



Prevalence of Cytomegalovirus in Iraqi Children

Sevan Najem Alwan^{1*}, Atheer Jawad Al-Saffar², Ali Hattem Bayati³,
Haider Sabah Kadhim⁴, Hala Sameh Arif⁵, Avan Hussein Ghaib⁶, Luay Ibraheem Alrawi⁷,
Saadoon Abed Abdulrudha⁸, Ahmed Hasan Mohammed⁹, Bestoon Muhammad Saeed¹⁰ and
Brian L. Wickes¹¹

¹ Department of Microbiology, College of Medicine, Baghdad University, Baghdad, Iraq

² Department of Community and Family Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

³ Department of Community Health, Technical College of Health, Sulaimani Polytechnic University, Sulaymaniyah, Kurdistan Region, Iraq

⁴ Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

⁵ Department of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

⁶ Department of Microbiology and Immunology, School of Medicine, University of Sulaimani, Sulaymaniyah, Iraq

⁷ Children's Welfare Hospital, Baghdad, Iraq

⁸ Alkarama General Hospital, Wasit, Iraq

⁹ Department of Pathology Analysis, College of Science, Dhi-Qar University, Dhi-Qar, Iraq

¹⁰ Department of Laboratory Analysis, Pediatric Teaching Hospital, Sulaymaniyah, Iraq

¹¹ Department of Microbiology and Immunology, Director of Advanced Nucleic Acids Core Facility, University of Texas Health Science Center, San Antonio

*Corresponding e-mail: dr.alkarkhi@gmail.com; sevan.alwan13@gmail.com

ABSTRACT

The majority of children with congenital cytomegalovirus are born to cytomegalovirus seropositive women. However, the likelihood of congenital infection with disability is highest for children whose mothers were cytomegalovirus seronegative acquired infection during pregnancy. **Objectives:** to provide first nationally estimate of cytomegalovirus seropositivity among Iraqi children under five years of age. Materials and Methods was used total of 1000 hospitalized children under five years of age from different geographical area in Iraq were enrolled in this study. The numbers of children were collected by proportional allocation for each selected governorate according to total number of participant children. Kuppusswamy scale has been used to measure the socioeconomic status for children. Serum samples were obtained from each subject participate in this study, cytomegalovirus infection was defined as IgM antibody positive by electro-chemiluminescence Immunoassay techniques. The results show that the current study revealed a prevalence of cytomegalovirus specific IgM as a serum marker was 5.4% among children under five years of age. Positive cytomegalovirus was associated with low socioeconomic status, maternal bad obstetric history, and lower age of children, while the infection was not associated to geographical distribution and gender. By stratified the children into symptomatic and asymptomatic according to the signs and symptoms of cytomegalovirus congenital and acquired infection at time of sample collection, 15% and 0.9% proved to have positive specific IgM among symptomatic and asymptomatic children, respectively. Jaundice was the most predominant clinical sign 56% among symptomatic infected children, followed by hepatosplenomegaly 41.7%. **Conclusion:** The data provide in the current study strongly encourage routine testing for cytomegalovirus Antibodies among pregnant women in Iraq. Infants diagnosed to be sub-clinically infected with cytomegalovirus, considered being at a risk for the developmental sequelae and they should be observed closely in order to detect the consequences of congenital infection and to allow treatment to occur as early as possible.

Keywords: Prevalence, Cytomegalovirus, Hepatosplenomegaly, Iraqi Children, IgM

INTRODUCTION

Human Cytomegalovirus (HCMV) is a member of Herpesviridae family with a wide distribution [1]. Human is the only reservoir for the virus, which is the largest human viral pathogens [2,3]. Like other herpesviruses, after primary infection HCMV has the ability to establish latency in various different types of cells [4]. HCMV infection may occur in individual without prior exposure to the virus (primary infection), or in those with previous exposure (recurrent infection) [5]. Recurrent infection may result from reactivating of endogenous latent virus or from reinfection with an exogenous virus [6,7]. Primary or recurrent infection (active infection) may be asymptomatic or cause mild or severe illness [8].

