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Seroepidemiology of cytomegalovirus infections in Croatia

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Abstract

Objectives: Cytomegalovirus (CMV) is endemic worldwide, with marked differences in the seroprevalence rates between countries. The aim of this study was to analyze the seroprevalence of CMV infection in Croatia.

Methods: During a three year period (2013-2015), 2438 consecutive serum samples collected from Croatian residents were tested for the presence of CMV IgM and IgG antibodies using enzyme-linked immunoassay. IgM/IgG positive samples were further tested for IgG avidity.

Results: The overall seroprevalence rates for CMV IgG and IgM antibodies were 74.4% and 4.3%, respectively. The IgG seroprevalence differed significantly between population groups: children/adolescents 54.6%, adult general population 77.2%, hemodialysis patients 91.4% ($p<0.001$). CMV seropositivity was strongly age-dependent with prevalence ranging from 53.0% in children less than 10 years to 93.8% in persons above 60 years ($p<0.001$). There was no difference in the prevalence between women with normal pregnancy and women with bad obstetric history. Gender and place of residence was not associated with CMV seropositivity. Using IgG avidity, current/recent primary CMV infection was confirmed by low/borderline avidity index (AI) in 46.7% participants, while in 53.3% high AI indicated CMV reactivation or reinfection. Primary infections were detected mainly in children and adolescents (83.2% and 70.5%), while reactivations/reinfections were common in persons older than 40 (77.0-100%). Reactivations/reinfections were most commonly detected in hemodialysis patients (92.3%). Logistic regression showed that older age and being on hemodialysis were significant predictors of CMV seropositivity.

Conclusions: CMV is widespread in the Croatian population. Older age and being on hemodialysis appeared to be main risk factors for CMV infection.

Key words: cytomegalovirus, seroprevalence, Croatia

Introduction

Cytomegalovirus (CMV) is a ubiquitous virus with high worldwide prevalence ranging from 34%-80% in developed countries to 100% in some parts of Africa [1,2]. Virus is transmitted by close personal contacts through infected body fluids, usually saliva, urine, blood or genital secretions [3]. Primary CMV infections occur mainly in early childhood or adolescence and are usually asymptomatic in otherwise healthy children and adults. Symptomatic CMV infections are typically manifested as a non-specific febrile disease or a mild self-limiting mononucleosis-like syndrome [4]. However, there are many reports of severe or prolonged symptomatic CMV infection in immunocompetent patients [5-7]. After primary infection, CMV establishes a lifelong latent infection that can periodically reactivate [3]. Immunocompromised individuals such as HIV-infected patients, hemodialysis patients and transplant recipients may develop severe CMV disease with a wide spectrum of clinical symptoms including retinitis, hepatitis, colitis, pancreatitis, pneumonitis and encephalitis [8-10]. In addition, pregnant women also represent a risk-group for CMV infection. In seronegative pregnant women, CMV transmission can occur following primary maternal CMV infection. In seropositive women, CMV can cross the placenta during non-primary maternal infection (reactivation of virus or re-infection with a different strain) resulting in congenital CMV infection [11].

In Europe, there is wide range of CMV seroprevalence among countries and different population groups. Prevalence rates are reported to be 41%-92% in children and adolescents [12-15], 45%-94% in adult general population [14, 16-19], 30%-91.5% in pregnant women [20-24] and 68%-99% in hemodialysis patients [25-27].

In Croatia, there are few published studies on the prevalence of CMV infection in selected population groups such as childbearing-aged women and hemodialysis patients [28,29]. The

aim of this study was to analyze the prevalence of CMV infection in the Croatian general population.

Materials and methods

During a three-year period (January 2013-December 2015), a total of 2438 consecutive serum samples were tested for the presence of CMV specific IgM and IgG antibodies. Samples were collected from patients residing all Croatian counties testing at two large medical institutions (Croatian National Institute of Public Health and Clinical Hospital Center Zagreb). There were 1064 males (43.6%) and 1374 females (56.4%) aged one month to 82 years (Figure 1). Patients enrolled in the study were admitted to a routine testing for a preoperative check-up (cardiac surgery, renal transplant program), elevated liver transaminases, lymphatic disorders, neurological disorders, antenatal and postnatal screening, and patients from infertility centers (couples undergoing medically assisted reproduction). Among pregnant women, 238 (76.5%) had a normal pregnancy and 73 (23.5%) had a bad obstetric history (history of intrauterine fetal death, intrauterine growth retardation, stillbirth, and habitual abortions).

