



Original Article

Prevalence of Cytomegalovirus Infection among Suspected Infants in Baghdad

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ABSTRACT

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Background: Cytomegalovirus (CMV) virus is a recognized important cause of congenital CMV infection which carries a significant risk for symptomatic disease and developmental defects in newborns. Its prevalence varies from place to other and time to time. This study is conducted to estimate its prevalence in Baghdad among infants suspected of having a congenital infection and to study the associated findings.

Subjects and Methods: The study was carried out in Al-Alwiyah pediatrics teaching hospital. Data were collected, and blood samples were taken for infants suspected to have intrauterine infections over a period of one year, from 1 October 2019 to 1 October 2020. Immunoglobulin M (IgM) tests for CMV were conducted for all collected samples. CMV-immunoglobulin G (IgG) was a further analysis if negative results were obtained for CMV-IgM testing. Samples were tested by Eliza method.

Results: The overall positivity for CMV-specific antibodies among suspected infants was 16.7% for IgM and 66 % for IgG. Males constitute 55% of CMV-IgM positive results. These results are statistically significant concerning age groups. 1-3-month age group was the largest (40 % of IgM positive infants) with a p-value of 0.000. This indicates delayed presentation of CMV-affected infants. With the same p-value, 74% of infants less than one month of age were IgG positive reflecting the maternal prevalence of CMV- IgG in an equivalent percentage.

Convulsion followed by delayed milestones was the most common presenting symptoms in congenital CMV infection with statistically significant associations.

Conclusions: The study indicates that infection with CMV constitutes a significant portion of suspected infants. The study recommends special attention to take steps concerning early management.

Introduction

Human cytomegalovirus (HCMV) is the type species of the virus genus Cytomegalovirus (CMV), a member of a family known as Herpesviridae or herpesviruses (1,2). HCMV is also called cytomegalovirus (CMV), although there are eight types of species are included in the genus CMV, and HCM is one type of these (2,3). The human cytomegalovirus (CMV) virus is recognized as an important cause of congenital infection leading to birth defects and

developmental disabilities (4). Congenital CMV infection is one of the TORCH infections which include: toxoplasmosis, syphilis, rubella, CMV, and HSV. These infections carry a risk of significant symptomatic disease and developmental defects in newborns.

Congenital CMV infection is the most common congenital infection worldwide, with an incidence ranging from 0.2% to 2.5% of all live births worldwide (5). Its incidence in developed countries ranges from 0.6 to 0.7% of all live births (6).

Not every maternal CMV infections lead to a congenital CMV since transmission to the fetus occurs in only 40% of primary maternal infections, which results in the delivery of 10-15% symptomatic and 85-90% asymptomatic congenitally-infected newborns (7). Asymptomatic disease in infants is not entirely benign as 10 to 15% go on to develop long-term morbidities (8). Seroconversion of CMV immunoglobulin M (IgM) happens in both primary and reactive forms of CMV, while seroconversion for immunoglobulin G (IgG) happens in primary infection.

For non-primary infections, the transmission rate is significantly low at 1.1–1.7% of infected mothers (6,9).

The seroprevalence rates of CMV among pregnant women in developed countries were lower than those of developing countries, which is estimated to be between 1 and 5% of all live births (6, 10).

Infection earlier in life is typical in developing countries, whereas up to 50% of young adults are seronegative in many developed nations (11). In general, estimated CMV IgG antibodies was (40-80%) in developed countries and (90 - 100%) in developing countries (12,13,14).

CMV is considered to be the second leading cause of mental retardation in the United States and is currently the leading cause of sensorineural deafness.

Congenitally infected infants are often divided into two groups: those with findings that are apparent in the neonatal period and those with signs of CNS damage that become apparent later in childhood. In addition to intrauterine growth restriction, over 70% have evidence of CNS involvement (15). The clinical manifestations of congenital cytomegalic inclusion disease include jaundice, splenomegaly, petechiae, intrauterine growth retardation, microcephaly, and retinitis, lethargy, hypotonia, optic atrophy, decreased hearing, and intracranial calcifications (11). Furthermore, they may have seizures or retinitis. Some babies with signs of congenital CMV infection at birth may have long-term health problems, such as developmental and motor delay, vision loss, seizures, and late-onset hearing loss even if normal at birth or passed the newborn hearing test (11,15,16).