The signs and symptoms of HCMV infection vary with age, route of transmission and immunocompetence of the patient [9]. Individuals at greatest risk for cytomegalovirus disease are those receiving organ transplants, malignant tumors, whom are receiving chemotherapy, and those with acquired immunodeficiency syndrome [10,11]. HCMV is the major viral etiology of congenital infection and birth defects [12], the transmission rates from infected pregnant women to the fetus are significantly higher than *Rubella* and *Toxoplasma gondii* [13,14].

HCMV circulates worldwide without seasonal variation and commonly infects people of all ages, races, and those from a variety of socioeconomic, cultural, and geographic backgrounds [8,15]. Seroprevalence of HCMV in developing countries is higher than seroprevalence in developed countries [16]. This observation is important for congenital HCMV epidemiology because women of childbearing age, are at major risk of giving birth to infants with congenital infection if the infection acquired during pregnancy [17].

In addition, congenital HCMV may indeed exert its greatest burden on developing countries due to high birth rates and high seroprevalence [18]. Because of that, incidence of congenital infection is directly correlated with the seroprevalence of HCMV antibodies in the population [19,20]. Many factors enhanced the magnitude of HCMV as a health problem such as, no specific antiviral therapy for HCMV infection and no licensed vaccine [21-23], a lack of awareness of congenital HCMV among health care workers and the public because most maternal and newborn infections are asymptomatic and, therefore, not recognized at birth [24].

Despite this accumulation of knowledge, however, we lack aggressiveness in dealing with this health problem concerning management in Iraq. Therefore, this study may inform the behavioral interventions that aimed to preventing infection in children and help identify target populations for future HCMV vaccine.

MATERIALS AND METHODS

Patients and Methods

Study setting

Cross-sectional nationally representative study design was conducted from September 13, 2014 to March 23, 2015. Total of 1000 children under five years of age were enrolled in this study. Inclusion criteria for selecting children were the age group (from zero time to 60 months). Children visited the emergency room, children admitted to the hospital for medical reasons, and neonates admitted to the neonatal intensive care unit (NICU) were selected by consecutive sampling from four Iraqi governorates (Bagdad, Wasit, Dhi Qar, and Sulaymaniyah).

The regions adopted in the study were represented by the following: Sulaymaniyah; northern region, Baghdad and Wasit; Central region, Dhi-Qar; southern region. The middle Euphrates region was not included due to logistical difficulties. The total number of samples was allocated proportionally to each governorate based on the number of children under five years it has at the time of samples collection. Serum samples were collected from one hospital per governorate except Baghdad in which three hospitals were included to reach the required number of samples within the study period as shown in Table 1.

Table 1 Targeted number of samples and hospitals for each governorate

Governorate	Targeted Number of samples for each governorate	Hospitals	Number of children/hospital
Baghdad	590	Children's Welfare Hospital	419
		Central Teaching Hospital of Child	61
		Imamein Kadhimein Medical City	110
Wasit	120	Alkarama General Hospital	120
Dhi-Qar	175	Bint Al-Huda Maternity and Children Hospital	175
Sulaymaniyah	115	Pediatric Teaching Hospital	115
Total	1000	-	1000

A structured questionnaire was developed by the investigator based on relative literature and research to cover six categories of information that were filled out through 2 phases, the field phase and laboratory phase. The field phase included collecting data regarding child information, clinical manifestations, maternal variables, parent's demographic information, and contact information. The field phase was completed during face-to-face interviews of child mother or child-caring relative that made by the investigator at the time when samples were taken. All the questionnaire information was successfully filled except data regarding household income of 54 participants due to parent's unwillingness to share the income information. Kuppuswamy scale has been used to get the most relevant outcome in terms of reliability regarding SES. The scale includes income, occupation, and education of the household as a variable that is used for SES measure. Kuppuswamy scale was adapted according Iraqi society by the investigator.

