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Cytomegalovirus Infection in Pregnancy - Our Experiences

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ABSTRACT

Introduction: Cytomegalovirus (CMV) infection is ubiquitous. It affects all age groups, and its clinical picture ranges from mild to severe, especially as a congenital infection in neonates. **Aim:** To determine frequency of CMV infection in pregnant women in Tuzla Canton (TC) and the risk factors that lead to the infection. **Methods:** This prospective study included 300 pregnant women from TC aged 18 to 42 years. CMV serology was performed on all participants, and in case of acute infection additionally IgG avidity test. Participants also completed the questionnaire on the risk factors for CMV infection. **Results:** The median age of the 300 women was 28 ±4.97 years. There were 161participants (53.6%) who classified their environment as urban and 295 (98.33%) were married. More than half of the women had completed secondary school 168 (56%). Positive IgG antibodies to CMV had 280 (93.0%) women. Positive IgM and IgG antibodies had 9 (3.0%) participants, but all of them had high IgG avidity, which indicates reinfection or recurrent CMV infection. There was a statistically significant higher number of seropositive participants living in rural areas than those living in urban areas (p= 0.048). Also, there was significantly higher percentage of positive anti-CMV IgG in pregnant women with lower education (p=0.04). **Conclusion:** In our region there is high seropositivity rates of IgG antibodies to CMV in pregnant women. No case of primary CMV infection was proven. The risk factors for CMV infection have been proven to be rural environment and lower level of education.

Keywords: CMV, pregnancy, risk factors.

1. INTRODUCTION

Cytomegalovirus is a member of the Herpesviridae family, which belongs to DNA viruses and is ubiquitous in the general population. Most CMV infections are mild, but the virus can cause a wide range of symptoms, especially in neonates and immunocompromised patients (1). About 5-10% of newborns with congenital CMV infection will have a symptomatic form of the disease, which causes severe central nervous system (CNS) damage including microcephaly, intracranial calcification, and ventriculomegaly (2).

Although CMV is present around the world, its epidemiology is different. CMV transmission occurs from person to person via body fluids, and requires close contact with contaminated secretions because the virus is not very contagious. The transmission mode is vertical and horizontal. In developing countries, most infections occur during childhood, while in developed countries up to 50% of young adults are seronegative to CMV. The seroprevalence of CMV among women of reproductive age ranges from 35 to 95%, depending on the country in which they live (3). It also increases with womens age, and it is believed that depends on the sexual activity, in particular occupations that involve close contact with children residing in collective accommodation, like kindergartens. In parents, contact with the urine or the saliva of their child is a major source of infection (4). In developed countries, 40-50% of women reproductive age is CMV seronegative, but on average about 0.5 to 1% will become positive in one year (5).

Congenital infection is present from 0.6 to 4% of neonates, depending on the studied population (6).

Acute CMV infection can be detected, and the virus isolated from urine, saliva, bronchial secretions, breast milk, vaginal secretion, and tissue biopsy sample. CMV antigen detection, as well as CMV DNA, is possible from different samples (1).

Screening for CMV is not one of the routine tests done in antenatal screening (7). CMV testing is only performed on demand, despite the

serious impact of CMV on the development of the fetus (8).

2. AIM

To determine the presence and frequency of CMV infection in pregnant women in Tuzla Canton and the risk factors that lead to the infection.

3. METHODS

In this prospective study, conducted from June 2017 to August 2018, 300 pregnant women from the Tuzla Canton (TC) area were interviewed in a specialist outpatient clinic for infections in pregnancy, at the Clinic for Infectious Diseases,University Clinical Center Tuzla (UCC Tuzla),with the cooperation of gynecologic dispensaries in the health centers in the TC region.

Pregnant women had a CMV serology testing done, which could have shown an acute or past infection, and be negative. In the case of a positive finding in terms of suspected acute infection, an IgG avidity test was performed on CMV to confirm the "age" of the acute infection. Also, these women completed a questionnaire for CMV infection risk factors (age, place of living, gestational age, parity, previous miscarriages, children younger than 5 years at home, educational level, job with close contact with small children, marital status, more than 1 sexual partner, chronic diseases, history of blood transfusion and organ transplant, history of fever during pregnancy, child with congenital disorder, previous knowledge of CMV, outcome of pregnancy).