Of infants who are asymptomatic at birth, 10 to 20% eventually will have CNS involvement (17).

CMV excretion is common in children with congenital infection and may represent a reservoir for infection in other children and daycare workers (11).

The IgM level is elevated in patients with recent CMV infection, or there is a 4-fold increase in IgG titers. CMV- IgM antibodies may be found as early as 4-7 weeks after initial acquired infection and may persist as long as 16-20 weeks (11).

The drug of choice for the treatment of CMV disease is intravenous ganciclovir, although valganciclovir may be used for non-severe CMV treatment in selected cases (11). CMV immune globulin can be used in combination with ganciclovir to treat CMV pneumonia.

The purpose of this study is to shed light on the prevalence of congenital CMV among suspected infants living in Baghdad and to study some associated characteristics.

Subjects and Method

Ethical Approval: -

This study was approved by the research ethical committee/ research unit in the Baghdad Al-Risafa Health Directorate as part of the 2020 research plan. This plan was also approved by the training and planning directorate / MOH-Iraq.

As far as patients involved in this study, parent/s approval was taken for every infant involved in this study. The procedures and information taken during this study are part of the routine work in the hospital.

A cross-sectional study design was conducted from 1 October 2019 to 1 October 2020. All children who were suspected of having congenital infection consulting al-Elwya pediatric teaching hospital were enrolled in the study. Criteria for inclusion included the presence of any of the following: neonatal or persistent hepatosplenomegaly, jaundice, rash, congenital malformations, various CNS manifestations like convulsions or delayed milestones, and hearing or ophthalmological abnormalities.

Samples of blood were taken from all infants and sent to the virology laboratory as a part of routine diagnostic services as well as for research purposes.

Sample collection:

About 1.5 ml to 3 ml of venous blood was obtained as a part of the required investigation for these children. The blood samples were placed in a sterile plain tube, allowed for clotting at room temperature for 30 minutes and then centrifuged at 1500 rpm for 5 minutes. All sera were stored at -20°C pending testing. Samples were tested for CMV-specific IgM antibodies by commercially available IgM capture ELISA kits (Bioactiva, Germany). CMV negative samples were also tested for CMV-specific IgG antibodies. The manufacturer's instructions were strictly adhered to in the performance and interpretations of the tests and results.

Statistical Analysis:

The following statistical data analysis approaches were used to analyze and assess the study's results under the application of the statistical package (SPSS) ver. (22.0): 1) descriptive data analysis. 2) inferential data analysis to accept or reject the statistical hypotheses, which included the contingency coefficients (C.C.) test for estimating correlations.

Results

Figure. (1) Prevalence of CMV IgM and IgG seropositivity:

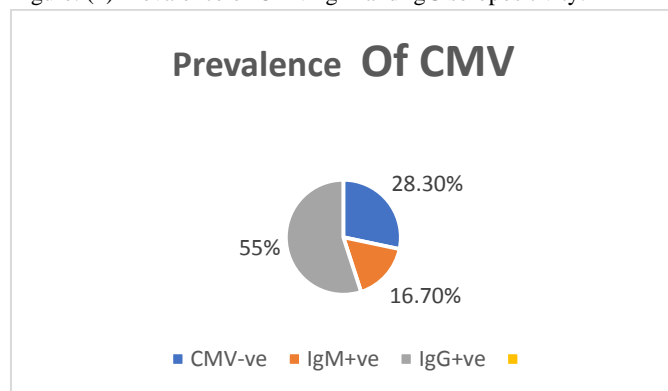


Figure.1 shows that CMV IgM and IgG seropositivity were 16.7% and 55% respectively among the studied sample.