Anti-CMV IgM and IgG antibodies were detected using a commercial enzyme-linked immunosorbent assay (ELISA; Vircell, Granada, Spain) and enzyme-linked fluorescent assay (ELFA, Vidas, Biomerieux, Marcy l'Etoile, France). Results were interpreted according to the manufacturer's recommendations: Vircell CMV IgM/IgG, antibody index <9 negative, 9-11 equivocal, >11 positive; Vidas CMV IgM <0.70 negative, 0.70-0.90 equivocal, >0.90 positive, CMV IgG <4 aU/ml negative, 4-6 aU/ml equivocal, >6 aU/ml positive. IgM/IgG positive samples were further tested for IgG avidity to confirm or to rule out primary CMV infection using ELISA (Euroimmun, Lübeck, Germany) or ELFA (Vidas, Biomerieux, Marcy l'Etoile, France).

Statistical analysis

The frequencies are shown with 95% confidence intervals (CI). Difference between groups was assessed using Fischer' exact test. The strength of association between dependent and independent variables was assessed using logistic regression (crude odds ratios; OR, odds ratios adjusted for age and gender; AOR). Due to mother-to-child passive immunity, the youngest age group (<6 months), was excluded from logistic regression models. For statistical analysis, software package STATA/IC ver 11.2 (StataCorp LP, USA) was used. The level of statistical significance was $\alpha= 0.05$.

Results

CMV IgM/IgG seroprevalence according to the characteristics of participant's is presented in Table 1. CMV IgG antibodies were detected in 1815 (74.4%) participants. Women were more often seropositive than men (76.3% vs. 72.0%, $p=0.015$). Of children aged less than 6 months, 84.4% were IgG seropositive indicating transplacentally derived maternal antibodies. A significant progressive increase in IgG seroprevalence with age was observed from 53.0% in children aged 6 months to 9 years to 93.8% in persons older than 60 years ($p<0.001$). There was no difference in the CMV prevalence among participants residing urban regions (74.4%) and participants residing suburban/rural regions (74.3%, $p=0.952$). According to population group, seropositivity was lowest in children and adolescents (54.6%) compared to adult general population (77.2%) and hemodialysis patients (91.4%, $p<0.001$). In pregnant women, there was no difference in the prevalence in women with normal pregnancy and bad obstetric history (78.2% vs. 78.1%, $p=0.999$). Univariate logistic regression showed a steady increase in the strength of association between belonging to an older age group and CMV IgG seropositivity (ORs 2.03-13.3). In addition, adult general population and hemodialysis patients had higher risk for being CMV IgG seropositive compared to children and

adolescents (OR 2.82; 95%CI=2.28-3.48 and 8.83; 95%CI=5.73-13.60, respectively). After standardization for age and gender, being on hemodialysis remained the main predictor of CMV IgG seropositivity (AOR=0.95, 95%CI=1.15-3.41) (Table 2).

CMV IgM antibodies were documented in 105 (4.3%) participants. IgG avidity was low in 38 (36.2%) and borderline in 11 (10.5%) participants indicating current/recent primary CMV infection. In 56 (53.3%) participants, high IgG avidity was found suggesting CMV reactivation or reinfection. Acute infections were most frequently detected in participants aged 6 months-9 years (7.6%) and 20-29 years (6.7%), however they occurred in all age groups (2.4%-4.3%, $p=0.005$) (table 1). Avidity indices (AI) according to age groups are presented in the Figure 2. Acute primary infections (low AI) were reported mainly in persons younger than 30 years (83.2% aged 6 months-9 years, 75.0% aged 10-19 years, 50.0% aged 20-29 years). In contrast, majority of CMV reactivations/reinfections (high AI) were detected in persons above 40 (76.9%-100%). According to the population group, acute infections were most common in hemodialysis patients (8.6%) compared to 5.0% in children/adolescents and 3.3% in adult general population ($p<0.001$). Among CMV IgM positive hemodialysis patients, 92.3% showed high AI indicating reactivation or reinfection. In children/adolescents and adult population, prevalence of current/recent primary infection was 80.0%/8.0% and 33.3%/13.0%, respectively (Figure 3).

Discussion

The results of this first large seroprevalence study have demonstrated a high seroprevalence of CMV infection the Croatian general population (74.4%) with significant differences among population groups. Data from European countries showed wide geographical variability. In the general population, seroprevalence in Croatia is comparable to that of Portugal (77%) [14]. Lower prevalence rates were reported in the Netherlands (45.6%) [19], France (49.5%)

[18], Germany (57.25%) [17] and Spain (62.8%) [30], while Hungary, Turkey and Russia showed higher seroprevalence rates (86% and 80.9-94.8%, respectively) [10,13,15,16].