The study involved pregnant women aged 18-45 years, of any gestational age, living in region of TC. The study did not include pregnant women under the age of 18, or older than 45 years, as well as pregnant women living outside of the TC area. The CMV serology test, as well as the IgG avidity test, were performed at the Department of Microbiology of the Polyclinic for Laboratory Diagnosis UCC Tuzla. The serology on CMV was performed by the ELISA method. The results were obtained by calculating the index value, and the interpretation was done according to the manufacturer's instructions. The Cutoff value for the determination of IgM/IgG is greater than 0.4 IU / ml, the border line is between 0.39 and 0.41 IU/ml and negative when their index is< 0.39 IU/ml.

Examples that were tested with ELISA and IgM and IgG were both positive, were also tested for IgG avidity for CMV. Avidity was obtained by performing a second set of ELISA test with microwells on the test plate for specific CMV IgG. Interpretation of the results: if the index <35% is considered to be low, and if it is> 35% high. Low IgG avidity indicates an infection acquired within 3 months, while high avidity excludes it, and indicates on reinfection or recurrent CMV infection.

Statistical analysis

In the statistical processing of the results, standard methods of descriptive statistics (measures of central tendency, dispersion measures) were used. For each laboratory finding, as well as for each risk factor, sensitivity, specificity, and positive and negative predictive value were tested. To test the statistical significance of the differences among samples, parametric and nonparametric significance tests, as well as the linear correlation method, were used. Statistical hypotheses were tested at a significance level of α 0.05, i.e. the difference between samples was considered significant if P <0.05.

4. RESULTS

In this study, a total of 300 pregnant women from the TC were examined for the presence of CMV infection. The median age of the subjects was 28 ± (SD) 4.97 years and the range of 18 to 42 years.

The pregnant women were divided into five age groups, with frequency distribution and percentage in the total participation given in Figure 1. The highest number of pregnant women was in age groups of 24-28 (36.3%) and 29-33 years (32.0%).

Immune response	N	%	Interpretation
IgG (+) IgM (-)	271	90,3	Previous exposure
IgG (+) IgM (+)	9	3,0	Acute infection
IgG (-) IgM (-)	20	6,7	Susceptibility to infection
IgG (-) IgM (+)	0	0,0	Recent, Primary Infection
Total	300	100	

Table 1. Seroprevalence of CMV specific IgG and IgM antibodies in the examined pregnant women (n = 300).

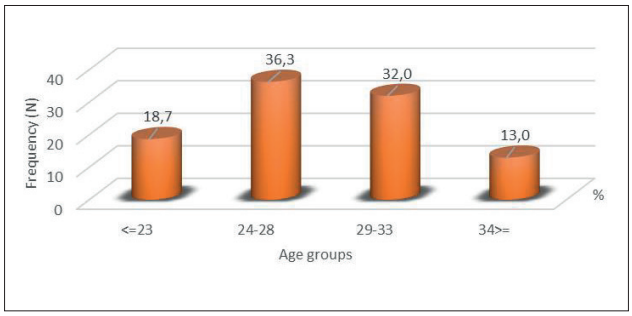

Figure 1. Distribution of pregnant women by age groups

Of the total sample of 300 pregnant women, 161 (53.6%) classified their environment as urban and 139 (46.3%) as rural. Two hundred and ninety-five (98.33%) of pregnant women were married, while a significant minority of 5 (1.67%) was unmarried.

More than half of the women had completed secondary school 168 (56.0%), 108 (36.0%) of pregnant women had completed college, 22 (7.3%) had completed primary school, and without any education were 2 (0.7%),

When it comes to the positivity of CMV immunoglobulin, the incidence of CMV IgG and CMV IgM positivity as an indicator of infection is shown in Table 1.

Two hundred and eighty of 300 pregnant women (93.3%) had positive IgG antibodies, which implies an earlier infection, and only 20/200 (6.7%) of pregnant women had IgM and IgG antibodies negative.

Acute infection based on serology, positive IgM and IgG antibodies had 9/300 (3.0%) pregnant women. In these subjects, an IgG avidity test on CMV was performed, and high IgG avidity had all 9/300 (3%) pregnant women - this subpopulation was considered to have evidence of a recent (3 months ago) acute CMV infection, or recurrent infection, or reinfection.