Table 1: Gender distribution of the sample distributed according to seropositivity and seronegativity with comparison significance

Gender	No. & %	Outcomes			Total	C.S. (*) P-value
		IgG +ve	IgM +ve	-ve test		
Male	No.	36	11	18	65	C.C. = 0.016 P=0.985 NS
	%	55.4%	16.9% (55%)	27.7%	100%	
Female	No.	30	9	16	55	
	%	54.5%	16.4% (45%)	29.1%	100%	
Total	No.	66	20	34	120	
	%	55%	16.7% (100%)	28.3%	100%	

(*) NS: Non Sig. at P>0.05

Table 1 shows that the constructed contingency's coefficient had reported a weak relationship with no significant difference at p>0.05 for the studied CMV results (IgG +ve, IgM +ve, and -ve results). Furthermore, Table 1 shows that 55 % and 45% of patients were males and females, respectively had CMV-specific IgM results (Figure 2).

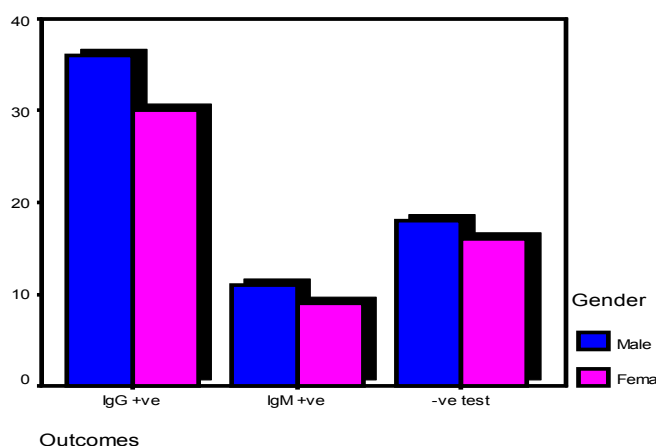


Figure. 2: Gender distribution of the sample

Table 2: Age distribution of the sample distributed with comparison significance

Age Groups	No. & %	Outcomes			Total	C.S. (*) P-value
		IgG +ve	IgM +ve	-ve test		
Neonatal period	No.	20	3	4	27	C.C. = 0.524 P=0.0 HS
	%	74.1%	11.1% (15%)	14.8%	100%	
1-3 M	No.	24	8	3	35	
	%	68.6%	22.9% (40%)	8.6%	100%	
4-6 M	No.	18	3	4	25	

Age Groups	No. & %	Outcomes	Total	C.S.		
7-9 M	No.	1	3 (15%)	12	100%	
	%	6.3%	18.8%	75.0%		
10-12 M	No.	3	3	11		
	%	17.6%	17.6% (15%)	64.7%		
Total	No.	66	20	34		120
	%	55%	17% (100%)	28%		100%

(*) HS: Highly Sig. at P<0.01

The results show that the constructed contingency coefficient reports a strong relationship with a highly significant (a value p-value =0.000) association of the age groups with (IgG +ve, IgM +ve and -ve results). There was a constraint association since 74% of neonates were IgG +ve and to some extent the IgM +ve marker at 1-3 months of life constituted 40 % of IgM positive infants. The maximum seronegative percentage was in the 7-9-month age group constituting 75% of seronegative infants (Table 2, Figure 3)

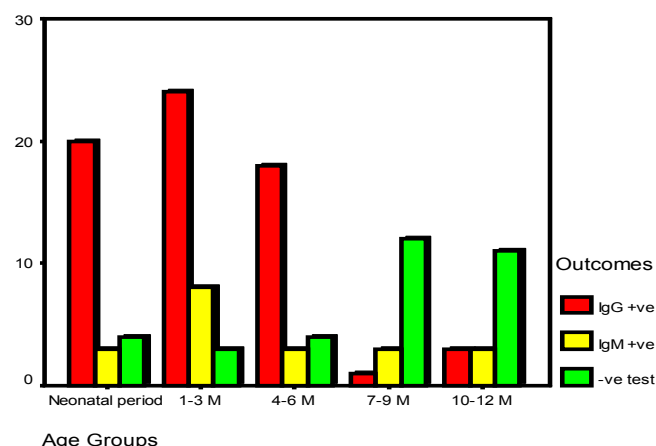


Figure.3: Age distribution of the sample

Table 3: Distribution according to maternal age with comparison significance.

