World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.008

THE ANTI-HUMAN HERPESVIRUS TYPE 6 IGM POSITIVITY RATE IN CHILDREN WITH ROSEOLA INFANTUM IN DIYALA PROVINCE

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Article Received on 09/09/2018 **Article Revised on 30/09/2018** **Article Accepted on 21/10/2018**

ABSTRACT

Background: Human herpesvirus type 6 (HHV-6) has been shown to infect almost all children by 4 years of age. Primary infection causes an undifferentiated febrile illness, with approximately 30% of children exhibiting the classic clinical manifestations of roseola infantum. **Objectives:** The current study was design to explore the anti-HHV-6 IgM as a marker of recent infect ion among children with roseola infantum and the effect of certain child and mother characteristics. **Materials and methods:** This is a cross sectional study conducted in Diyala province during the period from August 2017- July 2018. A total of 180 children who were clinically suspected as having roseola infantum were enrolled. They aged 1-24 months with a mean age ± SD 12.23 ± 6.11 months, consisting of 105 males with mean age ± SD 11.90 ± 5.91 months and 75 female with mean age ± SD 12.94 ± 6.41 months. Venous blood samples were collected. Sera were separated and tested for the anti-HHv-6 IgM (Sunlong Biotech, China) by Enzyme Linked Immunosorbant assay (ELISA) technique. Statistical analysis was done using SPSS version 23 and P value < 0.05 was considered significant. **Results:** The overall anti-HHV-6 IgM positivity rate among children with clinically suspected roseola infantum was 45.6% and the highest IgM positivity rate was insignificantly higher among the age group 1-6 months (P= 0.958). Again, the positivity rate was insignificantly higher among males compared to females (49.5% *vs* 40.0%, P= 0.207). Additionally, the positivity rate was insignificantly higher among children on breast milk compared to other feeding categories (P= 0.217). Furthermore, children who had negative history of hospitalization had higher but insignificant positivity rate compared to their counterpart (46.0% *vs* 43.9%, P= 0.809). Ultimately, an insignificantly higher positivity rate was found among children whom their mother complain an infection during pregnancy period compared to those with negative counterpart (48.5% *vs* 44.9%, P= 0.709). **Conclusion:** Detection of anti-HHV-6 IgM is fundamental beside the clinical features for the primary diagnosis of roseola infantum in children.

KEYWORDS: Human herpesvirus-6, Roseola infantum, Diyala.

INTRODUCTION

The HHV-6 is double stranded DNA enveloped virus first isolated in 1986. Like other human herpesviruses, HHV-6 is ubiquitous and capable of establishing a lifelong latent infection of its host particularly infants and young children with subsequent viral reactivation in the immunocompromised host (Miszczak *et al.*, 2013). The HHV-6 infection is usually acquired very early in life, between 6 months and 2 years of age, following the loss of protective maternal antibodies (Tesini *et al.*, 2014). Additionally, congenital infection following intrauterine transmission has been reported for about 1% of children (Hall *et al.*, 2006). The HHV-6A and HHV-6B are ubiquitous viruses detected in all human populations around the world, as reviewed by (Knipe *et al.*, 2013). HHV-6 infection is detected in more than 90% of adult populations in developed countries, with significant differences according to geographic location, age, and sensitivity and specificity of serologic assays (Ward *et al.*, 2005).

Roseola infantum (RI) is a common disease of childhood caused by a primary infection with HHV-6 and less frequently, by HHV-7, it also known as exanthema subitum or the sixth disease, because it ranks as the sixth condition following measles, scarlet fever, rubella, Duke's disease, parvovirus B19, in causing skin rash in infants. RI is an acutely developed high fever and often a febrile seizure, followed by a rapid defervescence after 3 days and a morbilliform rash appears predominantly among 9- to 12-month-old infant (Tesini *et al.*, 2014; Arnez *et al.*, 2016). Transmission occurs most frequently

through the shedding of viral particles into saliva. Studies reported varying rates of HHV-6 in saliva (3% - 90%) and have also described the salivary glands as an *in vivo* reservoir for HHV-6 (Suga *et al*., 1998).Vertical transmission was also described, and occurs in approximately 1% of births (Hall *et al*. 2006*;* Flamand *et al*., 2010).Direct close contact is required for transmission, supported by the observations that having older siblings and parents who share saliva are associated with virus acquisition (Zerr *et al*.,2005, Rhoads *et al*.,2007). The HHV-6 viral DNA was identified in nasal mucosa and olfactory bulb specimens (Brenda *et al*., 2014).

