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Seroprevalence of Cytomegalovirus in the North-Eastern Bulgarian Population, 2003-2015

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Abstract

The prevalence of cytomegalovirus (CMV) infections is between 50% and 85% in adults in different parts of the world. Its epidemiology varies on socioeconomic and age groups. The present retrospective study has been performed to determine the seroprevalence of CMV among the population in North-Eastern Bulgaria. For the period 2003–2015, the prevalence of individuals with antibodies to CMV was estimated, using indirect enzyme-linked immunosorbent assay (ELISA) to detect virus-specific IgG and IgM. The population sample included 7879 randomly chosen hospitalized patients of both sexes and different ages. The total seroprevalence of CMV was determined to be 78.4% (CI 95% 77.5; 79.3), and the relative proportion of acute CMV infections 22.9% (CI 95% 21.9; 23.7). The proportion of CMV IgG and IgM by sex and by age was also analysed. The results of the study reveal that CMV infection is highly prevalent among the population and occurs mainly in the first years of life.
Key words: Cytomegalovirus (CMV), seroprevalence, anti-CMV IgM, anti-CMV IgG.

Резюме

Цитомегаловирусната инфекция е широко разпространена и в различни области на света е между 50% и 85% според наличието на специфични антитела във възрастната популация. Епидемиологичното разпространение на цитомегаловирус (CMV) варира в зависимост от възрастта и социално-икономическия статус на населението. Настоящото ретроспективно сероепидемиологично проучване има за цел да уточни серопревалентността спрямо CMV в общата популация в Североизточна България и честотата на първичното заразяване в зависимост от възрастта. За периода 2003 – 2015 г. беше определяно наличието на специфични anti CMV антитела от IgG и IgM тип в единична серумна проба и стандартизирана ELISA. Изследвани са 7879 пациенти от двата пола, разпределени във възрастови групи. Статистическият анализ беше направен с помощта на McCallum Layton calculators (www.mccallum-layton.co.uk) and Social Science Statistics (www.socscistatistics.com). В нашето проучване средната серопревалентност на общата популация е 78,4 %. Относителният дял на острите инфекции е 22.9 %. Според нашите данни CMV-инфекцията е широко разпространена и заразяването става най-често в ранното детство.

Introduction

Cytomegalovirus (CMV) is an ubiquitous large enveloped dsDNA β-herpesvirus with no known seasonal predominant distribution and with a prevalence that ranges between 50% and 85% of adults (Staras *et al.*, 2006; Bate *et al.*, 2010). The epidemiology of CMV varies in different regions of the world and in different socioeconomic and age groups (Chandler *et al.*, 1985; Fowler *et al.*, 1993). According to previous data in North-Eastern Bulgaria, people over 15 years of age are infected in 85%, including women of childbearing age, and the average seroprevalence is 69.8% (Ivanova, 2007)

CMV infections are most often asymptomatic

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or with nonspecific presentations in healthy population, but when symptomatic, can lead to a syndrome resembling infectious mononucleosis or acute hepatitis. The virus is excreted through all body fluids, and the most common route of transmission is close or sexual contact, although transmission can also occur through breast milk, solid organ transplant or blood transfusions (Ross and Boppana, 2005; Stagno and Britt, 2006). CMV primary infection occurs mostly in childhood and adolescence, but primary infections can also be observed in adults (Stagno and Britt, 2006). The virus establishes lifelong latency in monocytes and lymphocytes after the primary infection (Schrier *et al*., 1985).

The infection is important and dangerous in certain risk groups such as immunocompromised patients and pregnant women. In immunocompromised individuals, CMV infection, reactivation or re-infection is the leading cause of morbidity and mortality, especially in association with transplants, haemodialysis, cancer, immunosuppressive therapy and infection with human immunodeficiency virus (HIV), as well (Pass, 2001).