Definition of variables

Clinical manifestations were determined by consultation of a pediatric specialist and verification of the information in the medical record. The children, having the signs and symptoms of HCMV congenital and acquired infection at time of sample collection were labelled as symptomatic children. The clinical manifestations of symptomatic congenital infection were hepatosplenomegaly, jaundice, rash, and various congenital malformations, especially those involving the central nervous system and ophthalmological abnormalities. Mononucleosis-like syndrome and mononucleosis complications, such as prolonged fever of unknown origin, hepatitis, pneumonitis, central nervous system involvement, ocular involvement, pericarditis, and myocarditis were the most common signs for symptomatic acquired HCMV infection.

Children mothers were stratified by mothers with bad obstetric history (BOH) and without BOH. Mothers that classified as a mother with BOH were those mothers with previous unfavorable pregnancy and/or delivery outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine fetal death, intrauterine growth retardation, preterm deliveries, early neonatal death, and deliveries with congenital defect. A consent letter was signed by each child parent's, and the study was approved by the Research Ethical Committee at College of Medicine of Al-Nahrain University.

Sample collection

About 1.5 ml to 3 ml of venous blood was obtained as a part of the routine investigation for sick children. The blood samples were placed in a sterile plain tube, allowed for clotting at room temperature for 30 minutes then centrifuged 1500 rpm for 5 minutes. All sera were stored at -20°C pending until testing.

Diagnostic test applied for detection of human cytomegalovirus infection

The serum samples of children were screened for HCMV-IgM antibodies using electro-chemiluminescence Immunoassay (Roche, Germany) according to manufacture protocol.

Data analysis

The collected data were compiled in a Microsoft Excel spreadsheet. Statistical analysis was performed in SPSS software (Version 24, IBM licensed), frequency analysis was used to calculate rate and ratios. Chi square test and Fisher's exact test was used to determine any significant difference between the categorical data. $P < 0.05$ was considered as a cut-off value for significance.

RESULTS

The present study enrolled 1000 children from four governorates in Iraq, involving 443 females and 557 males making male-to-female ratio of 1.26:1, 213 (21.3%) mothers of studied children had bad obstetric history (BOH), (Figure 1). Kuppaswamy socioeconomic scale discriminated a total of 946 families into high SES level 58 (6.1%), middle SES level 711 (75.2%), and low SES level 177 (18.7%). SES for 54 of them was missing due parent's unwillingness to share such information (Figure 1).

Human Cytomegalovirus infection was detected in 54 (5.4%) children under five years of age with no significant difference in the rates of IgM positive cases among the four Iraqi governorates. However, Baghdad reported the highest rates of positive cases of HCMV (6.1%), as shown in Table 2.

Table 2 The distribution of IgM-Anti HCMV results according to geographical regions

Geographical regions in Iraq		IgM-Anti HCMV				Total
		Negative		Positive		
		No.	%	No.	%	
Center	Baghdad	554	93.9	36	6.1	590
	Wasit	114	95	6	5	120
Southern	Dhi Qar	167	95.4	8	4.6	175
Northern	Sulaymaniyah	111	96.5	4	3.5	115
Total	-	946	94.6	54	5.4	1000

$X^2=1.673$; $df=3$; $P=0.643$

No significant difference between gender of participant and IgM results. Close rate of positive HCMV was seen in females 25 (5.6%) to males 29 (5.2%), as shown in Table 3.

Table 3 The distribution of study group of IgM-Anti HCMV results to gender

Gender	IgM-Anti HCMV				Total
	Negative		Positive		
	No.	%	No.	%	
Female	418	94.4	25	5.6	443
Male	528	94.8	29	5.2	557
Total	946	94.6	54	5.4	1000

$X^2=0.092$; $df=1$; $P=0.761$

According to age group, HCMV active infection was higher among neonates 25 (8.4%), than children 29 (4.1%) from 1 month to 5 years of age with significant difference, as shown in Table 4.

