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The Role of Cytomegalovirus in Congenital and Early Postnatal Infections in Northeastern Bulgaria

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Background: Human cytomegalovirus is a ubiquitous, large enveloped DNA β -herpesvirus that, like other herpesviruses, establishes lifelong latency following primary infection. It is the most frequent cause of congenital, neonatal and early postnatal infections with long lasting sequelae.

Aim: The aim of the present study was to assess the prevalence of cytomegalovirus among a cohort of newborns and 1-3-month-old children with neurological symptoms, physical retardation, prolonged jaundice, thrombocytopenic purpura and other disabilities.

Materials and methods: The study was a retrospective cross-sectional analysis of serological screening data for detection of specific anti-cytomegalovirus IgM and IgG in children from Northeastern Bulgaria.

Results: Between 2003 and 2015, average prevalences of 18.8% (95% CI: 15.4 to 22.2) for anti-CMV IgM antibodies (suggesting acute infection) and 84.7% (95% CI: 81.6 to 87.8) for anti-CMV IgG antibodies were measured in a total number of 517 samples. The prevalence rate of anti-CMV IgM in 1-3-month-old children was 4-fold higher than that in newborns [25.8% (95% CI: 21.1 to 30.5) and 6.4% (95% CI: 2.9 to 9.9, respectively)]. In contrast, no significant difference was found for anti-CMV IgG positivity between newborns and 1-3-month-old infants (84% and 85%, respectively).

Conclusions: The data obtained strongly encourage screening of pregnant women for anti-CMV IgG and IgM to avoid transmission of the infection and severe complications of congenital infection.

BACKGROUND

Human cytomegalovirus (CMV) is a large enveloped DNA β -herpesvirus that, like other herpesviruses, establishes lifelong latency after the primary infection.¹ Despite its ubiquity in the general population, most CMV infections are inapparent in healthy individuals.

CMV can be transmitted vertically and horizontally, and infections are classified as being acquired before birth (congenital), at the time of delivery (perinatal), or later in life (postnatal).¹ The infection causes a wide range of severe disabilities in neonates and could be manifested as intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenic purpura, myocarditis, pneumonitis, central nervous system abnormalities or chorioretinitis.

As the virus remains in latency after the primary

contamination and reactivates periodically, congenital CMV infection may follow either primary or recurrent infection of mother.² Primary CMV infections are more likely to cause damages as they are transmitted more frequently to the fetus³, but the correlation between newborn disease severity and the type of mother's infection (primarily or not primarily) still remains unclear.⁴ The time of maternal primary infection is also important and earlier pregnancy infections are associated with worse outcome.^{3,5} The birth prevalence of congenital CMV infection is estimated to be 0.64%, but it varies considerably among different study populations.³ The permanent neurological and neurodevelopmental sequelae is as high as 60% in infants with symptoms at birth and even infants without symptoms at birth will have sequelae in up to 13.5% of cases, most commonly manifested as sensorineural hearing loss.⁶

AIM

Little is known about the infant seroprevalence and the relative deal of CMV in congenital and early postnatal disorders in Bulgaria as national and regional trends in CMV infection have not been examined recently. Universal screening during pregnancy does not exist (as in most of the countries) and it is difficult to recognize primary CMV infection in newborns and babies. With the present study we aim to assess the relative deal of CMV in congenital and early postnatal infections in selected group of children with undefined cause of different disorders after birth. We also try to determine the possibility of early age acquisition of CMV infection in North-Eastern Bulgaria.

MATERIALS AND METHODS

From January 2003 to December 2015, a total of 517 children (187 newborns and 330 1-3-month-old children) with neurological symptoms, physical retardation, prolonged jaundice, thrombocytopenic purpura and other disabilities were studied in single serum samples. For clinical purposes, the diagnosis was based primarily on serological screening data.

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

The proportion of CMV seropositive infants and their corresponding confidence intervals, chi-squared distributions and p-values were calculated with McCallum Layton calculators (www.mccallum-layton.co.uk) and Social Science Statistics (www.socscistatistics.com). $P < 0.05$ was considered significant for all statistical tests.