In primary maternal infection, the probability of transmission of CMV to the fetus is approximately 30% to 40%. In women with pre-existing CMV immunity during pregnancy the probability of fetal CMV transmission decreases to approximately 0.5% to 1.4% (Stagno *et al*., 1982; Schleiss, 2008) and congenital infections in such cases can be less severe (Fowler *et al.,* 1992). According to the data, the overall birth prevalence of congenital CMV infection is reported to be 0.64%, but varies considerably among different study populations from 0.3% to 2.3% of all live births (Kenneson and Cannon, 2007). Congenital CMV infection is defined by either the detection of CMV-DNA or anti-CMV IgM in infant body fluids or sera within the first 21 days post-partum (Mosca and Pugni, 2007). Approximately 10% to 15% of infants with congenital CMV infection are symptomatic at birth, with manifestations including growth retardation, prolonged jaundice, thrombocytopenic purpura, hepatosplenomegaly, microcephaly, CNS calcifications and abnormalities, and retinitis. The risk of long term neurodevelopmental disabilities is high in these children and include microcephaly, hearing loss, motordeficits, cerebral palsy, mental retardation, seizures, ocular abnormalities and learning disabilities (Kylat *et al.*, 2006; Sharon and Schleiss, 2007).

Little is known about the seroprevalence and the relative proportion of CMV infections in Bulgaria as national and regional trends in CMV infection have not been examined recently. The current work aimed to assess the seroprevalence of CMV and to determine the relative proportion of recent CMV infections in different age groups among the North-Eastern Bulgarian population.

Material and methods

Study population

From January 2003 to December 2015, a total of 7879 individuals of both sexes, distributed in 12 age groups: 0-6 months, 7 months-1 year, 2 - 5, 6 - 10, 11 - 15, 16 - 20, 21 - 25, 26 - 30, 31 - 40, 41 - 50, 51 - 60, and ≥ 61 years were studied in single serum samples for the presence of CMV-specific antibodies.

Method

Commercial ELISA test kits for detection of specific anti CMV IgM and IgG antibodies (EUROIMMUN – Germany, VIRCELL – Spain, Dia Pro – Italy, Adaltis – Italy) were performed according to the producer's recommendations. Anti-CMV IgM positive samples were considered as acute/recent infections.

Statistical analyses

The proportion of CMV seropositive individuals and their corresponding confidence intervals (CI), chi-squared distributions and p-values were calculated with McCallum Layton calculators and Social Science Statistics. A p-value < 0.05 was considered significant for all statistical tests.

Results

In a total of 7879 individuals, 6176 had IgG antibodies to CMV (78.4%, 95% CI: 77.5; 79.3) and 1703 were seronegative (21.6%, 95% CI:20.7%; 22.5). Among the male participants, 3224 of 4220 (76.4%, 95% CI: 75.1; 77.7) were CMV IgG-positive and among the female participants 2952 of 3659 (80.7%, 95% CI: 79.4; 82.0) were CMV IgG-positives (Table 1). This difference in the proportions of seropositive males and females was statistically significant with chi-square of 21.2 and p-value of < 0.001.

By distribution of CMV IgG-positive individuals (male and female) by age, 82.9% (95% CI: 79.9; 85.9) of children in the youngest age group (0 – 6 months) were shown to have antibodies (Fig. 1). It is consistent with the level of anti CMV IgG positivity (84.5% - (95% CI: 82.7; 86.3) found in women of childbearing age from the same population (Table 1), and most of them probably are transplacental. The percentage of seropositive individu-

Table 1. Individuals IgG-positive for cytomegalovirus, distributed by sex, 2003-2015

	Study participants (N)	N CMV-IgG positive	% CMV-IgG positive	95% confidence interval
Male	4220	3224	76.4	75.1 - 77.7
Female	3659 (1612*)	2952 (1362*)	80.7 (84.5*)	79.4 - 82.0 (82.7 - 86.3*)
Total	7879	6176	78.4	77.5 - 79.3

