Japanese Encephalitis IgM among Patients with Acute Encephalitis in Karachi, Pakistan –Implications of Laboratory Diagnostic Results

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Abstract

Japanese encephalitis (JE) is a mosquito-borne illness and a major cause of viral encephalitis in southern Asia and Southeast Asia. Infection is symptomatic in less than 1% of all infected cases. Past studies from the 1980s identified JE as an infrequent cause of viral encephalitis in Pakistan. We conducted Enzyme-linked immunosorbent assay (ELISA) based laboratory testing for Japanese encephalitis IgM in cerebrospinal fluid (CSF) and serum samples among patients with acute encephalitis presenting to a tertiary care hospital in Karachi, Pakistan. Among 117 patients hospitalized with encephalitis of unknown etiology at the Aga Khan University Hospital whose sera or CSF were tested, 4 (n=4; 3.4%) were positive for JE IgM. Further testing of the samples against dengue and West Nile IgM is warranted to ensure whether these are true positive cases of JE or cross-reactive with other flaviviruses.

Introduction

Japanese encephalitis (JE), a mosquito-borne illness, is a major cause of viral encephalitis worldwide. JE virus (JEV) is a positive sense single-strandedÊ virus belonging to family Flaviviridae, which is maintained in a cycle involving mosquitoes and vertebrate hosts, mainly pigs and wading birds. Infection is symptomatic in less than 1% of all infected cases,[1] however, post-infectious neuropsychiatric sequelae are observed in 30-50% of patients and a high mortality of up to 25% has been reported.[2] The disease is epidemic in temperate regions of Asia and is endemic in many tropical countries in Southeast Asia, with an estimated rate of 67,900 cases reported annually from the region.[3]

According to the WHO, borders of the region of endemicity extend from Western Pacific islands in the east to the Pakistan border in the west. Pakistan itself, however, is considered a 'negligible-risk' region for acquisition of JE.[4, 5] This risk categorization is based on infrequent reports published in 1985 and 1994. In 1983, Sugamata *et al* undertook a seroepidemiological study of West Nile virus (WN) encephalitis (caused by another mosquito-borne flavivirus that is closely related to JE virus) in Karachi and found high JEV antibody titers in 2 out of 156 patients.[6] In 1992, Igarashi *et al* found JE viral genome sequences in 1 of 24 patients with encephalitis from Karachi.[7] High antibody titers have also been reported form healthy volunteers.[8] Indirect evidences of possible JEV activity has been reported very recently by Khan *et al* where 16.1% of cases with suspected West Nile Virus infection showed some neutralizing antibodies for JEV on Plaque Reduction Neutralization Assay (PRNT) therefore it is important to have focused study on JEV as the current situation on the prevalence in southern Pakistan is uncertain.[9]

To detect the presence of JE antibodies among patients with encephalitis hospitalized at Aga Khan University Hospital Karachi, we tested serum and cerebrospinal fluid (CSF) samples for JE IgM.

Materials and Methods

Study Design, Study Setting, Study Period

This was a hospital-based surveillance of acute encephalitis cases, conducted at The Aga Khan University Hospital (AKUH) Karachi during the period of May 2015-August 2017. Laboratory testing was done at the Infectious Disease Research laboratory (IDRL) at the AKUH.

Sample Size

According to a study published in northern India in 2011,[10] Japanese encephalitis viruses (JEV) has been the major cause of outbreaks in the Uttar Pradesh State, accounting for 10% to 15% of total AES cases annually. Assuming an average of 12.5% as the estimated prevalence, a sample size of 117 patients with encephalitis was required determine the presence of JE in our sample, with expected 6% desired level of absolute precision (d), 95% confidence interval, and 5% level of significance.

$$n = \frac{1.96^2\, p\,(1\text{-}p)}{d^2} = n = \frac{1.96^2 \text{x} (0.125)(1\text{-}0.125)}{(0.06)^2} = 117 \text{ Total number of samples}$$

Selection Criteria

Inclusion Criteria

Patients with acute encephalitis fulfilling the following criteria were included: patients of any age, any gender, at any time of year, with the acute onset of fever and a change in mental status

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(including symptoms such as confusion, disorientation, coma, or inability to talk) and/ or new onset of seizures (excluding simple febrile seizures).[11]

Exclusion criteria

Patients with acute uncorrected dehydration, cirrhosis and suspected hepatic encephalopathy or hepatorenal syndrome, uremic encephalopathy, prolonged (> 3 months) undiagnosed systemic illness, known cerebrovascular etiology/ stroke, recent rash (e.g. measles or varicella), and brain or spinal tumors causing neurological deficits were excluded.

