Presence of Japanese Encephalitis Virus Specific IgM and IgG Antibodies in Suspected Pediatric Febrile Illness Cases in South Western Saudi Arabia - A Transitory Experience

Saleh M. Al-Qahtani* Ayed A. Shati* Riyad Al-Moosa** Ali Alsuheel Asseri* Youssef A. Alqahtani* Asma Muhyi *** Ahmed M. Al-Hakami** Ibrahim Al zaydani Asiri **** Harish C. Chandramoorthy**

ABSTRACT

Presence of Japanese encephalitis (JE) have not been documented in Saudi Arabia. There has been no systemic screening of JE in both vectors (Mosquito) and vulnerable populations. In the current study for the first time in southern Saudi Arabia, JE virus specific IgM and IgG antibodies were evaluated in blood samples from children attending emergency room, Abha Maternity and Children hospital. The results showed 20% seropositivity for JE with 19 (17.7%) IgM and 2(1.8%) IgG. There was no double positivity of IgM or IgG reported. The mean age for JE seropositive were 3 and 2 for male and female respectively. Convulsion was one single statistically significant clinical presentation that was observed in JE seropositive cases compared to the un-known viral etiology group. Paralysis and altered consciousness were only observed in JE seropositive individuals with majority of cases showing JE positivity from 1 to 3 days' date of illness compared to 1 day to 4 weeks among un-known viral etiology individuals. From the results it is evident that there is presence of JE among the community causing febrile illness and can be escalated to be screened in suspected viral meningitis and encephalitis cases. Further investigation including cerebrospinal fluid (CSF) and larger seroprevalence study can throw light on the incidence and prevalence of the JE in this part of the Kingdom.

Keywords: JE, Japanese encephalitis, Seropositivity, JE IgM, JE IgG, Prevalence

INTRODUCTION

The Japanese encephalitis virus (JEV) is known to be well associated with encephalitis[1] and meningitis[2] throughout Asia, western Pacific countries and northern Australia[3]. It belongs to genus Flavivirus with positive strand RNA[4]. Most of these viruses are vector borne with other common features like symmetry, size etc. Most of the Flavivirus are associated with encephalitis or meningitis with ordinary febrile illness[5]. The presence of JEV in common population or vectors or reported case of encephalitis have not been reported or much documented in Middle East particularly Saudi Arabia[6,7]. To add, the presence of mosquito *Culex tritaeniorhynchus* known to be the vector for the transmission of JEV have been reported in many provinces of Saudi Arabia with highest infestation in Jazan without much investigation as host for JEV rather it has been implicated to be the vector for Rift Valley Fever (RVF) virus[8]. The presence of JEV has been widely reported in endemic areas of other Flavivirus in which Saudi Arabia has reported WNV, RVF, tick borne encephalitis virus etc[9]. There is no report on the laboratory diagnosis of JE (Japanese encephalitis) among pediatric or adult suspected encephalitis cases in Saudi Arabia[10]. Most of the available literature represents human herpes virus (HHV), enterovirus[11] and other flaviviruses like west Nile virus or Alkhumra virus etc[12,13] associated with encephalitis or aseptic meningitis cases. JEV was not given much attention though there is abundant presence of amplifying host (Culex mosquitos) in the south western Saudi Arabia due to its large land area under agriculture and water bodies[14]. Further, reason for JEV getting not much attention among clinicians was to its asymptomatic infections resulting in non-specific febrile illness to encephalitis in worst cases. Therefore, it was decided to screen JEV specific IgM and IgG antibodies in children visiting Pediatric clinic during late winter in Abha.

MATERIALS AND METHODS

Study Design: The current transitional study was conducted over four months during late winter period (January to April 2021) at Abha, capital of Asir province, south western Saudi Arabia. The sample population were from pediatric age group (birth to 14 years of age)[15] who visited with various clinical symptoms at emergency room. The study was approved by the Research Ethics Committee at King Khalid University (HAPO-06-B-001) vide approval # ECM#2020-3310 dated 04/01/2021. Written informed consent was obtained from the patients' parents following the relevant national regulations and institutional policies. No compensation was provided to the study participants. Clinical history was collected using questionnaire designed for this study.