Several European studies have reported a higher CMV seroprevalence in females, although in most instances these differences were small [19,30,31]. Results of this study revealed similar results (76.3% in females *vs.* 72.0% in males). No gender-specific differences were recognized in German adolescents aged 13-16 years [32].

CMV seroprevalence in the Croatian population tends to increase progressively with age from 53.0% in 6 months-9-year-olds to 93.0% in participants older than 60. The only exception to this trend was observed in children less than 6 months who had high prevalence (84.4%) reflecting transient presence of transplacentally derived maternal IgG antibodies. Similar results were found in majority other studies [10,15,18,19,33]. Comparing seroprevalence in the similar age groups, some differences were found in children and adolescents. For example, in Portugal the overall seroprevalence rate (77%) is similar to that of Croatia (74.4%), however, seroprevalence in children/adolescents was higher than in the similar population group in Croatia (64.9%-71.3.5% *vs.* 53.0-55.4%) [14]. Moreover, 82.1% Turkish children were infected with CMV by the age of 6 and 92% by the age of 13 [13].

Several studies published in 1990s-2000s reported higher CMV seroprevalence in hemodialysis patients ranging from 83% to 99.3% [25,26,34]. Croatian hemodialysis patients showed significantly higher seroprevalence rate (91.4%) than adult general population as well (77.2%). In contrast, a Dutch study found the percentage of CMV-seropositive hemodialysis patients within range of the reported prevalence in the general population [27]. Higher prevalence in hemodialysis patients could be explained by the acquisition of CMV through repeated blood transfusions as well as exposure to CMV through contaminated equipment during hemodialysis procedures.

Studies on the prevalence of CMV in childbearing-aged, pregnant and parturient women showed seroprevalence rates of 42.3% in Germany [32], 49% in the United Kingdom [35], 56.3% in Finland [36], 57% in France [22], 62.4% in Poland [37] and 91.5%-97.3% in Turkey [24,38]. Regional differences in CMV seropositivity were observed in Norway (58.5%-72.1%) [23,39] and Belgium (30%-54%) [21,31]. A very low prevalence rate was found in Irish pregnant women (30.4%) [40]. A Russian study reported slightly higher seroprevalence in women with current abortions (81.1%) compared to women with normal pregnancy (78.0%) [20]. There was no difference in CMV seropositivity in Croatian pregnant women with normal pregnancy (78.2%) and pregnant women with bad obstetric history (78.1%).

Place of residence was not found to be a risk factor for CMV seropositivity in Croatia which is consistent with results from Finland [36] and Turkey [13].

In this study, IgM antibodies were detected in 4.3% participants indicating acute CMV infection. Since IgM antibodies could be false positive in some population groups such as hemodialysis patients and pregnant women, serology results should be interpreted with caution. Distribution of acute CMV infections in the Croatian population was bimodal. The highest prevalence of acute infections was reported in children between 6 months and 9 years (7.6%) and young adults between 20 and 29 years (6.7%). A high prevalence documented in young children is probably due to starting attending day-care centers. A study from Belgium demonstrated that probability of CMV seroconversion is significantly associated with the contact with children aged less than 3 years [31]. In addition to contact with young children, a higher prevalence in young adults could be attributable to sexual CMV transmission.

According to population group, acute infections were common in hemodialysis patients (8.6%). Using IgG avidity, recent primary infection (borderline AI) was documented in only 7.7% patients, while in 92.3% high AI indicated CMV reactivation or reinfection.

In conclusion, the results of this study indicate that CMV is widespread in Croatia. More than half of the population (54.6%) is infected by age of 20. Older age and being on hemodialysis appeared to be main risk factors for CMV infection.

Conflict of interests

Authors report no conflict of interests regarding this manuscript.