Maternal Age Groups	No. & %	Infant test results			Total	C.S. (*) P-value
		IgG +ve	IgM +ve	-ve test		
Less than 20 yrs.	No.	8	3	4	15	C.C. = 0.120 P=0.9
	%	53.3%	20.0%	26.7%	100%	
20 - 30 yrs.	No.	38	13	19	70	
	%	54.3%	18.6%	27.1%	100%	

	No.	%	%	%	%	NS
31 - 40 yrs.	18	60.0	10.0	30.0	100	
> 40 yrs.	2	40.0	20.0	40.0	100	
Total	66	55.0	16.7	28.3	100	

(*) NS: Non Sig. at P>0.0

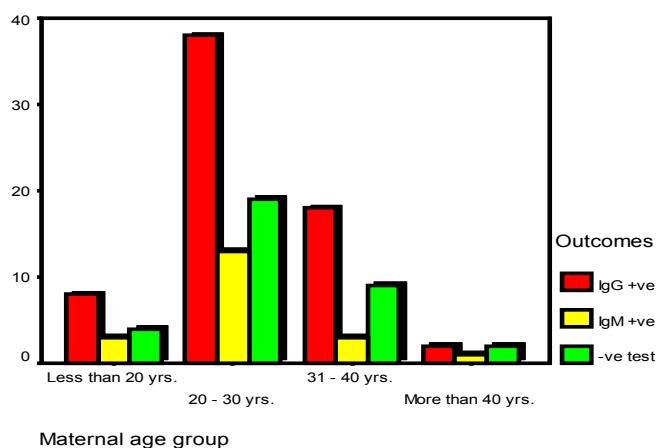


Figure.1: Distribution according to maternal age.

The results in Table 3 show that the constructed contingency coefficient reported a weak relationship with no significance at p>0.05 for the studied CMV results (IgG +ve, IgM +ve, and -ve tests) according to the mother's age groups variable. The maximum seroprevalence of infant CMV specific IgM was high in infants whose mother were within < 20-year age group (20%). The maximum CMV-specific IgG was high in infants whose mothers were within the 31-40 year age group (60%) (Table 3 and Figure 4):

Table 4: Distribution of presenting complaints across studied infants CMV immunoglobulins results (IgG +ve, IgM +ve and -ve) with comparison significance

Complaint	Res p.	No. & %	Outcomes	Total	C.S. (*)	P-value	C.C. =
Hypotonia/lethargy	Absent	No. 47 % 71.2	16 80%	31 91.2%	94 78.3%	0.206	
	Present	No. 19 % 28.8	4 20.0%	3 8.8%	26 21.7%	NS	
Abnormal features	Absent	No. 49 % 74.2	17 85.0%	32 94.1%	98 81.7%	0.220	
	Present	No. 17 % 25.8	3 15%	2 5.9%	22 18.3%	S	
Poor feeding	Absent	No. 52 % 78.8	17 85.0%	31 91.2%	103 83.3%	0.144	
	Present	No. 14 % 21.2	3 15%	3 8.8%	20 16.7%	NS	
Delayed mile stones	Absent	No. 52 % 78.8	14 70%	34 100%	103 83.3%	0.282	
	Present	No. 14 % 21.2	6 30%	0 0.0%	20 16.7%	HS	
Jaundice	Absent	No. 50 % 75.8	18 90%	32 94.1%	113 83.3%	0.222	
	Present	No. 16 % 24.2	2 10%	2 5.9%	20 16.7%	S	
Microcephaly	Absent	No. 62 % 93.9	17 85.0%	32 94.1%	111 92.5%	0.26	
	Present	No. 4 % 6.1	3 15%	2 5.9%	9 7.5%	NS	

Complaint	Res p.	No. & %	Outcomes	Total	C.S. (*)	P-value
Hydrocephaly	Absent	No. 61 % 92.4	20	33	114	95.0%
	Present	No. 5 % 7.6	0	1	6	2.9%
Failure to thrive	Absent	No. 64 % 97.0	18	33	115	95.8%
	Present	No. 2 % 3.0	2	1	5	4.2%
Skin rash petechial	Absent	No. 63 % 95.5	19	34	116	96.7%
	Present	No. 3 % 4.50	1	0	4	3.3%
Hearing impairment	Absent	No. 63 % 95.5	20	34	117	97.5%
	Present	No. 3 % 4.50	0	0	3	2.5%
Visual impairment +/- glaucoma	Absent	No. 64 % 97.0	20	33	117	97.5%
	Present	No. 2 % 3.0	0	1	3	2.5%
Trauma	Absent	No. 65 % 98.5	20	34	119	99.2%
	Present	No. 1 % 1.5	0	0	1	0.8%

(*): HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; testing based on the contingency coefficient.