* Department of Child Health
College of Medicine, King Khalid University
Abha Saudi Arabia.
E-mail: smuadi@kku.edu.sa
** Department of Microbiology & Clinical Parasitology
College of Medicine, King Khalid University
Abha, Saudi Arabia.
*** Department of Pediatrics
King Khalid University Medical City
Abha, Saudi Arabia.
**** Pediatric Infectious Diseases Unit,
Department of Pediatrics, Abha Maternity and Children Hospital, Abha, Saudi Arabia

Blood samples were collected by qualified pediatric nurse and were allowed to clot for 30 minutes at room temperature. The samples were then centrifuged at 1000 X g speed for 10 minutes and serum were separated, aliquoted and stored at -80 °C till further analysis. The samples were analyzed for specific antibodies of IgM and IgG origin against JEVby using commercially available IgM and IgG ELISA kits (MyBioSource company, CA, USA).

JEV IgM ELISA

50 µL of negative, positive controls and diluted serum samples (1:5) were added to test wells with one well was used as a blank. The plates were incubated for 30 minutes at 37 ℃ followed by 5 cycles of washing. 50 µL of horse radish peroxidase were added to all wells except blank and were incubated 30 minutes at 37 ℃. The wells were washed as before followed by addition of 100 µL of substrate mixture and incubated for 15 minutes at 37 ℃. Reaction was stopped by addition of 50µLof stop solution to all wells and the plates were read at 450nm using ELISA reader, Humareader (Human, Wiesbaden, Germany). The patients' results were calculated as a cut off value (Negative control optical density+0.15). If the patient's optical density is < cutoff value, the patient is considered positive while if patient optical density is less than cutoff value it is considered negative.

JEV IgG ELISA

100 µL of negative, positive controls and diluted serum samples (1:10) were added to test wells with one well left as blank, and incubated for 30 minutes at 37 ℃ followed by 5 cycles of washing. 100 µL of horse radish peroxidase were added to all wells except blank and were incubated 30 minutes at 37 ℃. The wells were washed as before followed by addition of 100 µLof substrate mixture and incubated for 15 minutes at 37 ℃. Reaction was stopped by addition of 50µL of stop solution to all wells and the plates were read at 450nm using ELISA reader, Humareader (Human, Wiesbaden, Germany) The patient's results were calculated as a cut off value (Negative control optical density+0.1). If the patient's optical density is < cutoff value, the patient is considered positive while if patient optical density is less than cutoff value it is considered negative.

Statistical Analysis

Variables of definite origin were presented in numbers (n) and percentage (%) whereas, continuous variables were presented with median since the number of samples were limited. Chi-square univariate analysis testing was performed and *p*-value and significance were calculated using the Fisher exact test for definite variables.

RESULTS

The suspected cases were clinically examined for serology of JE viral etiology. Other cases which turn out to be bacterial or other systemic diseases were excluded from the current screening. Out of 107 samples collected, 66 (62%) were from male and 41 (38%) from female children. 21 (20 %) samples tuned out to be positive for both JE IgM and IgG while rest 86 (80%) were classified as samples of unknown viral etiology (Table 1). This was first time an investigation for JE serology was undertaken in this part of country with 20% positivity. There was no double positive sample reported from the current observation. Out of 21 JE seropositive samples, 11 (17%) were male and 10 (24%) were female (Table 1). Mean age for JE seropositive were 3 and 2 years for male and female respectively while a mean age of 5 years were recorded for samples from un-known viral etiology (Table 1). Only two samples were positive for JE IgG compared to the 19 JE IgM positives (Table 2). There was no significant correlation observed with short date of infection (DOI) compared to the results of IgM or IgG positivity from our results.

