Congenital Cytomegalovirus Infection in Israel: Screening in Different Subpopulations

Yechiel Schlesinger MD[1], Dan Reich MD[3], Arthur I. Eidelman MD[2], Michael S. Schimmel MD[2], Jamal Hassanin MD[3] and Dan Miron MD[4]

[1]Infectious Diseases Unit and [2]Department of Neonatology, Shaare Zedek Medical Center, Jerusalem, Israel
Affiliated to Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
Departments of [3]Neonatology and [4]Pediatrics, HaEmek Medical Center, Afula, Israel
Affiliated to Technion Faculty of Medicine, Haifa, Israel

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Abstract

Background: The incidence of congenital cytomegalovirus in Israel has never been determined, either in general or in relation to various population subgroups. We recently proved the utility of newborn urine polymerase chain reaction as a screening tool for congenital CMV.

Objectives: To define the incidence of congenital CMV infection in two different subpopulations, as a model for the entire population of Israel.

Methods: Urine specimens were randomly collected from 2,000 newborns in Shaare Zedek Medical Center, Jerusalem, and HaEmek Medical Center, Afula (1,000 specimens each). These hospitals have many characteristic differences, presumably representing the diverse population of Israel. Urine specimens were subjected to a CMV PCR reaction and positive specimens were validated by urine viral culture. Maternal seroprevalence was determined in a representative sample of the mothers in each hospital. Epidemiologic characteristics of the mothers were extracted from hospital records and compared.

Results: The population in Shaare Zedek Medical Center was mostly Jewish (97.7%) and urban (87.0%), as compared to that of HaEmek Medical Center (49.2% and 61.0%, respectively, $P < 0.01$). Nevertheless, CMV seroprevalence was similar: 81.5% and 85%, respectively. Ten (1.0%) and 4 (0.4%) newborns, respectively, were found to have congenital CMV infection (not significant).

Conclusions: The combined incidence of congenital CMV infection in the study population was 0.7% (95% confidence interval 0.3–1.0%). If this rate is extrapolated to the entire population of Israel, then a total of 945 cases of congenital CMV can be expected among the 135,000 annual deliveries. A nationwide screening program for congenital CMV should be considered.

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Congenital cytomegalovirus infection is currently the leading cause of congenital infection worldwide, occurring in 0.2–2.2% of live births [1–4]. Ten percent of neonates with congenital CMV infection resulting from a primary maternal infection in pregnancy will be symptomatic at birth, and the majority (90%) of these will be left with permanent neurologic handicaps [1]. The other 90% appear normal at birth, but 10% will suffer from late central nervous system sequelae, primarily sensory-neuronal hearing loss. In addition, as reported recently, newborns whose mothers had reactivation of previous CMV infection during pregnancy may also be born with CMV infection [5,6]. This yet unknown number should be added to the total number of both symptomatic and asymptomatic congenital CMV infection cases. Early detection of hearing deficits and intervention is mandatory to minimize subsequent neurodevelopmental dysfunctions. Therefore, there is a potential benefit to routine screening for asymptomatic congenital CMV. One of the parameters that should be considered when addressing this question for the Israeli population is the current rate of annual congenital CMV in Israel. This rate was estimated by Stein et al. [7] to be 280 per year, with 56 (20%) symptomatic patients. Since this number was based on a wide maternal seroprevalence study and the assumption that symptomatic congenital CMV may occur only after primary infection and not reactivation, they concluded that such a screening program is not justified. Nevertheless, actual measurement of the true rate of congenital CMV in Israel has never been performed.

Classically, the diagnosis of congenital CMV infection is made by urine viral culture within the first 3 weeks of life. Polymerase chain reaction that would detect CMV DNA in urine specimens of newborn infants is a new and attractive method that might replace viral cultures. We recently described our method of screening for congenital CMV by urine PCR in two medical centers in Israel [8]. Other investigators successfully used this method as well [9,10]. In the future, screening may become even simpler with the option of DNA extraction from Guthrie filter paper and automated amplification of CMV sequences [11,12].

We discuss the potential role of routine screening for congenital CMV in Israel, based on our results in two different populations, by means of a PCR-based screening method. Special attention was given to the populations studied and to their epidemiologic characteristics. The results of this study may serve as a database for the future decision regarding routine screening for congenital CMV in Israel.

