.1% samples were reported positive and negative for CMV immunoglobulin G (IgG), respectively. The positivity for anti-CMV immunoglobulin M (IgM) antibody was found in 0.75% samples, while 99.25% were negative for the anti-CMV IgM antibody. Anti-IgG against rubella seropositivity was found in 95.8% and rubella IgM seropositivity in 0.93%. Anti-IgG against rubella seronegativity was found in 4.2% and rubella IgM seronegativity in 99.07%.**Conclusions:** According to the results, primary and secondary infections are usually symptomless or inconspicuous, but they can cause serious fetal damage. Routine antenatal care should therefore include serological testing to check the immune status of the woman and to diagnose any fresh virus infections that may arise.

1. Introduction

Children born with some disabilities, deformities, or who develop disabilities later in life have been documented in the world over and this has become a senior public health concern[1]. These pregnancy-related disabilities may be the result of exposure to congenital infections from certain viral infections during pregnancy[1,2].

Cytomegalovirus (CMV) is a member of the Herpesviridae with a double-stranded DNA (235 kb) that infects people. During active infection, CMV can be found in most organs and tissues, as well as in most bodily fluids especially saliva and urine[3]. CMV is

found throughout all geographic groups and locations, but it is more widespread in communities with lower socioeconomic status and developing countries[4,5]. This virus's clinical manifestations range from asymptomatic forms in 90% of cases to severe fetal damage and, in rare cases, death due to abortion[6]. Worldwide CMV infection prevalence is reported to be approximately 40% to 80%, but it has been estimated to vary from about 45% in developed countries to 100% in developing nations[4]. Rubella virus is a member of Paramyxovirus[1]. It is a single-stranded RNA virus enveloped by a capsule and causes infections only in humans[7]. It is the only member of the genus *Rubivirus* and the cause of rubella, a childhood disease commonly known as German measles[1,7]. Clinically, it appears with mild measles-like symptoms. During the prodromal period, fever and flu-like symptoms accompanied by a maculopapular rash are common[7]. During pregnancy, the virus may have potentially devastating effects on fetus[8]. The incubation period of virus is generally 12 to 19 days and the infection is

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commonly asymptomatic. Fever, rash, malaise and conjunctivitis are the most common symptoms in affected patients. Particularly in young women, a self-limiting arthritis may be occurring. Encephalitis, thrombocytopenic purpura and orchitis are other rare complications[7]. The World Health Organization calculated that each year, more than 110000 cases of congenital rubella syndrome are found worldwide and most of them are in developing nations[9].

Antenatal screening tests are an important part in the diagnosis of these infections[7]. Automated immunoassay analyzers and chemiluminescent immunoassay kits have been developed and commercialized by several major diagnostics companies. The comprehensive test menus cover analytes routinely measured in clinical immunology, toxicology, endocrinology and virology laboratories for the assessment of thyroid function, cancer, anemia, therapeutic drug levels, fertility and infectious diseases[10]. Survey with chemiluminescent method has greater specificity[10]. We conducted this study for the determination of seroprevalence of CMV and rubella virus infection by quantitative chemiluminescent immunoassay in Tabriz City, Iran.

2. Materials and methods

The cross-sectional study was accomplished according to the women who attended the pathological laboratories of Tabriz. A total of 26 618 women aged 18–35 years were enrolled in this study. Among them, 5044 were examined for CMV IgG test, 5554 for CMV IgM test, 7676 for rubella IgG test and 8344 for rubella IgM test. Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies from whole blood samples collected were measured using a chemiluminescent kit (DiaSorin LIAISON®, Turin, Italy). First, a number of samples were poured to the cuvette by the left arm of chemiluminescent LIAISON (Germany), then the solution containing magnetic particles was added by right arm and finally, they were incubated. The solution was washed three times by the washing solution buffer, and then rubella antigen and CMV antigen were added to the cuvette. In the next step, the conjugating solution was added and was re-incubated. Again, the solution was washed three times and a starter solution was applied for measurement. First, Starter 1 was poured into the vial and the indicator was injected to start quantitative chemiluminescent reactions. After about one-tenth of a second, the measurement signal was detected and the measurement was completed in 3 s. Also the age, education level, residential status and marital status of patients were investigated by a questionnaire. The research results were analyzed by *Chi*-square test using SPSS software (version 16). Statistically significant difference was considered at $P < 0.05$ [10].