Table 4 The distribution of IgM-Anti HCMV results to age group

Age group	IgM-Anti HCMV				Total
	Negative		Positive		
	No.	%	No.	%	
Less than one month	272	91.6	25	8.4	297
More than one month	674	95.9	29	4.1	703
Total	946	94.6	54	5.4	1000

$X^2=7.407$; $df=1$; $P=0.006$

Highest rate of HCMV infection was seen among children from families with low SES level 15 (8.5%) and 39 (5.5%) from families with middle SES level, while no positive HCMV was found among children from families with high SES level. A significant difference was found between the positive HCMV and SES level as shown in Table 5.

Table 5 The relation of IgM-Anti HCMV results according to socio-economic state

Socioeconomic state	IgM-Anti HCMV				Total
	Negative		Positive		
	No.	%	No.	%	
High	58	100	0	0	58
Low	162	91.5	15	8.5	177
Middle	672	94.5	39	5.5	711
Total	892	94.3	54	5.7	946

$X^2=6.09$ $df=3$ $P=0.048$

Children of mothers with BOH had significantly higher frequency of IgM positivity 30 (14.1%) than positivity among children of mothers without such history 24 (3%) as shown in Table 6.

Table 6 The relation of IgM-Anti HCMV results according to maternal BOH

Maternal obstetric history	IgM-Anti HCMV				Total
	Negative		Positive		
	No.	%	No.	%	
No	763	97	24	3	787
Yes	183	85.9	30	14.1	213
Total	946	94.6	54	5.4	1000

$X^2=39.959$; $df=1$; $P=0.00001$

Among the 302 studied children who were symptomatic for HCMV infection clinically, 48 (15.9%) proved to have positive specific IgM, while only six (0.9%) children out of 698 without clinical signs for HCMV infection were proved to have positive specific IgM. A significant association was present between positive IgM and clinical manifestations (Table 7).

Table 7 The relation of IgM-Anti HCMV results according to symptomatic cases

Clinical findings	IgM-Anti HCMV				Total
	Negative		Positive		
	No.	%	No.	%	
Symptomatic children	254	84.1	48	15.9	302
Asymptomatic children	692	99.1	6	0.9	698
Total	946	94.6	54	5.4	1000

$X^2=93.272$; $df=1$; $P=0.000$

Jaundice, hepatosplenomegaly, hydrocephaly, microcephaly, vision problems, pneumonitis, and petechiae showed significant relation with HCMV active infection, while feeding difficulties, heart diseases, mental retardation, convulsions, and developmental problems have failed to prove such relation, as shown in Table 8.

Table 8 The distribution of HCMV infection in studied children according to clinical findings

Clinical finding	IgM Positive (54)				P-value
	Absent		Present		
	No. (total)	%	No. (total)	%	
Jaundice	27 (834)	3.2	27 (166)	16.3	0.00001**
Hepatosplenomegaly	34 (934)	3.6	20 (66)	30.3	0.00001*
Hydrocephaly	48 (960)	5	6 (40)	15	0.017*
Microcephaly	47 (961)	4.9	7 (39)	17.9	0.004*
Vision Problems	47 (980)	4.8	7 (20)	35	0.00001*
Hearing Problems	52 (994)	5.2	2 (6)	33.3	0.037*
Petechiae	48 (959)	5	6 (41)	14.6	0.019*

Pneumonitis	43 (915)	4.7	11 (85)	12.9	0.04*
Feeding Difficulties	52 (974)	5.3	2 (26)	7.7	0.647*
Heart Diseases	52 (973)	5.3	2 (27)	7.4	0.653#
Mental Retardation	52 (989)	5.3	2 (11)	18.2	0.115#
Convulsion	50 (955)	5.2	4 (45)	8.9	0.299#
Developmental Problems	53 (986)	5.4	1 (14)	7.1	0.543#
Prolonged Fever	51 (997)	5.1	3 (3)	100	0.00001*

*Significant (Fisher's Exact test); **Significant (Pearson Chi-Square); #Not significant

Jaundice was the most predominant clinical finding 27 out of 48 (56%), followed in order of frequency by hepatosplenomegaly 20 out of 48 (41.7%) and pneumonitis 11 out of 48 (22.9%). Other clinical findings were less frequent (Figure 1).