In children with primary infection, anti-HHV-6 antibody is detectable from 3–7 days (Dockrell *et al*., 1999). IgM production peaks in the second week and continues detectable for 2 months after infection. IgG antibodies rise by 2 weeks post infection and are detectable for life in 90% or more of adults (Yamanishi *et al*., 1988; Robinson, 1994). In contrast, in children who experienced HHV-7 followed by HHV-6 infection, the IgM response was firstly directed against HHV-6, suggesting that cross-reactive responses to heterologous virus infection (Yoshida *et al*., 2002). Since no previous studies on HHV-6 as a causative virus of RI among infants in Diyala province, this study was suggested which is also aimed to explore the effect of certain child and mother characteristics on the positivity rate.

PATIENTS AND METHODS

This is a part of larger cross sectional study conducted in Diyala province during the period from August 2017-July 2018. A total of 180 children who were clinically suspected as having roseola infantum from those attending the primary healthcare centers were enrolled. They aged 1-24 months with a mean age ± SD 12.23 ± 6.11 months, consisting of 105 males with mean age ± SD 11.90 ± 5.91 months and 75 female with mean age ± SD 12.94 ± 6.41 months. Venous blood samples were collected. Sera were separated and kept frozen at -20 ^{0}C till testing. The anti-HHv-6 IgM (Sunlong Biotech, China) was applied for all sera by Enzyme Linked Immunosorbant assay (ELISA) technique. Human privacy was respected by taken child's parent consent. Statistical analysis was done using the Statistical Package of Social Science (SPSS) version 23 and P value < 0.05 was considered significant.

RESULTS

The results showed that the overall anti-HHV-6 IgM positivity rate among children who were clinically suspected as having roseola infantum was 45.6% (82/180), table (1) also showed that the highest IgM positivity rate was among the age group 1-6 months (50%) compared to other age groups. The difference among these age groups was statistically insignificant compared to reference group (P= 0.958, P=0.326, P= 0.958 and P= 0.435) respectively.

Table 1: Anti-HHV-6 IgM positivity rate by age groups of patients.

Age group (Ms)	Total No.	Anti- IgM positive (%)	Odd ratio	Inverse OR	95% CI	P value	PF
1-6	30	15 (50%)	Ref	1.02	(0.43 - 2.24)	0.958 [NS]	0.011
7-12	89	44 (49.4)	0.98	1.70	(0.2 - 1.7)	0.326 [NS]	0.206
13-18	27	10 (37)	0.59	1.62	(0.23 - 1.68)	0.345 [NS]	0.190
19-24	34	13 (38.2)	0.62	1.02	(0.43 - 2.24)	0.958 [NS]	0.011
Total	180	82 (45.6)					

The distribution of anti-HHV-6 IgM positivity rate by gender revealed that the positivity rate was insignificantly higher among males (49.5%) compared to females (40.0%) with a statistically insignificant difference (P= 0.207), table (2).

Table 2: Anti-HHV-6 IgM positivity rate by gender of patients.

Gender	Total No.	Anti- IgM positive (%)	Odd ratio	Inverse OR	95% CI	P value	EF
Female	75	30 (40.0)	Ref				
Male	105	52 (49.5)	1.47	**	(0.81 - 2.68)	0.207[NS]	0.159

The distribution of anti-HHV-6 IgM positivity rate according to the type of feedings was revealed that the highest positivity rate was found among children who were fed on breast milk (54.0%) compared to other feeding categories; However, the difference was statistically insignificant (P= 0.217 and P= 0.206) respectively, table (3).

Table 3: Anti-HHV-6 IgM positivity rate by type of feeding.

Type of Feeding	Total No.	Anti- IgM positive (%)	Odd ratio	Inverse OR	95% CI	P value	PF
Breast feeding	50	27 (54.0)	Ref				
Bottle feeding	86	37 (43.0)	0.64	1.55	(0.32 - 1.3)	0.217[NS]	0.193
Mixed feeding	44	18 (40.9)	0.59	1.70	(0.26 - 1.34)	0.206[NS]	0.222

Table (4) revealed that the highest positivity rate was among those who had negative history of hospitalization compared to their counterpart (46.0% *versus* 43.9%), but the difference was failed to reach the levels of statistical significance (P= 0.809).

Table 4: Anti-HHV-6 IgM positivity rate according to previous hospitalization.

Previous hospitalization	Total No.	Anti- IgM positive (%)	Odd ratio	Inverse OR	95% CI	P value	PF
Negative	139	64 (46.0%)	Ref				
Positive	41	18 (43.9%)	0.92	1.09	(0.45 - 1.85)	0.809 [NS]	0.038

It is obvious from table (5) that the higher positivity rate was found among children whom their mother complain an infection during pregnancy period compared to those with negative counterpart (48.5% *versus* 44.9%); However, the difference was statistically significant (P= 0.709).

Table 5: Anti-HHV-6 IgM positivity rate according to history of maternal infection.