Cytomegalovirus (CMV IgG) n:300				
			95% C.I. za OR	
Predictive factors	p-value	OR	Lower limit	Upper limit
Age	0,288	0,952	0,870	1,042
Age group	0,223	0,776	0,477	1,263
Younger (<=35)/older pregnant women	0,658	0,730	0,159	3,360
Environment	0,048	0,363	0,129	1,026
Gestational age	0,537	1,028	0,942	1,122
Trimester	0,558	1,311	0,530	3,241
Parity	0,717	0,919	0,581	1,454
Number of abortions	0,693	0,846	0,368	1,943
Number of spontaneous abortions	0,565	1,530	0,360	6,507
Number of intentional abortions	0,239	0,555	0,208	1,479
A child younger than 5 years old	0,742	0,851	0,328	2,207
Education level	0,042	0,422	0,184	0,967
Contact with young children	0,624	0,872	0,545	1,395
Marital status	0,293	3,632	0,391	34,12
Number of sexual partners	0,342	0,545	0,116	2,559
Blood transfusion history	1			
Chronic Diseases	0,504	0,631	0,076	5,245
A previous child with congenital malformation	1			
Fever	1			
Previous knowledge of CMV	0,162	2,333	1,371	3,295

Table 2. Risk factors for CMV infection in pregnant women, dependent variable CMV IgG (n = 300).

We analyzed the impact of the environment for the presence of CMV IgG antibody and there was a statistically significant higher number of seropositive subjects in rural areas than those of the urban environment (p = 0.048) (Table 2).

There is a significantly higher percentage of positive anti-CMV IgG antibodies in pregnant women with lower education level, and there is an increase in the percentage of negative CMV IgG antibodies with an increase in education (p = 0.042) (Table 2). For pregnant women with completed college, this percentage is 10.2%, and for those with secondary education, 5.4%.

Analyzing the impact of predisposing risk factors: contact with small children going to a kindergarten, age, parity, abortion information, blood transfusion or organ transplantation information, data on multiple sex partners, marital status, gestational age, trimester, prior knowledge of CMV, fever during pregnancy, a child with congenital malformation, it was not found to affect the incidence of cytomegalovirus infection in pregnant women (Table 2).

5. DISCUSSION

CMV infection is present worldwide and can be presented from a mild clinical picture, similar to cold, to an extremely severe form of illness in immunocompromised individuals, and in neonates with congenital CMV infection. In pregnant women, CMV infection usually appears with a mild clinical picture, and therefore is this condition difficult to recognize,so if the serology testing on CMV is not done as common analysis in pregnant women in the first trimester of pregnancy, the infection can easily be overlooked, and lead to infection of the fetus and cause congenital disease.

In our study, 300 pregnant women participated whose average age was 28 years and ranges from 18 to 42 years. Pregnant women were divided into five age groups, with the highest percentage in the age groups 24 to 28 and 29 to 33 years (with 36.2% and 32% respectively). In the study conducted in Turkey, the largest number of respondents were in age groups 20-24 and 25 to 29 years (with 29.23% and 31.16%) (9), in the second study the percentage was 38.5% for the age group 21-25 years, and 36.5% for the age group 26-31years (10), and in the third study in Kenya 36% for the age group 26-30 years, and 21% for the age group 31-35 years (11). As can be seen, there is a similar percentage of pregnant women divided by age groups, with the largest number of participants being between 20 and 30 years of age.

Out of our 300 pregnant women, 161 (53.6%) classified their environment as urban and 139 (46.3%) as rural. Similar to our results, we also have data from Yemen (12) where 71.1% of pregnant women were from urban areas, also in Kenya, most pregnant women were from urban areas, 57.3% (11). This data shows us that more pregnant women living in urban areas are tested for CMV compared to those living in rural areas, which may indicate a better education of women in urban areas on the impact of CMV on the fetus, and their insistence on testing on CMV.

In our study, 295 out of 300 pregnant women (98.33%) were married. We have almost identical data in a study conducted by Mamuye, where 98.5% of the participants in the survey were married (10). Even today, although we are a modern society, this shows that the vast majority of pregnant women are married, or perhaps that single-handed pregnant women are less likely to do test for CMV (maybe because of stigma).