3. Results

All the women subjected to this study were between the age of 18–35 years. From 5044 examined cases for CMV IgG test, 4988 (98.9%) individuals were positive and 56 (1.1%) individuals were negative. From 5554 examined cases for CMV IgM test, 42 (0.75%) individuals were positive and 5512 (99.25%) individuals were negative. From 7676 examined cases for rubella IgG test, 7356

(95.8%) individuals were positive and 320 (4.2%) individuals were negative (or non-immunized). From 8344 examined cases for rubella IgM test, 78 (0.93%) individuals were positive and 8266 (99.07%) individuals were negative. According to the results, the prevalence of CMV between women was higher than that of rubella. About 98.9% individuals of IgG antibodies against CMV were positive and this prevalence of IgG was higher than IgM antibody against CMV. The lowest rates for both virus infection were noted in women younger than 20 years old (Table 1).

Table 1

Demographic characteristics of CMV and rubella infected women in Tabriz, 2013. %.

Parameters		CMV	Rubella
Age groups	< 20	2.40	1.02
	20–25	23.70	22.05
	25–30	50.20	52.35
	30–35	23.60	24.57
Education	Less than high school	60.86	58.69
	High school graduate	26.08	29.34
	University graduate	13.04	11.95
Residential status	Urban	70.40	74.10
	Rural	29.60	25.80
Marital status	Single	0.60	0.50
	Widowed	1.40	1.80
	Divorced	1.20	1.60
	Married	96.70	95.90

4. Discussion

In pregnancy period, almost every system in the body is structurally or functionally changed[7]. During pregnancy, the immune system is suppressed, which makes women more susceptible to infections[7]. A highly efficient placental barrier prevents some microorganisms present in the mother from crossing from maternal into fetal circulation[11]. Nevertheless, some microbes are known to cross the placenta with devastating consequences to the fetus. Maternal IgG1, IgG3 and IgG4 cross the placenta to provide the fetus with a spectrum of molecules differing in specificity[11]. Two viral infections in this period are CMV and rubella virus infection[11]. The prenatal screening tests can play an important role in the diagnosis of these infections. In this study, 4988 cases were reported positive and 56 cases negative for CMV IgG. Positive CMV IgG results indicate past or recent CMV infection. These individuals may transmit CMV to susceptible individuals through tissue products and blood. Individuals with negative CMV IgG results are assumed to have not had prior exposure or infection with CMV and therefore considered susceptible to primary infection. A negative CMV IgM result proposes that the patient is not experiencing a recent infection. Positive CMV IgM results demonstrate a recent infection: primary, reactivation or reinfection. For rubella, the seropositivity was 95.8% for IgG and 0.93% for IgM. IgG-positive and IgM-negative, indicating previous infection with the rubella virus is vaccination[12]. The rubella and CMV IgG positivity were observed to be high in all of the groups. Interpretation of high rate of seropositive CMV (98.9%) requires to understand the fact that CMV has the capability to persist in its human host indefinitely as latent infection in the

kidneys and several glands. CMV has the ability of escaping host defense specially, when CMV settled dormancy in host cells allowing the virus to endure without triggering immune responses. In addition, the virus has the ability to encode proteins which qualifies itself to regulate the myosin heavy chain and inhibit natural killer cell[13].

Şevki *et al.* demonstrated that for rubella, the seropositivity was 93.9% and 0.4% for IgG and IgM, respectively[7]. In a study, the incidence of rubella and CMV infection in 86 pregnant women in the presence of IgG, IgM and immunoglobulin antibodies was assessed using ELISA kit. Sadik *et al.* indicated seropositivity for IgG for anti-rubella and CMV between 23% and 29%[14], which was much less than the results of this study. The highest frequency rates were noted in 25–30 age group for CMV and rubella infections. Based on the study of Afzali *et al.*, the highest frequency of hepatitis B virus infection was noted in 20–29 age group, married cases and residents of urban areas, and our findings were almost similar to the those results[15]. According to results of Aljumaili *et al.* in Iraq, CMV IgG seropositivity was 100% in women aged < 20 years, and then declined in women aged 20–29 years, but subsequently raised to 100% in women aged 40–48 years. The CMV IgM seroprevalence was the lowest (1.4%) in women aged < 20 years and the highest (10.1%) in women aged 20–29 years. The CMV IgM seroprevalence then declined to 3.7% in women aged 40–48 years[6]. Another similar study in 2012 showed that 72.1% of women were CMV IgG-positive[4]. Furthermore, the rate of positive CMV-IgM, primary and recurrent infection was 2.5%, 0.83% and 1.67%, respectively and the rate of CMV infection in pregnant women living in rural area was higher than those living in urban area. In addition, the first group had lower education level than the latter group. Both factors may increase the risk of CMV infection among rural population. In their study, women with primary school (73.3%), diploma (75%), academic (64.6%) and secondary education (75%) were seropositive for anti-CMV IgG and seronegative for anti-CMV IgM[4]. Tamer *et al.* had similar study carried out in West Turkey on the sera of pregnant women[16]. The results showed that 1 896 (96.1%) and 4 (0.2%) pregnant women were found serum-positive for anti-rubella IgG and IgM, respectively[16]. Seropositivities for anti-CMV IgG and IgM were found in 1 900 (96.4%) and 13 (0.7%) pregnant women[16], and this is in line with findings in the present study in Iran. Falahi *et al.* studied 42 cases (14.28%) and reported that 12 patients (28.58%) had positive anti-CMV IgG and IgM in Kosar Hospital, Ilam, Iran (2007–2008), with ELISA method[17]. Results demonstrate that there is a significant relationship between level of antibody with CMV and rubella infections[17]. Results of Ghazi *et al.* studied in 2002 in Saudi Arabia showed that frequency of IgG antibodies (anti-CMV, 92.1% and anti-rubella, 93.3%) was similar with our results for anti-CMV IgG, but the rate of rubella infection was higher in this study. All serum samples were screened using a sandwich ELISA[11].