Patients and Methods

Study population

The study was conducted between 1 May 1998 and 31 August 1999 in two hospitals: Shaare Zedek Medical Center in Jerusalem and HaEmek Medical Center in northern Israel. These two facilities were chosen because their populations differ in many epidemiologic

CMV = cytomegalovirus
PCR = polymerase chain reaction

aspects and may represent the heterogeneous population typical of the country. The study participants numbered 2,000 neonates aged up to 3 days (1,000 newborns each). The ethics committees of the two participating hospitals approved the study. Informed consent was obtained from the parents of each newborn after verbal and written information on the nature of the study was provided. Basic data on the participating mothers were extracted from hospital records, including maternal age, ethnic group (Jewish or non-Jewish), and residency (urban or rural).

Methods

The selection of newborns from whom specimens were collected was based on convenience. Urine specimens were collected into a sterile urine-bag attached to the perineum after thorough cleaning. All the urine specimens were processed in the Infectious Diseases Molecular Diagnostic Laboratory at Shaare Zedek Medical Center. The specimens from HaEmek Medical Center were stored at 4°C, and transported on ice to the laboratory within 1 week.

Pre-study urine-PCR sensitivity assessment to assure the specificity and sensitivity of the method was performed prior to the study [8]. All the urine specimens were processed and used for CMV PCR reaction as previously described [8]. Specimens found by PCR to be positive were validated by conventional tube culture method.

Measurement of cord blood immunoglobulin G antibodies against CMV was performed on 333 (33.3%) consecutive mothers in Shaare Zedek, and 283 (28.3%) consecutive mothers in HaEmek, reflecting maternal CMV seroprevalence. Newborns with either positive urine PCR or tube culture for CMV in their urine specimen had their sera tested for the presence of CMV IgM. The assay performed was antibody-capture enzyme-linked immunosorbent assay (DiaSorin S.R.L., Saluggia, Italy).

The clinical assessment of newborns with positive urine PCR infection included physical examination, blood tests (complete blood count, kidney and liver function tests, CMV IgM), head ultrasound or computerized tomography scan, retinal examination, and brain stem evoked response audiometry.

Statistical analysis

Statistical analysis was performed using EpiInfo 6.0 software package. *P* values were calculated by chi-square, and by Fisher's exact test, where indicated, due to small numbers. $P < 0.05$ was considered statistically significant.

Results

A total of 2,000 newborns was included in the study. Maternal epidemiologic data were available for 987 mothers in Shaare Zedek and for 952 mothers in HaEmek. As shown in Table 1, there were statistically significant differences in epidemiologic aspects between the two hospitals regarding ethnic group (Jews vs. non-Jews) and residency (urban vs. rural). However, there was no significant difference in CMV seroprevalence between the two hospitals (80.5% in Shaare Zedek and 85% in HaEmek). Seroprevalence was similar in Jews and non-Jews in both hospitals (data not shown). Ten of the 1,000 newborns (1.0%) in Shaare Zedek and 4 of the 1,000 newborns (0.4%) in HaEmek were found to have CMV DNA in their urine, consistent with congenital CMV infection. This difference was not statistically significant. The combined incidence of congenital CMV was 0.7%, 95% CI 0.3–1.0%. The distribution of the infected infants among the various epidemiologically distinct subgroups is shown in Table 2. As seen, all the 10 infected newborns in Shaare Zedek were

Table 1. Demographic characteristics in the two population groups

	Shaare Zedek, Jerusalem (n=987*) No. (%)	HaEmek, Afula (n=952*) No. (%)	*P*
Origin			
Jewish	964 (97.7)	468 (49.2)	<0.01
Non-Jewish	23 (2.3)	484 (50.8)	
Residency			
Urban	859 (87.0%)	581 (61.0%)	<0.001
Rural	128 (13.0%)	371 (39/0%)	
Jews			
Urban	843 (85.4)	360 (37.8)	<0.01
Rural	121 (12.3)	108 (11.3)	
Non-Jews			
Urban	16 (1.6)	221 (23.2)	
Rural	7 (0.7)	263 (27.6)	

* Mothers with available epidemiologic data. Urine specimens collected from 1,000 newborns