More than half of our respondents (56.0%) had secondary school education, 36.0% of them had college education. In other studies, pregnant women were slightly different according to the finished school, so in the Kenya study (11), most of the respondents completed a college degree of 63.8%, while in the study conducted by Mamuye 32.0% had completed secondary school. This again shows that women with higher education are more tested than those with lower education, or no schooling.

A large percentage of seroprevalence on CMV is found in almost all developing countries, so in Nigeria there is 97.2% (13), in Turkey 96.4% (14), Iran 97.69 % (15). Regarding the countries in Europe, 75.3% seroprevalence in Croatia (16) and in developed countries ranges from

30.4% in Ireland to Irish women (17), 45,6% in The Netherlands (18), 51.5% in France (19), 72% in Sweden (20).

Of the total of 300 subjects in our study, 280 (93.0%) had positive IgG antibodies to CMV, which means that these respondents had a previously reported CMV infection, with only 20 (6.7%) who had IgM and IgG negative antibodies. According to this data, our region, and probably the rest of the country, has a very high percentage of pregnant women with positive IgG antibodies to CMV, and with these results belongs to group of developing countries. This can be explained by lifestyle, great intimacy of the population, a large number of household members, and poorer hygiene (especially in rural environment).

The incidence of primary infection among pregnant women ranges from 0.5% to 4% (21, 22). Active or recent infection high prevalence rate in pregnant women was reported for Poland, 13% of 1332 pregnant women (23), while the lowest rate was reported for Turkey, 0% of 249 pregnant women (24). In our case, acute infection based on serology, with positive IgM and IgG antibodies had 9/300 (3.0%) pregnant women. However, after performed IgG avidity test on CMV, all 9/300 (3.0%) had high IgG avidity, which means that either the infection was primary, but which occurred three months ago, or was a recurrent infection, or reinfection. In a study conducted in Egypt (25) in 40 women with positive IgM and IgG antibodies, an IgG avidity test was also performed on CMV, all of which were high. In a study in Iran, the prevalence of active CMV infection in pregnant women was 4.35%, of which the incidence of primary infection was 34.4%, and recurrent 65.6% (15).

Pregnant women in this study had a statistically higher number of subjects with positive IgG antibodies to CMV from rural areas compared to those from urban areas ($p<0.05$). A similar result was found in our neighboring Croatia, where IgG antibodies positive to CMV in women from rural areas was more frequent than those living in urban areas (85.0% vs. 73.1%, p–0.018) (16).

Also, in our study, a significantly higher percentage of pregnant women with positive IgG antibodies to CMV was with a lower level of education, and with a growth in the percentage of negative CMV IgG antibodies with an increase in education ($p = 0.04$). In the Yemen research, the majority of respondents were illiterate, but there were no statistically significant differences between those of the CMV seropositive and those with low education (12). While research in Nigeria has shown that the degree of seronegativity in CMV increases significantly with the level of education (26).

In our research, we did not find the statistical significance of certain risk factors, like contact with small children going to kindergarten, age, parity, abortion information, blood transfusion or organ transplantation information, information on a number of sexual partners, marital status, gestation age, trimester, prior knowledge of CMV, fever, child with congenital malformation, having an impact on the incidence of cytomegalovirus infection in pregnant women. Also, in the study conducted by Mamuye et al. (10), there was no significant correlation between seropositivity to CMV and education, employment, gestation age and parity. In in the research from Mexico, no socio-demographic characteristics of pregnant women including age, ethnic group, place of birth, housing, place of work, education level, low socioeconomic status were associated ($P> 0.05$) with seropositivity to CMV (27). While a study in Tanzania proved that older age, multiparity, and low socioeconomic status were significantly associated with CMV IgG seroprevalence ($p <0.01$) (33).

6. CONCLUSION

This study has proven that in our Tuzla canton there is a high percentage of positive IgG antibodies to CMV in pregnant women, as much as 93.0%. Three percent of pregnant women had positive IgM and IgG antibodies, which speaks in favor of acute infection, but in all of them IgG avidity test for CMV was high, so primary infection was excluded. Among the risk factors that had a statistically significant effect on the onset of CMV infection in pregnant women are the rural environment and lower education.

- **Author's contribution:** H. P-J gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. F.S., S.A., D.P., R.J. and J.P. had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they are agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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