*Women of childbearing age 15-49 years (according WHO classification)

als then decreased and was similar in age groups of 7 months -1 year-old and 2 - 5 years-old with 53.7% (95% CI: 48.1; 59.3) and 52.6% (95% CI: 49.4; 55.8), respectively. Subsequently the proportion of seropositive individuals progressively increased with age, reaching 62.8% (95% CI: 58.9; 66.7) in the age group of 6 – 10 years-olds, 69.4% (95% CI: 65.4; 73.4) in the 11 – 15 years-old, 70.8% (95% CI: 66.8; 74.8) in the 16 – 20 years-old, 78.5% (95% CI: 74.8; 82.2) in the 21 – 25 years-old, 83.8% (95% CI: 80.6; 87.0) in the 26 – 30 years-old, 85.4% (95% CI: 83.2; 87.6) in the 31 – 40 years-old, 92.2% (95% CI: 90.2; 94.2) in the 41 – 50 years-old, 92.5% (95% CI: 90.6; 94.4) in the 51 – 60 years-old and 94.9% (95% CI: 93.5; 96.3) in the oldest group (≥61years). The proportion of males and females with CMV IgG in each age group is shown in Figure 1.

By distribution of CMV IgM-positive individuals (male and female) by age, 20.5% (95% CI: 17.3; 23.7) of children in the youngest age group (0 – 6 months) were shown to have antibodies. The percentage of CMV IgM-positive individuals was 36.7% (95% CI: 31.3;42.1) in the 7 months -1 year-old, 38.4% (95% CI: 35.3; 41.5) in the 2 - 5 years-old and then progressively decreased with age, reaching 30.8% (95% CI: 27.1; 34.5) in the age group of 6 – 10 years-olds, 26.2% (95% CI: 22.4; 30.1) in the 11 – 15 years-old, 28.7% (95% CI: 24.8; 32.7) in the 16 – 20 years-old, 21.5% (95% CI: 17.8; 25.2) in the 21 – 25 years-old, 21.1% (95% CI: 17.6; 24.7) in the 26 – 30 years-old, 19.6% (95% CI: 17.1; 22.1) in the 31 – 40 years-old, 16% (95% CI: 13.3; 18.7) in the 41 – 50 years-old, 13% (95% CI: 10.6; 15.4) in the 51 – 60 years-old and 12.8% (95% CI: 10.7; 14.9) in the oldest group (≥61 years). The proportion of males and females with CMV IgM in each age group is shown in Figure2.