Table 2. Characteristics of infected newborns in the two medical centers

	Shaare Zedek, Jerusalem		HaEmek, Afula		Total	
	Total N (%)	Infected N (%)	Total N (%)	Infected N (%)	Total N (%)	Infected N (%)
Origin						
Jewish	964 (97.7)	10 (1.0)	468 (49.2)	0 (0)	1,432 (73.9)	10 (0.7)
Non-Jewish	23 (2.3)	0 (0)	484 (50.8)	4 (0.8)	507 (21.7)	4 (0.8)
Residency						
Jews						
Urban	843 (85.4)	10 (1.2)	360 (37.8)	0 (0)	1,203 (62%)	10 (0.8)
Rural	121 (12.3)	0 (0)	108 (11.3)	0 (0)	229 (11.8%)	0 (0)
Non-Jews						
Urban,	16 (1.6)	0 (0)	221 (23.2)	2 (0.9)	237 (12.2%)	2 (0.8)
Rural	7 (0.7)	0 (0)	263 (27.6)	2 (0.9)	270 (14%)	2 (0)
Total number (%)	987*	10 (1.0)	952*	4 (0.4)	1939	14 (0.7%, 95% CI 0.3 –1%)
CMV IgG seroprevalence (%)	80.5**		85.0***		82.6%****	

* Mothers with available epidemiologic data. Urine specimens collected from 1,000 newborns.
** Based on a representative sample of 333 mothers.
*** Based on a representative sample of 283 mothers.
**** Based on a combined representative sample of 616 patients.

Ig = immunoglobulin

Table 3. Characteristics of infants with congenital CMV infection

Pt.*	Gender	Ethnic group	Gest. age (wks)	SGA	Micro-cephaly	CMV IgM	Urine culture	Head CT/US	Retinal exam	BERA	Blood tests**
1	M	Jewish	40	N	N	Negative	ND	ND	ND	ND	ND
2	F	Jewish	40	N	N	Negative	Positive	Normal	Normal	Normal	Normal
3	M	Jewish	39	N	N	Negative	Positive	Normal	Normal	Normal	Normal
4	M	Jewish	40	N		Negative	Positive	Normal	Normal	Normal	Normal
5	M	Jewish	37	N	N	Negative	Positive	Normal	Normal	Normal	Normal
6	F	Jewish	41	N	N	Negative	Positive	Normal	ND	Normal	Normal
7	F	Jewish	40	N	N	Negative	Negative	Normal	ND	Normal	Normal
8	M	Jewish	39	N	N	Negative	ND	ND	ND	ND	ND
9	F	Jewish	40	N	N	Negative	Positive	Normal	Normal	Normal	Normal
10	M	Jewish	40	N	Y	Positive	Positive	Calcifications	Normal	Normal	Hepatitis Anemia platelets↓↓
11	M	Non-Jewish	33	N	N	Negative	ND	Normal	Normal	Normal	Hepatitis
12	M	Non-Jewish	40	N	N	Negative	ND	ND	ND	ND	ND
13	M	Non-Jewish	40	N	N	ND	Positive	Normal	Normal	Normal	Normal
14	M	Non-Jewish	28	N	N	Positive	Positive	Normal	Normal	ND	Normal

* Patients 1 through 10 are from Shaare Zedek, patients 11 through 14 from HaEmek.

** Including complete blood count, liver enzymes, renal function

SGA = small for gestational age, BERA = brain-stem evoked response audiometry

Jewish and their mothers were urban, whereas all 4 infected newborns from HaEmek were non-Jews. These differences are due to the distribution of the mothers in both hospitals but are not statistically significant because of the small number of patients in each group. Comprehensive clinical evaluation was carried out on all PCR-positive newborns, and is summarized in Table 3. As shown, only 1 of 14 (7.1%) infected newborns was symptomatic. He was small for gestational age with a birth weight of 2,510 g (third percentile), and his head circumference was 28.5 cm (<3%). He had hepatosplenomegaly and thrombocytopenia of 80,000/mm^3 with petechial rash. Eye examination and BERA testing were normal. Brain ultrasonography and CT did, however, reveal severe periventricular calcifications. Another baby had evidence of transient mild hepatitis.

Discussion

The effect of CMV seroprevalence in the population on the incidence and natural history of congenital CMV infection has been extensively studied [2,13–15]. It has consistently been shown that the higher the seroprevalence of maternal CMV, the higher the incidence of congenital CMV infection, albeit asymptomatic in most cases. We found a combined seroprevalence rate of 82.6%. This rate is somewhat higher than the 69% found by Isacsohn et al. [16] in the early 1980s in Jerusalem, and is similar to the 84.3% found by Stein et al. [7] in 6,126 women of childbearing age in various regions in Israel. It seems that despite major differences among subgroups in this country, the overall CMV seroprevalence is similarly very high, with a consequent incidence of CMV infection. If about 13–20% of the 135,000 annual pregnancies in Israel are in seronegative mothers, then 17,550–27,000 women are prone to experience primary CMV infection during pregnancy. Assuming a 1–4% risk of primary maternal CMV infection during pregnancy and a 40% risk of transmission of the infection to the fetus, we may expect any number between 175 and 1,000 cases annually of congenital CMV resulting from primary maternal infection during pregnancy. Since 20% of the infected newborns become symptomatic (10% at delivery and 10% later), about 35–200 symptomatic infections are expected per year.