Table 1: Demographics of the study individuals from whom blood samples were collected male and female distribution

Demographics	Male (N & %)	Female (N & %)
JE Seropositive	11 (17)	9 (22)
Unknown Viral Etiology	55 (83)	32 (78)
	Years	
Mean age - JE	3	2
Mean age - Unknown Viral Etiology	5	5

Table 2: Comparison of symptoms and clinical presentations between JE seropositive cases and other cases of un-known viral etiology

Symptoms	JE (n & %)	Unknown Viral Etiology (n &%)	ChiSq *p*-value	$p<0.05$
Fever	6 (28.6)	24 (28)	0.833533	NS
Coryza	2 (9.5)	2 (2.3)	0.119012	NS
Head ache	NIL	4 (4.6)	ND	ND
Trauma	2 (9.5)	2 (2.3)	0.119012	NS
Convulsion	7 (33.3)	5 (5.8)	0.00034	*
Neck Rigidity	NIL	NIL	ND	ND
Motor weakness	3(14.3)	7 (8.1)	0.385667	NS
Paralysis	1(4.8)	NIL	ND	ND
Hallucinations/ confusion	NIL	NIL	ND	ND
Aphasia	NIL	NIL	ND	ND
Altered Consciousness	1 (4.8)	NIL	ND	ND
Unconsciousness	NIL	NIL	ND	ND
Vomiting	5(23.8)	21 (24.4)	0.953475	NS
Shortness of breath	1 (4.8)	3 (3.5)	0.782693	NS
Abd/Joint/Back Pain	1 (4.8)	6 (7)	0.712876	NS

*Significant $p<0.05$, NS – Non-Significant, ND – Not Done

From the results it is evident that 20% of the cases were JE seropositive (Figure 1) within in which 86% of the cases showed one or more symptoms compared to the 80% of un-known viral etiology showing only 66% with symptoms which was not statistically significant. However, asymptomatic cases were about 14% in JE compared 34% cases of un-known viral etiology which was statistically significant (Figure 1). Most of the JE seropositive cases showed Convulsion compared to the cases of unknown viral etiology (Table 3) which was statistically significant. Other symptoms like fever, vomiting motor weakness, shortness of breath, joint pain etc were observed in both JE and un-known viral etiology cases. It may be noted that paralysis and altered consciousness were only observed in JE seropositive individuals only. Headache was not observed in JE seropositive individuals. Classical presentations of neck rigidity, hallucinations/ confusion, aphasia and unconsciousness were not observed in both the groups in the current study (Table 3). In order to check a comparison of clinical presentation and JE serology results, the DOI was compared, the results showed JE seropositivity in majority cases were well within first three days (Figure 2) of illness while the un-known viral etiology showed a distribution of 1 day to 4 weeks. The comparison however, was not statistically significant.

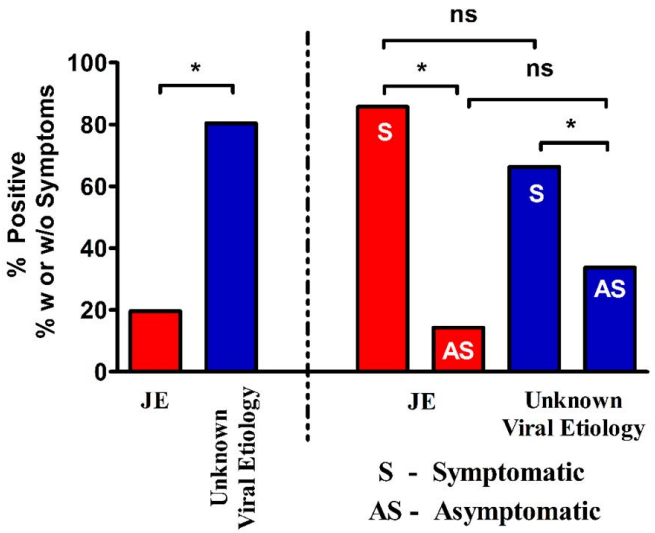

Figure 1: JE seropositivity and comparison of JE seropositive individuals with and without symptoms with cases of un-known viral etiology

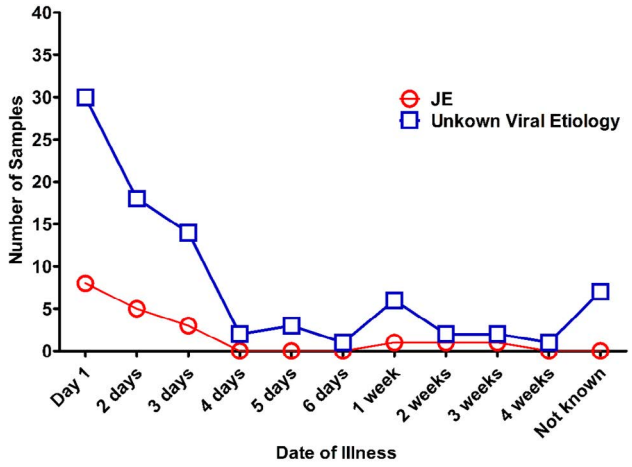

Figure 2: Comparison of Date of onset of illness (DOI) among JE seropositive cases and un-known viral etiology