In order of frequency the clinical findings were (Table 4):1) Convulsion accounted for 11/20 (55 %) of patients with CMV-specific IgM making it a major presenting symptom (HS). 2) DMS accounted for 6 /20 (30%) of patients with CMV specific IgM making it the second most common presenting symptom in our sample (HS). 3)Hypotonia and lethargy accounted as 4/20 (20%) making them at the third order (NS) (p- value 0.07) 4) Three CMV-infected infants (3/20 ;15%) of CMV infected infants were accounted for each of abnormal features , microcephaly, and poor feeding (NS) 5) Failure to thrive (FTT) (NS) and jaundice (S) were accounted for 2/20 (10 %). 6) Skin rash accounted for 1/20 (5%) (NS).

Table 4: Distribution according to associated conditions or finding with comparison significance

Associated Conditions or Finding	Res p.	No. & %	Outcomes			Total	C.S. P-value
			IgG +ve	IgM +ve	-ve test		
Hepatomegaly +/- Splenomegaly	Absent	No. 66 % 100	18	30	114	95.0%	
	Present	No. 0 % 0.0	2	4	6	5.0%	
CHD	Absent	No. 64 % 97.0	18	31	113	94.7%	
	Present	No. 2 % 3.0	2	3	7	6.1%	
Low birth weight	Absent	No. 66 % 100	20	33	119	99.2%	
	Present	No. 0 % 0.0	0	1	1	0.8%	
Prematurity	Absent	No. 66 % 100	20	33	119	99.2%	
	Present	No. 0 % 0.0	0	1	1	0.8%	
Laryngomalacia	Absent	No. 66 % 100	20	33	119	99.2%	
	Present	No. 0 % 0.0	0	1	1	0.8%	
PKU	Absent	No. 66 % 100	20	33	119	99.2%	
	Present	No. 0 % 0.0	0	1	1	0.8%	
Galactosemia	Absent	No. 66 % 100	20	33	119	99.2%	
	Present	No. 0 % 0.0	0	1	1	0.8%	

Associated	Res	No.	Outcomes	To	C.S.		
CHT	ent		100	100	97.	99.	P=0.27
		%	%	%	1%	2%	9
	Pre sent	No.	0	0	1	1	NS
		%	0.0	0.0	2.9	0.8	
	Abs ent	No.	65	20	34	11	C.C. =
		%	98.5	100	100	99.	0.083
	Pre sent	No.	1	0	0	1	2
		%	1.5	0.0	0.0	0.8	NS
			%	%	%	%	

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; testing based on the contingency coefficient.

Table shows that constructed contingency's coefficient reports a significant relation in the studied (IgG +ve, IgM +ve, and -ve) findings concerning to (hepatomegaly +/- splenomegaly) meanwhile , CHD had weak relationship (not significant at p>0.05).

Discussion

Some of positive CMV IgM infants could be due to postnatal infection. 13.3% of the infants at the age of one month were seroconverted to anti-CMV IgM at one study in china (18). Therefore, it is important to explore other possible causes regarding infant complaints.

CMV IgM seropositivity was 16.7% among the studied sample (Figure 1), comparable to 12.5%-20% of suspected children with congenital infection in different studies from India (7,19,20).

CMV-specific IgG was 66% in this study (Figure 1). The finding of these antibodies (Abs) among infants largely represents the transplacental passage of maternal Abs (as far as there is no evidence of recent infection). Maternal Abs can last for 9 months. Just 3 out of 120 cases (2.5 %) showed these Abs above 9 months of age, representing the most probable previous postnatal acquired infection. Seroprevalence of CMV-specific IgG varies greatly from country to country.

The highest seroprevalence was seen in the World Health Organization (WHO) Eastern Mediterranean region (90%) and the lowest in WHO European region (66%) (21).

In Ghana, the estimated IgG seroprevalence lies between 76 and 96% among the general population (22).