It has traditionally been accepted that newborns whose mothers were immune to CMV prior to conception were only very seldom symptomatic either at birth or later [1,17,18]. Recently, Boppana and colleagues [5] challenged this concept. In a series of 47 symptomatically infected newborns, the immune status of the mothers was known in 20. Eight of 20 symptomatic newborns were born to mothers with recurrent rather than primary infection. Furthermore, there was no significant difference in the severity of the infection either at birth or later. Other reports [19] confirmed this observation. On the other hand, conflicting findings were recently reported by Lozzaroto et al. [20,21] who used CMV IgG antibody avidity as an additional measure of primary infection versus reactivation. These authors found that the fetus was symptomatic only when born to mothers with primary infection and not with reactivation. These conflicting data support the conclusion that maternal serologic status is not a sufficiently reliable basis for the decision regarding which neonates should be screened. Thus, an additional unknown number of congenital CMV infections resulting from reactivation during pregnancy of previous CMV infection, with at least some of those who are expected to suffer symptomatic infection, should be added to the total number of annual cases of congenital CMV.

By performing an actual measurement of infected newborns, we found 14 of 2,000 (0.7%) newborns with congenital CMV infection as a result of either primary infection or reactivation. This combined

CI = confidence interval

BERA = brain stem evoked response audiometry

number was derived from the population of two medical centers: Shaare Zedek in Jerusalem and HaEmek in northern Israel. These two medical facilities were chosen because their populations differ in many epidemiologic aspects from each other [Tables 1 and 2] and probably represent the heterogeneous population of Israel. If the same rate applies to the remainder of the country, about 945 cases are expected to occur from the 135,000 annual deliveries.

Of note, the CMV IgM antibodies were negative in all but one of the asymptomatic CMV infected newborns. Melish and Hanshaw [22] also found that only 50% of culture-proven CMV-infected newborns were IgM-positive, and noted that the IgM results tended to be positive along with higher titers in the symptomatic newborns. These results are very important for clinicians, since IgM, although known not to be fully sensitive, is still considered a reasonable parameter for the diagnosis of congenital CMV infection.

In the United States, the spectrum of mass screening program has been widened in many states, far beyond the traditional two entities of hypothyroidism or phenylkethonuria [23]. Many states routinely screen for various metabolic and other diseases, including congenital infections, e.g., human immunodeficiency virus and toxoplasmosis [23]. Routine screening is classically justified only for conditions with an expected high rate, where the mode of screening is safe, reliable and inexpensive, and that have a proven mode of intervention [23]. The incidence of congenital CMV in our study was much higher than that of hypothyroidism or phenylketonuria – two diseases routinely screened for in many countries, including Israel. Therefore, regarding this aspect, routine screening seems justified. Our results also suggest that urine PCR screening is convenient and very reliable [8]. Nevertheless, the justification for routine screening of newborns for congenital CMV may be seriously questioned because of the lack of a proven mode of treatment. It may be argued that the knowledge per se of the risk of sensory-neuronal damage in the CMV-infected newborn, resulting in close monitoring and early diagnosis and intervention, may be a justification for such a screening program, much the same as the rationale for mandatory universal hearing screening [23,24]. Furthermore, the results of the recent randomized study by the National Institute of Allergy and Infectious Diseases noted the effect of ganciclovir in symptomatic patients in preventing hearing deterioration [25]. Although these results are premature and no firm recommendations have yet been made regarding the treatment of symptomatic congenital CMV infection, this potential mode of intervention may also be considered in the complex equation of the pros and cons of a mass screening program.

In summary, the incidence of congenital CMV in Israel – as determined for the first time in this study – is significant, and is well within the expected range found in studies from other countries. Routine screening for this condition should be considered, although serious cost-effectiveness issues need to be addressed on a nationwide level before such a major decision is made.

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Correspondence: Dr. Y. Schlesinger, Infectious Diseases Unit, Shaare Zedek Medical Center, P.O. Box 3235, Jerusalem 91031, Israel.
Phone: (972-2) 655-5147
Fax: (972-2) 666-6840
email: yechiel@szmc.org.il