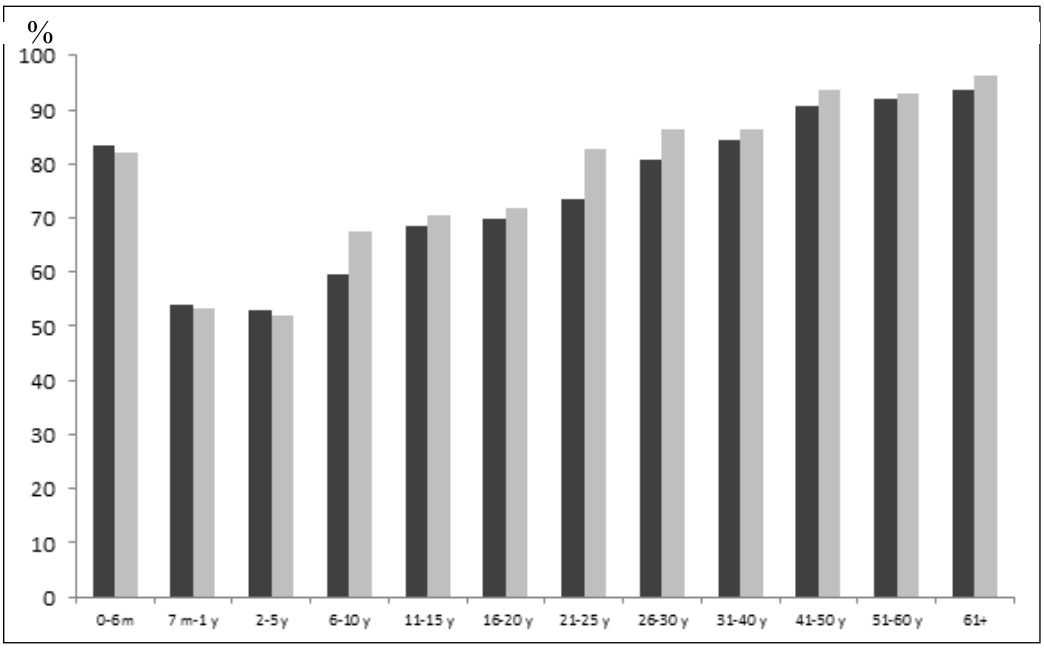

Fig. 1. Proportion of individuals IgG-positive for Cytomegalovirus, by sex (black – male and grey – female) and age, 2003-2015 (N=7879)

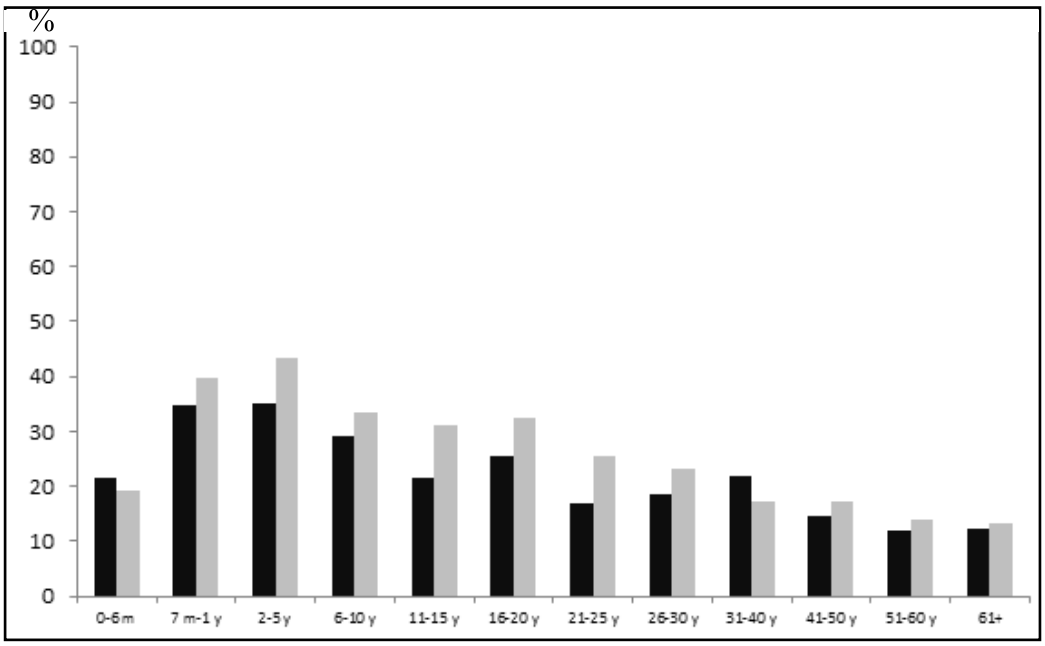

Fig. 2. Proportion of individuals IgM-positive for Cytomegalovirus, by sex (blacke – male and grey – female) and age, 2003-2015 (N=7879)

Discussion

The results of this study indicate that CMV infection is highly prevalent among the population (78.4%, 95% CI: 77.5; 79.3), similarly to reported results about other countries (Takeda *et al*., 2001; Kothari *et al.*, 2002; Staras *et al.*, 2006). Such high seroprevalence may have profound effects with respect to immunocompromised individuals and maternal infections.