Maternal IgG passes through the placenta and its presence in newborn infants reflects maternal CMV antibodies. These anti CMV Abs protect infants up to 8-9 months from CMV infection (18,23,24).

In general the prevalence of CMV IgG antibodies in pregnant mothers in developed countries is 40-80% and 90 - 100% in developing countries (12, 13). The reported figures vary between 80 and 90% in India (25), 98.9% in Turkey(5), 93-98 in China (18,26,27) ,and 95.6%. in Ghana (28). In conclusion, these immunoglobulins are found in newborn infants and last for several months as exogenous transplacental passive immunity. Compatible with this, we found (with HS association) that the seroprevalence of anti-CMV IgG was much higher in young infants (<6 months old) as compared to older infants denoting waned maternally acquired antibodies (table 2).

CMV-specific IgGs were present among 74.1% of infants of less than one month of age in this study; we suggest that maternal anti-

CMV IgG prevalence among childbearing mothers would be around 74 %. In one previous study in Thi-Qar / Iraq, it was 60%(29) while a seroprevalence of 85.9% in pregnant women was found in Duhok / Iraq (30).

Our results are statistically significant concerning age groups with a p-value of 0.000. 1-3 months' age group constitutes 40 % of IgM-positive infants. This indicates delayed presentation of CMV-affected infants beyond the neonatal period.

We recommend testing maternal IgG and IgM-CMV status during antenatal care and to take further measures after any observed seroconversion.

There was no significant difference between the gender of participants and IgM results in this study (males constituted 55% as shown in Table 1) which is compatible with a previous study in Iraq (31).

In this study, the seroprevalence of infant CMV, specific IgM, was more common in infants of the < 20-year maternal age group, decreasing with increases in maternal age (Table 3). Contrarily, IgG prevalence increased with an increase in maternal age, which reflects an increase in the prevalence of maternally-acquired CMV-IgG Abs, as seen in the infant CMV-IgG prevalence distribution (Table 3). A previous study in Iraq also showed an increasing prevalence of IgG with maternal age (30). Further studies abroad were in concordance with this as well (13,32,33). Maternal IgG seropositivity lessens the fetal risk for congenital infection since identifying maternal CMV IgG status for pregnant women is an important predictor of congenital CMV infection. Symptomatic CMV congenital disease is less likely with mothers with pre-existing immune responses to CMV than in CMV-naïve individuals (34). Annual seroconversion rates for pregnant women ranged from 1-7% (34).

In contrast to our findings, the most common clinical findings at birth for congenital CMV infection reported in literature included petechiae (71%), jaundice (67%), microcephaly (53%), and small size for gestational age (50%)(11). While in this study in order of frequency: Convulsion accounted as (55 %) of patients with CMV positive IgM (HS) followed by DMS (30%) (HS), Hypotonia and lethargy (20%) (S); abnormal features, microcephaly, and poor feeding (15% of each) (NS), failure to thrive ((10 %) (NS), jaundice (10 %) (S) and hepatomegaly +/- splenomegaly (10%)(S), congenital heart disease (10%)(N S) and skin rash 1/20 (5%). (NS).

In contrast to our findings, the most common clinical findings at birth for congenital CMV infection reported in the literature included petechiae (71%), jaundice (67%), microcephaly (53%), and small size for gestational age (50%) (11).

The variances are attributed to the small size of the sample and the quality of our sample with delayed presentation.

Hearing impairment was not a presenting symptom in this study because Iraq has not yet implemented a universal neonatal hearing screening program.

Hearing loss can occur later on at any point during childhood, even those with normal hearing at birth, and accounts for 20% of sensorineural hearing loss in children (15,35,36,37).

A frequent hearing assessment is advised for our patients during initial presentation and follow-up visits.

One of the study's limitations is that diagnosis depends on an IGM assay rather than PCR. The gold standard for diagnosing CMV infection in early life is to detect the virus in urine or saliva by viral culture and/or PCR. Another limitation is the difficulty in ascertaining (to 100%) whether some infants were infected during

intrauterine life. Furthermore, this study involved symptomatic infants whose parents were looking for treatment and never involved asymptomatic infants who could develop certain squally at a later age.

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Conflict of Interest

No conflict of interest

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