Maternal infection	Total No.	Anti- IgM positive (%)	Odd ratio	Inverse OR	95% CI	P value	EF
Negative	147	66 (44.9)	Ref.				
Positive	33	16 (48.5)	1.16	**	(0.54 - 2.46)	0.709[NS]	0.065

DISCUSSION

The primary outcome of this study is that the overall anti-HHV-6 IgM positivity rate, as detected by ELISA technique, among children who are clinically suspected as having roseola infantum was 45.6%, and the highest infection rate (71.9%) was among children 1-12 months old. It was well documented that roseola infantum is a common sporadic disease or even outbreaks of childhood caused by a primary infection with HHV-6 and less frequently, by HHV-7 (Freitas *et al*., 2000; Stone *et al*., 2014). Furthermore, there was a wide variability in HHv-6 infection around the world, with significant differences according to geographic location, age, and sensitivity and specificity of serologic assays (Ward *et al*., 2005; Knipe et al., 2013). Several previous studies are consistent with the present one, in that the most primary infection with HHV-6 occurred in early life between 6 months and 2 years (Ongradi *et al*., 1999; Ward, 2005; Tesini *et al*., 2014). In this regard, HHV-6 was found as the cause of roseola infantum in 40 % of children by the age of 12 months, 77 % by the age of 24 months, and the peak age of acquisition was 9 - 21 months, concluding that acquisition of HHV-6 in infancy is usually symptomatic (Zerr *et al*., 2005). In a Brazilian study, 43.5% of the children had primary HHV-6 infection, the age of onset was peaked at 6-11 months and 75% of infection occurred in children between 6 and 17 months (Vianna *et al*., 2008).

It is worthy to mention that in patients with primary infection, the serologic studies had shown the appearance of specific HHV-6 IgM antibodies during the In primary HHV-6 infection, the IgM antibodies appeared 5 to 7 days after onset of exanthem subitum, reached maximum titers at 2 to 3 weeks, and tended to decline to undetectable levels after 2 months., while IgG antibodies are detected later and persist indefinitely (Suga *et al*., 1992; Flamand *et al*., 2014; Becerra *et al*., 2014). Confirming that, the anti-HHV-6 IgM was efficiently used as a marker of recent infection in children since several studies had affirmed the high sensitivity, specificity, accuracy and validity of anti-HHV-6 IgM immunofluorescence assay for detection of primary infection (Ward et *al*., 2002; Vianna *et al*., 2008). Furthermore, the anti-HHV-6 ELISA technique was found to be highly predictable without cross reactions to EBV or CMV IgM positive antibodies (Nielsen and Vestergaard, 2002; Agut *et al*., 2015).

It is unsurprisingly to find that the primary HHV-6 infection is peaked in children after the 6 months of age as most of previous studies affirmed that (Cermelli *et al*., 1996; Stone *et al*., 2014; Tesini *et al*., 2014). This is largely related to the cessation of transplacently transferred maternal antibodies. Moreover, it is worthy to mention that the maternal transferred neutralizing antibody against HHV-7 were higher and remained longer after birth than those of HHV-6, and these findings were in accord with the clinical observation that HHV-6 infection usually occurs earlier than HHV-7 infection (Cermelli *et al*., 1996; Yoshida *et al*., 2002). Probably the presence and shedding of HHV-6 in the saliva of older siblings and parents may have a role for dissemination and initiate the primary infection of HHV-6 in the newly born infants (Suga *et al*., 1998; Miyazaki *et al*., 2017).

The remaining infants who expressed clinical signs of roseola infantum but their results for HHV-6 IgM were negative are probably infected with HHV-7 or less commonly other variant of HHV-6 (Ward, 2005; Magalhaes *et al*., 2011; Stone *et al*., 2014; Agut *et al*., 2017). In this regard, it was documented that the clinical manifestations of primary and reactivated HHV-7 infections were similar, except that seizures occurred more frequently in reactivated infections, suggesting that HHV-7 viremia could represent primary or reactivated infection and may be affected by the interaction between HHV-6 and HHV-7 (Hall *et al*., 2006). Thus a molecular study to discriminate these viruses in infants with primary infection of roseola infantum is recommended.

Concerning the gender, the HHv-6 IgM positivity rate was found to be insignificantly higher in male versus female. Similar results were obtained by other workers (Ongradi *et al*., 1999). On the contrary, other studies had reported dissimilar results (Fre+itas *et al*., 2004; Zerr *et*

al., 2005). Furthermore, the present study found that infants on breast feeding had slightly higher HHV-6 IgM positivity rate versus other types of feeding. It was previously reported that HHV-6 transmission through blood transfusion and breast feeding had never been reported to be origins of primary infections (Kusuhara *et al*., 1997). Thus, the increased HHV-6 infection rate among breast feed infants may be related to bad maternal sanitation. Ultimately it can be concluded that detection of HHV-6 IgM is fundamental beside the clinical picture for the diagnosis of roseola infantum in infants.

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