With respect to distribution by sex, women were found to be more infected than men, 80.7% (95% CI: 79.4; 82.0) and 76.4% (95% CI: 75.1; 77.7), respectively. The calculated risk ratio shows that females are exposed to a greater risk of CMV seropositivity by 6% (RR = 1.06, 95% CI: 1.03; 1.08) when compared to males . A possible explanation of this fact is that women more often become infected by sexual contact in addition to the contact route in the society, and are in close contact with the virus-containing secretions (urine, saliva) of their infected children. This horizontal route of transmission presents a risk to young mothers, pregnant women and those with occupations associated with exposure to children, such as teachers and day careworkers (Staras *et al.,* 2008). To support this, the study showed that 32.4% of the 16 – 20-year-old young women and 25.6% of the 21 - 25year-old show evidence of acute infection. According to Italian and Brazilian authors, due to the high CMV seroprevalence in women of childbearing age, actually transmission in previously seropositive women through reactivation or re-infection happens more often and is responsible for most congenital CMV infections (Barbi *et al.,* 2006; de Vries *et al.*, 2013). According to our data about CMV seroprevalence in women of childbearing age, the total risk of congenital infection is still higher in cases of primary maternal infection. In our population, 15.5% of women of childbearing age were found to be susceptible to primary CMV infection, which, combined with the fact that in this case the risk of transmission to the newborn is 40% (Stagno and Britt, 2006), gives a total population risk of 6.2%. Assuming that the risk of congenital infection after reactivation of maternal CMV infection during pregnancy is around 1% (Stagno *et al.*, 1982) and taking into consideration that 84.5% of women of childbearing age in our population were CMV IgG-positive, the total risk of congenital CMV transmission for the population when the mother is immune, is 0.8%, which is approximately 8 fold lower than after the primary infection.

With respect to age-sex distribution, women showed higher seroprevalence than men in all age groups after age 5. The seroprevalence in women is considerably higher than in men in the age group 21 - 25 years old (82.9% versus 73.6%, p=0.01). After this age group, the seroprevalence values for both sexes converge, but remain higher in women, although not statistically significant.

A tendency for prolonged presence of IgG antibodies in the 0 - 6 months age group (82.9%, 95% CI: 79.9; 85.9) was also observed, probably due to complementary postnatal infection. Our conclusion

is confirmed by a relatively high proportion of acute infections (IgM anti CMV positivity) in the 0 - 6 month's group (20.5%), which continues to grow in the next age groups - 7 - 12 months (36.7%) and 2 – 5 year-old (38.4%). Our data indicate that more than 1/3 of the children under 5 years of age acquire CMV infection. Anti CMV IgG seropositivity gradually increases in the older age groups. For our study population, more than 90% of people above 40-ies are infected.

According to our study, seroprevalence increased with age and concurrently the proportion of the acute infections decreesed as a general tendency. A considerably high proportion – 25.9% (95% CI: 23.1; 28.7) of children in the youngest age groups (0 – 12 months) were shown to have IgM antibodies. The proportion of infants who become infected with CMV during the first year of life is directly related to the seroprevalence of women of reproductive age and to the proportion of mothers who breastfeed. In countries where breastfeeding is widely practiced and the majority of mothers are seropositive, for example in south Europe, some regions of Asia, Africa and Latin America, more than 50% of infants acquire CMV within the first year of life (Stagno *et al*., 1982). The highest proportion (38.4%, 95% CI: 35.3; 41.5) of acute infections (anti-CMV IgM positive) was obtained in the 2 - 5 age group, when children are admitted in day care centers (de Mello *et al*., 1996).

Conclusions

1. CMV infection is highly prevalent (78.4%) and has increased in comparison with a previous study (69.8%) (5) in the North-Eastern Bulgarian population, probably due to the more sensitive modern ELISA tests, and the larger number of tested individuals.
2. Primary CMV infection occurs mainly in the first 5 years of life.
3. Serological screening and viral load testing should be performed for immunocompromised patients and pregnant women or women who intend to become pregnant.
4. The main preventive measures against CMV infections and reinfections should be aimed at immunocompromised patients and women who intend to become pregnant, and women who work with children. To these individuals the exposure to CMV can be most detrimental and even fatal. They are the target groups for administration of a future effective vaccine.

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