According to seroepidemiological study in 2015, sera were screened for rubella specific IgM and IgG antibodies by recomLine TORCH. In this study, 10%–20% women were in the childbearing ages in India and susceptible to rubella infection[18]. Worldwide, there is considerable variation in the prevalence of rubella antibodies among women of childbearing age. Results of another study indicated that IgM and IgG seropositivity for rubella and CMV were 4.6% and 90.8%, and 9.2% and 95.4%[19], respectively, and their findings are consistent with our results. Uyar *et al.* in Turkey screened the sera of 600 pregnant women aged 17–40 years, for anti-rubella and anti-CMV IgG and IgM antibodies by a chemiluminescent enzyme immune assay method[20]. Results indicated that seropositivity of anti-IgG against rubella was found in 566 (94.3%) participants and rubella IgM seropositivity in 10 (1.7%). The positivity for anti-CMV IgG antibody was found in 584 (97.3%), while 6 (1.0%) were positive for the anti-CMV IgM antibody[20]. These results are compatible with the results of our research. Thus, CMV prevention strategies should take into account these age-related racial/ethnic differences. The observed disparities in CMV seroprevalence by race/ethnicity cannot be explained fully by education, marital status, area of residence, census region, family size, type of medical insurance or country of birth[21].

A rational screening program should be based upon the detection of intrauterine transmission of virus to identify fetuses at risk. This has been shown to be possible in anecdotal cases by culturing CMV from amniotic fluid or by detecting specific IgM antibodies in fetal serum[20]. In this study, the incidences of rubella infections in urban women were higher than those in rural women. According to study of Sabouri Ghannad *et al.* in Hamadan Province, Iran, it was showed that 77.1% of referred females were the urban residents[22]. This may be attributable to differences in the access of the residents in urban areas to this centre or maybe because of the presence of more educated people in the cities. In addition, the rate of married and single women who referred to the hospital consultants in genetic center were almost the same as 55% and 45% respectively. This may show the importance of medical consultation before and after marriage for females in this province. The presence of 95.5% of non pregnant females in the center may show the raising awareness of maternal-fetal conflict[22]. Cannon *et al.* found that CMV infection was relatively common among women of reproductive age, with seroprevalence ranging from 45% to 100%[23]. CMV seroprevalence generally increased with age in all 32 studies that examined this risk factor, as would be expected since antibody tests measure cumulative past exposure to infection. In most of the studies that were stratified by age, seroprevalence reached 60% or more in persons older than 50. To better understand the epidemiology of CMV infection, more and different seroprevalence studies are needed. Many studies have been done which look at CMV seroprevalence in special populations

such as parents of children in daycare, pregnant women or sexually transmitted diseases clinic attendees. More studies are needed which are population based and nationally representative. Currently, these types of studies have only been done in United States and Spain[23].

This study has established that CMV and rubella virus infections play a role in adverse fetal outcome in current pregnancy. We recommend that all antenatal cases, even if asymptomatic, should be routinely screened for CMV and rubella virus, as early diagnosis and appropriate intervention will help to manage these cases and reduce adverse fetal outcome, diminishing the morbidity and mortality. Further studies with more stringent design and building the new diagnostic technologies are recommended. In conclusion, serologic screening before pregnancy is important to diminish morbidity and mortality caused by rubella and CMV.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Present project was supported by Tabriz University of Medical Sciences, Tabriz, Iran. Authors are thankful to their cooperation.

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