RESULTS

From a total of 517 symptomatic children investigated, 97 (18.8%, 95% CI: 15.4 to 22.2) were anti-CMV IgM positive and 420 (81.2%, 95% CI: 77.8 to 84.5) were anti-CMV IgM negative (**Table 1**). Respectively, 438 (84.7%, 95% CI: 81.6 to 87.8) were anti-CMV IgG positive and 79 (15.3%, 95% CI: 12.2 to 18.4) were anti-CMV IgG negative. Out of anti-CMV IgM positive children, 91 were anti-CMV IgG positive too (17.6% of the total, 95% CI: 14.3 to 20.9), while out of anti-CMV IgM negative children, 347 (67.1%, 95% CI: 63.1 to 71.2) were anti-CMV IgG positive (**Table 1**).

In the group of newborns (aged < 1 month), twelve babies (6.4%, 95% CI: 2.9 to 9.9) were anti-CMV IgM positive (**Table 2**), while in the group of 1-3-month-old children, 85 (25.8%, 95% CI: 21.1 to 30.5) were anti-CMV IgM positive (**Table 3**). The risk ratio (1-3-month-old children versus newborns) is 4.0 and after accounting for sampling variability, the age could be considered to increase the risk of recent CMV infection after birth from 2.2 to 7.1 times (chi-square = 29.3; $p < 0.05$). In contrast, no significant difference was found for anti-CMV IgG positivity between newborns and 1-3-month-old infants – 84% and 85%, respectively (chi-square = 0.1; $p = 0.7$).

Ten of 12 anti-CMV IgM positive newborns were anti-CMV IgG positive too (5.3%, 95% CI: 2.1 to 8.5), while anti-CMV IgG negative were only 2 (1.1%, 95% CI: 0.4 to 2.6) of the IgM positive newborns (**Table 2**). In the group of 1-3-month-old children, 81 (24.5%, 95% CI: 19.9 to 29.1) were positive for both anti-CMV IgM and anti-CMV IgG and 4 (1.2%, 95% CI: 0.03 to 2.4) were anti-CMV IgG negative and anti-CMV IgM positive (**Table 3**).

DISCUSSION

Transmission of CMV occurs from person to person through body fluids and viral particles can be found in blood, urine, semen, cervical secretion, saliva, breast milk and transplanted organs.^{1,7} The presence of specific anti-CMV IgG is indicative for an accomplished infection. The seroprevalence rate varies in different population and regions, and usually increases with age. According to previous studies in Northeastern Bulgaria, about 85% of the people over 15 years of age are CMV infected, including women of childbearing age.⁸ Similar seroprevalence rates were found in other European countries.^{9,10} In accordance, 84.7 % of the children investigated in our study were anti-CMV IgG positive (suggesting a passive transmission of anti-CMV IgG antibody only).

Almost 19% of the tested children in the present study were found to be anti-CMV IgM positive, indicative for recent CMV infection. Primary infection during pregnancy could lead to intrauterine transmission, although a transmission in previously seropositive women due to reactivation or reinfection is the major route.^{2,9,10} CMV is the most common cause of congenital infection in newborns with a reported incidence of 0.2 - 2.5%¹¹ and is usually asymptomatic at birth but with cumulative effect on morbidity and mortality.¹² Symptomatic infants

Table 1. Seroprevalence of CMV in children aged 0-3 months (n=517)

	Number	Proportion
IgM+ IgG-	6	1.2% (95% CI: 0.3-2.1)
IgM+ IgG+	91	17.6% (95% CI: 14.3-20.9)
IgM- IgG+	347	67.1% (95% CI: 63.1-71.2)
IgM- IgG-	73	14.1% (95% CI: 11.1-17.1)

Table 2. Seroprevalence of CMV in newborns 0-1 month (n=187)

	Number	Proportion
IgM+ IgG-	2	1.1% (95% CI: 0.4-2.6)
IgM+ IgG+	10	5.3% (95% CI: 2.1-8.5)
IgM- IgG+	147	78.6% (95% CI: 72.7-85.4)
IgM- IgG-	28	15% (95% CI: 9.9-20.1)

Table 3. Seroprevalence of CMV in children 1-3 months old (n=330)