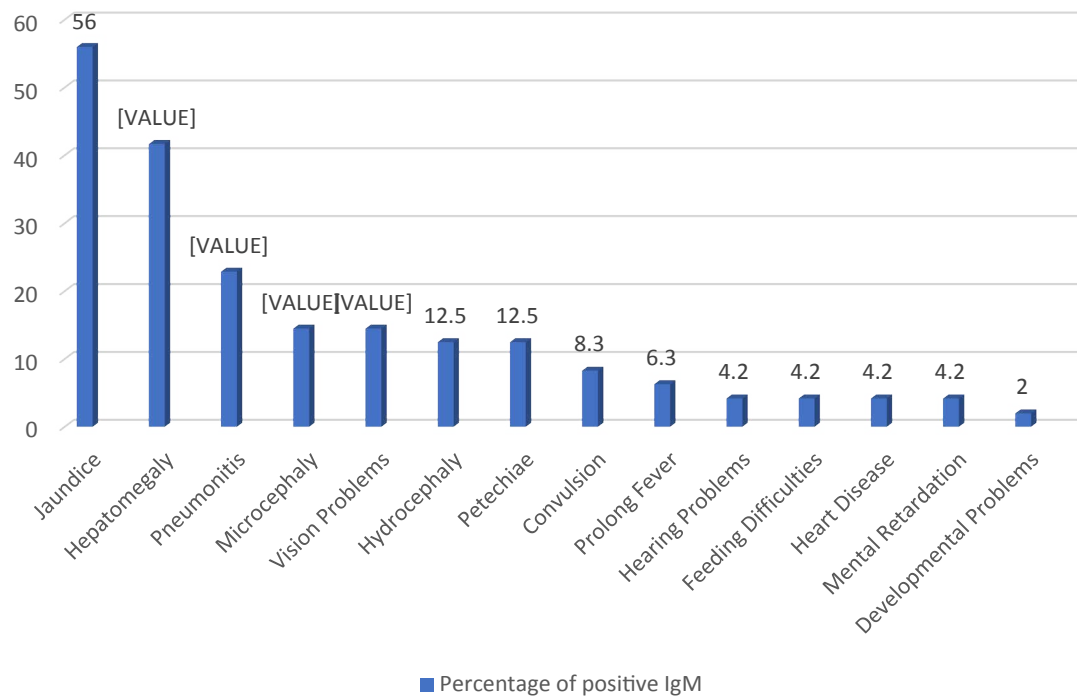


Figure 1 Clinical finding of HCMV infected children under five, listed in order of percent of positive IgM

DISCUSSION

This study provides the first national estimates of prevalence for HCMV in children less than five years of age. There are three main reasons why it is important to study HCMV infection in children under five years. In the first place, mortality rate of Iraqi children under five years is one of the highest in the middle east region [12]. Second, there was a lack of reliable data on the prevalence of HCMV infection [20]. Third, identifying the active infection in children may aid in the understanding and prevention of viral transmission to pregnant women. Shedding the virus for a long time in the saliva and urine of children after infection appears to be the leading source of primary infection in pregnant women [25].

HCMV infection exhibits significant geographic variability within countries among adults [26]. The observation in this study suggests no association between geographical distribution and prevalence of HCMV infection among children in Iraq under five, which might reflect a homogeneity in Iraqi population.

In the last five years, multiple nationally representative cross-sectional studies with adequate sample sizes were estimated the prevalence of HCMV infection in children by serology or molecular methods. In the United States a study conducted on children from one to five years old found that the seroprevalence of IgM and IgG were 1.1% and 20.7% respectively [27]. In Germany, children and adolescents aged one to seventeen years were screened for

HCMV specific IgG, the seroprevalence was 27% [28]. Another study in Netherlands found that the prevalence of HCMV infection among children with hearing problem from three to five years, using real-time polymerase chain reaction from dried blood spots, was 8% [29]. All of those studies reported a significant difference in HCMV infection according to geographical variability that reflects the difference in race and ethnicity within countries, a difference that not found in the current study. Ethnic and racial diversity highlights differences in the factors that influence the HCMV transmission. Examples include breastfeeding duration and frequency, day-care attendance and childcare arrangements [28,30].