Table 3: IgM and IgG seropositivity among suspected meningoencephalitis cases

Total suspected	Positives for Viral Specific IgM & IgG (n &%)		
cases (n)	JE IgM	JE IgG	Non-JE
107	19 (17.7)	2 (1.8)	86 (80.5)

DISCUSSION

According to the yearly report of WHO/UNICEF Joint Reporting Form on Immunization (JRF) Saudi Arabia has no reported cases or incidence of Japanese Encephalitis (JE). There is also no historical record that has documented JE in Saudi Arabia. To the best of our knowledge this is the first study done JEV specific antibody screening among pediatric cases. On the other hand, antibody prevalence to West Nile virus (WNV) the other vector borne Flavivirus which share the same environmental features including seasonality and vector of JEV has been reported in this part of the Arabian Peninsula[12]. This observation did not coincide with other studies done elsewhere which too speculates absence of JE while consistent presence of WNV is evidenced[16]. The current screening has shown some seropositivity among pediatric population which warrants further and large investigation across various sections of the populations. To add, the presence of vector Culex mosquitos have been well documented across Saudi Arabia[14] correlates with the JEV specific IgM or IgG seropositivity amongst the study group. Still there are no studies that was undertaken to screen the vectors for JEV has to be noted. Few studies have reported aseptic meningitis and encephalitis[11,17,18] in Saudi Arabia and other areas of Arab world[19]. There is scanty reference to the prevalence of Human Herpes virus (HHV)[10,20], Enterovirus (EV)[21] documented in the aseptic and meningitis and encephalitis cases in Saudi Arabia. However, it may be noted that there is no study pertaining to prevalence of viral etiology especially of Flavivirus in the asymptomatic individuals[22] in much of the Middle eastern countries. Reports from countries like USA and Romania show 1 in 150 flavivirus infected cases turn out to be meningitis or encephalitis while others are asymptomatic[23]. This was the major reason for our investigation of JEV amongst children visiting for pediatric clinic for various ailments with or without clinical signs of meningitis or encephalitis. Seroprevalence rate of 21% in current study was well in agreement with similar study done in India which showed 16% JEV positivity[23] along with other Flavivirus like WNV.

It is to be noted that discussion of anti JEV antibodies does not go alone as it shares cross reactivity with other Flavivirus especially WNV[24]. However, in this investigation the samples were not screened for WNV or dengue or ZIKA viruses as it known that there will be at least 30% samples cross reacting with either one of it[24,25]. Therefore, we took the samples which showed high titer values and was reconfirmed subsequently using another kit (data not shown). Hence the samples have to undergo confirmatory tests usually plaque reduction neutralization test ($PRNT_{50}$) specific for JEV[26]. There are studies which show JEV antibody prevalence in Flavivirus endemic countries at large due to its cross reactivity. The alarmingly high number of IgM seropositive in the current study may be due to cross reactivity or the samples were collected at early stage *i.e* short date of infection (DOI) which could be really asymptomatic JEV infection which requires confirmatory tests both clinically and diagnostically.

CONCLUSION

From the current observation it is clear that there is JEV specific seropositivity in asymptomatic Pediatric cases. The positivity has to be confirmed by JEV specific neutralizing antibodies which was not undertaken while literature evidence shows cross reactivity to JEV with other Flavivirus endemic regions. To add the sample collection, DOI and specific clinical picture in the JEV seropositive cases inclines the presence of JEV in this population and could not just be ruled out as Flavivirus cross reactivity. Therefore, a larger study on JEV specific antibody prevalence among vulnerable population has to be undertaken along with surveillance for JEV in the host. Additionally, investigation among aseptic meningitis and encephalitis for JEV will throw the light on symptomatic cases.

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