	Number	Proportion
IgM+ IgG-	4	1.2% (95% CI: 0.03-2.4)
IgM+ IgG+	81	24.5% (95% CI: 19.9-29.1)
IgM- IgG+	200	60.6% (95% CI: 55.3-65.9)
IgM- IgG-	45	13.6% (95% CI: 9.9-17.3)

identified at newborn screening generally have milder disorders than those referred for follow-up studies based on clinical abnormalities at birth. Prospective studies have shown that approximately half of the children born with symptomatic infection will develop sequelae.⁴ Our data reveal that 6.4% of newborns with consistent clinical symptoms have anti-CMV IgM positivity, probably indicating congenital infection. According to other study, symptoms were present in 11% of CMV infected neonates and 42% of them had sequelae.¹³ A study in Brazil confirmed congenital CMV infection in 1.08% of the screened newborns.¹⁴ Previous study in our region showed frequency of 1.1% in randomly chosen newborns¹⁵, while frequency among European countries was found to vary between 0.18% – 0.48%^{9,16}. Infants with symptomatic congenital CMV infection, identified on the base of clinical representation, have more frequent and severe disease at birth and more serious sequelae.¹⁷

The investigated symptomatic 1-3 month-old chil-

dren were suspicious for intranatal or early postnatal infection and their positivity was 25.7% – a 4-fold higher seroprevalence rate when compared with this of newborns. To existing data most of the term-born infants with perinatal infection are asymptomatic, because of maternal-transmitted CMV IgG antibody. In contrast, 15% - 20% of infected preterm infants may develop clinical disease.¹⁸

Finally, we should like to point out one of the limitations of our study – CMV infection was diagnosed based on consistent clinical symptoms and presence of specific anti-CMV IgM in the patient's serum. Although the strong positive anti-CMV IgM immune response may be useful for finding suggested infection, serologic reactions are not recommended for diagnosis of CMV infection in neonates because they have sometimes both false positive and false negative results.⁷ According to our observation, in symptomatic children, the serological testing is the first predicting sign to examine virus excretion in urine or viral load in plasma. Testing with PCR

techniques after positive serological screening results will be our future objective for better diagnosis.

Screening of pregnant women for CMV infection is not a routine practice in Bulgaria, but the data obtained strongly encourage screening for anti-CMV IgG and IgM among pregnant women to avoid transmission of the infection and severe complications of congenital infection.

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Роль цитомегаловируса при врождённых и ранних послеродовых инфекциях в Северо-Восточной Болгарии

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Введение: Человеческий цитомегаловирус представляет собой повсеместно распространённый большой с ДНК оболочкой β -герпесвирус, который, подобно другим герпесвирусам, приобретает пожизненную латентность после первичной инфекции. Он является наиболее часто встречающейся причиной врождённых, неонатальных и ранних постнатальных инфекций с долговременными последствиями.

Цель: Целью настоящего исследования является выработка оценки распространения цитомегаловируса среди группы новорожденных и детей в возрасте от 1 до 3 месяцев с неврологическими симптомами, отставанием в физическом развитии, длительной желтухой, тромбоцитопенической пурпурой и другими отклонениями.

Методы: Исследование представляет собой ретроспективный перебранный анализ данных серологического скрининга с целью установления конкретных анти-цитомегаловирусных IgM и IgG среди детей в Северо-Восточной Болгарии.

Результаты: За время периода 2003-2015 было измерено среднее распространение в рамках 18.8% (95% CI: 15.4 до 22.2) анти-ЦМВ антител класса IgM (предполагающие острую инфекцию) и 84.7% (95% CI: 81.6 до 87.8) анти-ЦМВ антител класса IgG общим числом в 517 проб. Преобладающая частота анти-ЦМВ антител класса IgM среди детей в возрасте 1-3 месяцев была в четыре раза выше по сравнению с новорожденными и составляла 25.8% (95% CI: 21.1 до 30.5) против 6.4% (95% CI: 2.9 до 9.9). В отличие от данных результатов не была установлена значительная разница в положительных результатах анти-ЦМВ класса IgG среди новорожденных и младенцев в возрасте 1-3 месяцев – соответственно 84% и 85%.

Заключение: На основании полученных результатов нами настоятельно рекомендуется проведение скрининга анти-ЦМВ класса IgG и IgM для беременных женщин с целью избежать передачу инфекции и тяжёлых осложнений после врождённой инфекции.