Interestingly, the rate of HCMV infection among children from one month to five years of age, was 4.1%, while prevalence of active infection among neonates in NICU was 8.4%. This result revealed that prevalence of HCMV active infection among neonates was significantly higher than older one-month old children.

This result was an unexpected since many studies have reported increased HCMV infection with age due to additional sources of HCMV transmission [16,29]. The higher rate of active infection among neonates than children in this study may be related to two factors. First, there was a high rate of IgM among pregnant women in Iraq. A review identified 22 studies conducted in Arab countries found that, the highest rates of IgM-anti HCMV 57.2% and 60.2% were reported in Iraq among pregnant women and women with BOH respectively [31]. The higher rate of IgM might indicate that neonates should be considered to have a higher risk of acquired HCMV infection as a consequence of vertical transmission.

Many studies have reported a high risk of viral transmission to fetus and preterm neonates during recurrent infection [32-34]. Second, a preference for toddler home-care rather than day care in our population due to social habits and/or economic reasons may lead to a lower prevalence of HCMV active infection among children from 1 month to 5 years in the current study. One study reported a significant lower prevalence of HCMV excretion among children in home care compared to children in day care centers [35]. These Data may be helpful in identifying the main route of viral transmission to children in our country, informing the most appropriate approach to prevention that needs to be taken by healthcare services that could reduce the rate of HCMV seroprevalence.

Socioeconomic status is an important determinant of health status because of its influence on the prevalence and the incidence of various health-related conditions [36]. Many SES scales have been proposed to measure the SES classes. Selecting SES scales that are most relevant to the outcome of a study is a challenge in the terms of reliability and practicality [37]. The reasons behind selecting the Kuppaswamy scale in the current study as a measurement for SES levels are the following:

- 1) It is difficult to investigate SES by income only among families in Iraq because of cultural-based attitudes [38].
- 2) Kuppaswamy scale is widely used in communities [36].
- 3) The scale includes education as one of the variables that is used for SES measure. In addition, to income and occupation, which make the scale relevant to the outcome of the study, since good hygiene practices and attitudes, that taught in school are likely to reduce the risk of HCMV transmission [39,40].

Thus, an uneducated head of the family with a high income will not be in the highest SES level, even though he has high score of income and can afford good health care [37].

In the current study, a significant statistical association was found between HCMV infection and SES level, with higher rates of incidence among lower SES level. A similar observation was reported by Voigt et al. in Germany and de Vries et al. in Netherlands, they found HCMV acquisition significantly associated with low SES in children [28,29]. Another study in the United States, conducted on general population including children reported that low household income was a risk factor for HCMV acquisition [41]. In contrast, two studies found lower seroprevalence among lower SES levels [30,42]. The results of these two studies may be due to the small sample size. It seems that only a large cohort study might show the factors that influencing the prevalence of IgM-Anti HCMV [43].

The present study suggests an association between lower SES levels and HCMV acquisition in children under five years of age. Children from families with low SES levels may be more likely to have larger families and experience crowded, unsanitary living conditions, leading to more exposure of young children, facilitating viral transmission by close contact.

In the present study, a significant statistical association was found between HCMV infection and BOH. In Kirkuk, Iraq, Al-jumaili, et al. in 2013 reported that IgM was significantly higher (7.2%) among women with BOH than control groups (5.3%) [38]. In Baghdad, Iraq, Tuma, et al. reported that 12.4% of women with BOH had the HCMV IgM antibody [43]. It is more likely that an active HCMV infection in women with BOH provides a source for infection to their children during pregnancy and both studies reported less rates than the current study. However, not all current maternal infection leads to fetal transmission [44]. The risk of viral transmission prenatally during delivery and through breast milk is documented [45]. In addition, there is a risk of postnatal viral transmission to infants and young children of currently infected mothers through close contact [46].