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Table 1. Prevalence of CMV antibodies according to the participant's characteristics

Characteristic	Tested N (%)	CMV IgM			CMV IgG		
		N (%)	95%CI	p	N (%)	95%CI	p
Overall	2438 (100)	105 (4.3)			1815 (74.4)	72.7 - 76.2	
Gender				0.07			0.015
Male	1064 (43.6)	55 (5.2)	3.9 - 6.7		766 (72.0)	69.2 - 74.7	
Female	1374 (56.4)	50 (3.6)	2.7 - 4.8		1049 (76.3)	74.0 - 78.6	
Age				0.005			<0.001
< 6 mo	32 (1.3%)	1 (3.1)	0.1 - 16.2		27 (84.4)	67.2 - 94.7	
6 mo - 9 yrs	249 (10.2)	19 (7.6)	4.7 - 11.7		132 (53.0)	46.4 - 59.3	
10 - 19 yrs	271 (11.1)	7 (2.6)	1.0 - 5.2		150 (55.4)	49.2 - 61.4	
20 - 29 yrs	431 (17.7)	29 (6.7)	4.6 - 9.5		300 (69.6)	65.0 - 73.9	
30 - 39 yrs	584 (24.0)	14 (2.4)	1.3 - 4.0		420 (71.9)	68.1 - 75.5	
40 - 49 yrs	278 (11.4)	12 (4.3)	2.3 - 7.4		237 (85.3)	80.5 - 89.2	
50 - 59 yrs	305 (12.5)	13 (4.3)	2.3 - 7.2		279 (91.5)	87.8 - 94.4	
60+ yrs	288 (11.8)	10 (3.5)	1.7 - 6.3		270 (93.8)	90.3 - 96.3	

Setting				0.604		0.952
Urban	1991 (81.8)	83 (4.2)	3.3 - 5.1		1482 (74.4)	72.5 - 76.3
Suburban/rural	444 (18.2)	21 (4.7)	3.0 - 7.1		330 (74.3)	70.0 - 78.3
Population group				<0.001		<0.001
Children/adolescents	496 (20.4)	25 (5.0)	3.3 - 7.4		271 (54.6)	50.1 - 59.1
Adult general population	1626 (66.7)	53 (3.3)	2.5 - 4.2		1256 (77.2)	75.1 - 79.3
Hemodialysis patients	314 (12.9)	27 (8.6)	5.7 - 12.3		287 (91.4)	87.7 - 94.2
Pregnant women				0.999		0.999
Normal pregnancy	238 (76.5)	2 (0.8)	0.1 - 3.0		186 (78.2)	72.4 - 83.2
Bad obstetric history	73 (23.5)	0 (0)	0 - 4.9		57 (78.1)	66.9 - 86.9

Table 2. Univariate logistic regression for risk of CMV seropositivity

Characteristic	CMV IgM	95%CI OR	CMV IgM	95%CI AOR	CMV IgG	95%CI OR	CMV IgG	95%CI AOR
	OR		AOR*		OR		AOR	
Male vs. female (ref.)	1.44	0.98 - 2.14	NA**	NA	0.80	0.66 - 0.96	NA	NA
Age			NA	NA			NA	NA
6 mo - 9 yrs	1 (ref.)	-			1 (ref.)	-		
10 - 19 yrs	0.32	0.13 - 0.78			1.10	0.78 - 1.55		
20 - 29 yrs	0.87	0.48 - 1.59			2.03	1.47 - 2.80		
30 - 39 yrs	0.30	0.15 - 0.60			2.27	1.67 - 3.09		
40 - 49 yrs	0.55	0.26 - 1.15			5.12	3.38 - 7.76		
50 - 59 yrs	0.54	0.26 - 1.11			9.51	5.93 - 15.26		
60 + yrs	0.44	0.20 - 0.96			13.30	7.76 - 22.77		
Urban vs. suburban/rural setting (ref.)	0.88	0.54 - 1.43	0.89	0.55 - 1.46	1.01	0.79 - 1.27	0.94	0.73 - 1-20
Population group								
Children/adolescents	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-

Adult general population	0.63	0.39 - 1.03	1.57	0.81 - 3.06	2.82	2.28 - 3.48	0.94	0.69 - 1.29
Hemodialysis patients	1.77	1.01 - 3.11	6.16	2.57 - 14.75	8.83	5.73 - 13.60	1.98	1.15 - 3.41
Bad obstetric history vs. normal pregnancy (ref.)	NA	-	NA	-	1.00	0.53 - 1.88	0.95	0.50 - 1.81

*AOR=adjusted for age and gender; **NA=not applicable

FIGURE LEGENDS:

Figure 1. Distribution of study participants according to age and gender

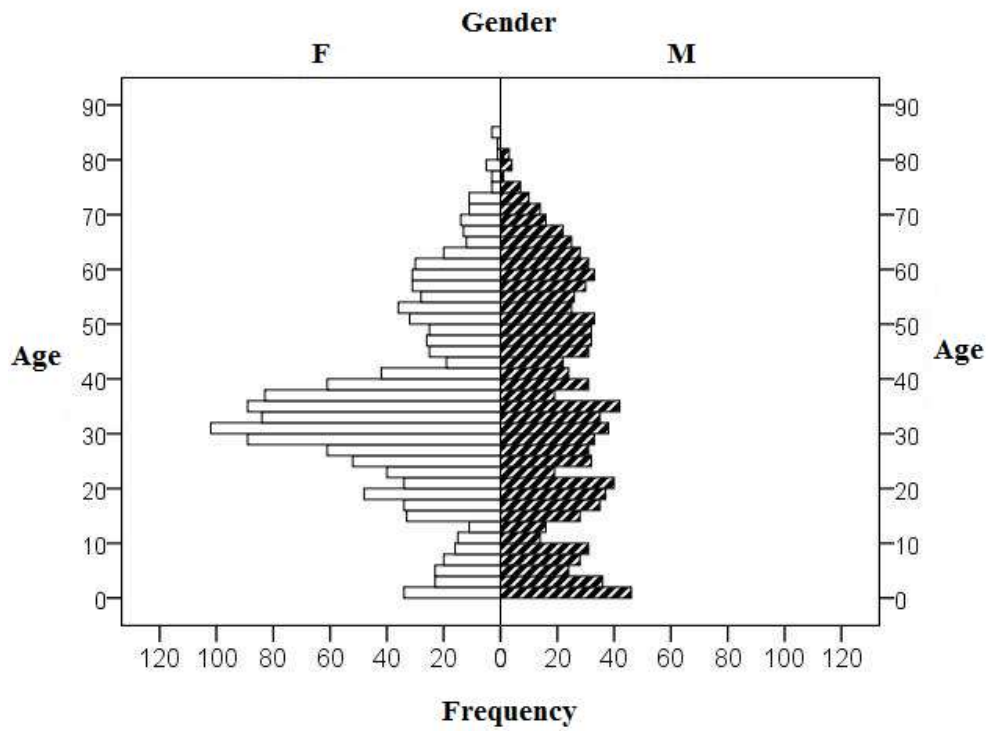


Figure 2. CMV IgG avidity according to participant's age

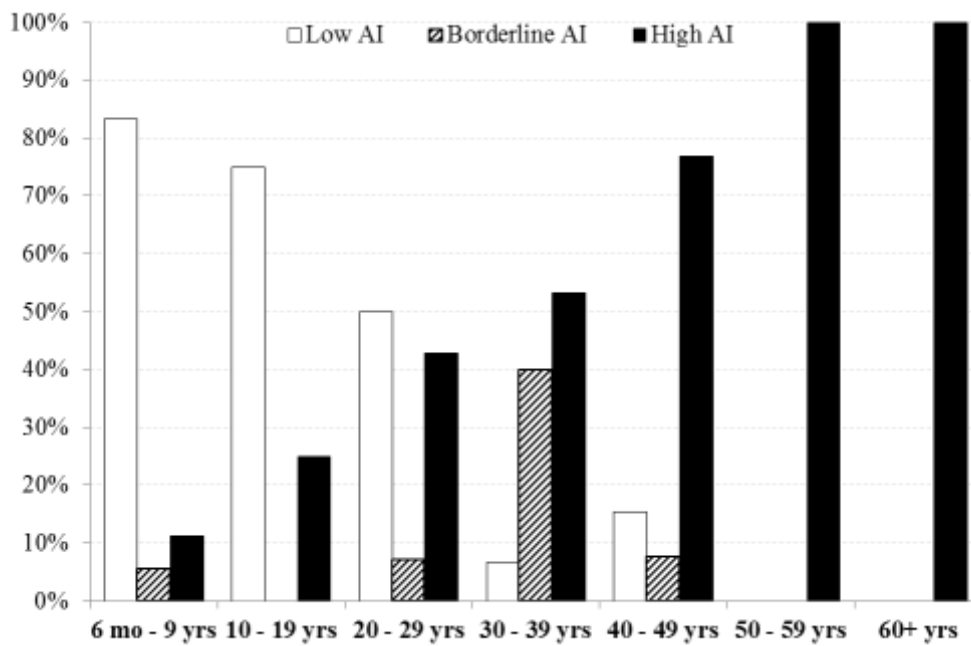


Figure 3. CMV IgG avidity according to population group