It should be emphasized that all children labelled as “symptomatic” were found with the usual signs and symptoms that overt HCMV congenital and acquired infection at the time of sample collection. In the current study, 15.9% of symptomatic children have positive specific IgM. Approximately close result was reported 16.1% in Baghdad by Habib, et al. among symptomatic infants using specific IgM for detection [47]. A lower result of 6% was recorded among symptomatic neonates in Iraq previously by Al- Ali and coworker in 1995. They conducted their work on cord sera only, so their results reflected the congenital HCMV infection only [48]. While the same author in 1999 reported 12.3% of HCMV infection among symptomatic live-born infants in Mosul, Iraq by using specific IgM as a screening test [17]. Lower results have been reported for positive IgM (11.7%) among symptomatic children in Palestine [49] and 1.6% among symptomatic infants in Iran [50]. Lower results (8%) have been reported also in Netherland among symptomatic children, even when the HCMV infection detection was made by using dry blood spot test, so their results reflect the active current infection and latent infection [29]. Higher results (20%) of HCMV infection as indicated by specific IgM among symptomatic infants were found in India [51]. The higher burden of HCMV disease in the current study in compare with the results of studies from other countries (except India) may be referred to the considerable rate of symptomatic congenital HCMV infection and reactivation or reinfection of HCMV in children with developmental sequelae of previous congenital or neonatal infection. However, a study involved Arab population reported that IgM-Anti HCMV was the highest among Arab countries [31]. The variation of congenital HCMV epidemiology could be related to the maternal seropositivity, because women of childbearing age, who are HCMV seronegative are at major risk of giving birth to infants with symptomatic congenital infection if primary infection is acquired during pregnancy [17].

In the present study, (0.9%) of children without clinical suggestion for HCMV infection were proved to have positive specific IgM. Approximately similar finding was reported (1%) in Baghdad, Iraq by Habib, et al. among asymptomatic infants using specific IgM for detection [47]. Positive IgM was also reported 1% in other study in Iraq among asymptomatic neonate with congenital infection by Al-Ali, et al. [48]. While IgM-Anti HCMV was not detected among asymptomatic children in fifty-three Egyptian and forty-six British children [52], who might result from the smaller sample size taken as compared to the current study.

HCMV-infected children have been described as being usually asymptomatic [9,53]. Additionally, about 90% of newborns with congenital HCMV infection have no clinical symptoms of disease at birth [34]. The association between having positive IgM and clinical manifestations may be due to the limitation of the current study as being hospital-based study. The medical conditions of a portion of those children may lead to development of HCMV disease and symptoms. Many studies reported an association between positive IgM and diseases [54-57]. In addition, the higher percentage of HCMV infection among symptomatic children may point to considerable number of children with developmental sequelae of previous congenital or prenatal infection. Higher burden of HCMV disease in the current study in compare with the results of studies from other countries may enhance magnitude HCMV infection as a health problem in our population.

The variations in the frequency of symptomatic and asymptomatic HCMV infection may raise a question of why some children develop symptomatic disease whereas others remain symptom free. Factors such age, genetics, immune response, route of transmission, and differences in the virulence of the viral strain are probably all related to the clinical outcome either singly or in combination [9,58].

CONCLUSION

In the present study, jaundice was the most frequently noted presenting among symptomatic children with HCMV infection, followed by hepatosplenomegaly. Those results are comparable to that documented in Iraq regarding the

most frequent signs and also the order of percent of positive IgM among symptomatic HCMV infected-infants. Habib et al. reported 65.2% for jaundice followed by 41.1% for hepatosplenomegaly [47]. Similar finding was reported in Palestine among hospitalized HCMV infected children regarding the most frequent clinical findings. Hepatosplenomegaly was the first sign in order percent of positive IgM 60% followed by jaundice 30% [49]. The variation in the order percent of positive IgM of jaundice and hepatosplenomegaly between hospitalized symptomatic HCMV-infected children in that study and the present study may be related to the variation in HCMV strains. Two studies have shown that gB1 genotype is associated with hepatosplenomegaly [59,60]. Jaundice was reported in 70% of symptomatic HCMV-infected infants by Boppana, et al. and his colleagues [61].

DECLARATIONS

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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