Informed Consent

Patients were enrolled after they (or their legal guardians) provided written informed consent. The study protocol was reviewed and approved by the Ethics Review Committee at Aga Khan University.

Laboratory Testing and Diagnostic Criteria

All patients satisfying the inclusion criteria were included in the study by non-probability consecutive sampling. CSF samples were collected in universal containers by the primary physician through lumbar puncture; serum samples were collected, where lumbar puncture was not possible, by phlebotomists through venipuncture. All samples were transported immediately at room temperature to the diagnostic laboratory. All CSF / serum samples were tested for JE IgM antibodies using InBios test JE IgM Capture ELISA as recommended by the manufacturer.

Results

Clinical Cases

Patient presenting to the study hospital from May 2015- August 2017 were recruited and maximum recruitment of cases was seen from August to September. Total 117 patients were recruited according to the clinical criteria and the patients were predominantly male (n=70; 59.8%). Median age of the patients was 19 years (Q3-Q1=37). Majority of the cases belonged to different areas of Sindh (n=110), followed by Baluchistan (n= 4) and Punjab (n= 3). The most common clinical diagnosis encountered upon discharge was meningitis (aseptic and viral both) (n=42; 35.9%) followed by encephalitis/ meningoencephalitis (n=36; 30.8%). Hypertension was a commonly encountered comorbid seen in 23 patients (19.7%) and diabetes in 17 patients (14.5%). Neurological symptoms were accompanied by fever in 71% of patients (n=83), followed by headache in 52% (n=61), and drowsiness in 36% (n=42).

Laboratory Testing

Among the 117 recruited patients, 100 CSF samples and 17 serum samples were obtained and tested. Four (n=4) of these samples (3 CSF and 1 serum) tested positive for JE IgM antibody. CSF culture was negative in all recruited patients and CSF was tested and was negative for Herpes Simplex Virus (1 and 2) nucleic acid in 101 of these patients (86.3%). All the positive cases were residents of Sindh. Median age of the patients with positive results was 56.5 years (Q3-Q1=42.25). All patients with positive results for JE IgM presented to the hospital between August and October. Further patient details are provided in table 1.

Discussion

Our results have established the presence of JE IgM in patients presenting with acute encephalitis in Pakistan. However, it is important to remember that significant antibody cross-reactivity exists between JE and other flaviviruses to warrant additional testing and confirmation of the etiological agent in such cases. Testing should especially be directed toward prevalent infectious agents in Pakistan such as dengue and West Nile virus, which can both cause acute encephalitis. Data available from studies performed in the recent past on patients with acute febrile illnesses in Pakistan reveal other cross-reacting flaviviruses such as the West Nile virus as a common cause.[9]

Similarity in the clinical symptoms along with serological cross-reactivity makes definitive diagnosis of JE difficult. Pakistan

Table 1. Summary of patient characteristics, clinical presentation and outcomes of cases tested positive for JE IgM

	Case 1	Case 2	Case 3	Case 4
Age	70 years	53 years	60 years	16 years
Gender	Male	Female	Female	Male
Presenting complaint	Fever, drowsiness, headache	Fever, drowsiness, headache, neck rigidity	Fever, drowsiness, seizure	Tonic clonic seizures
Comorbid	Hypertension	None	Hypertension	None
Outcome	Discharged	Discharged	Discharged	Discharged
Type of specimen tested	CSF	CSF	CSF	Serum

is endemic for dengue virus, and with increasingly reported WNV, the diagnosis of JE has become challenging due to coexistence of these flaviviruses. With increasing climatic changes, frequent human migration and global warming, various arboviral diseases are on the rise globally, and in Pakistan. It is therefore important to have adequate surveillance for all arboviral illnesses, including the ability to diagnose and differentiate between flaviviral infections in Pakistan.

Methods available for the definitive diagnosis of JE are limited. RT-PCR is not valuable in diagnosis of JE as viremia is usually low-level and transient. Therefore, confirmation of these preliminary results requires a testing algorithm including testing for Dengue virus IgM, Westnile virus IgM, to exclude cross reactivity, and the reference Plaque Reduction Neutralization Test (PRNT) for these flaviviruses.[12] We plan on performing further confirmatory testing in the near future.

Given the lack of peripheral and intermediate level laboratory diagnostic services for extensive serological and virological testing, JE and other arboviral encephalitides remain difficult diagnoses to establish as a cause of infectious syndromes. To inform the true prevalence and risk posed by arboviruses and flaviviruses, active human and animal surveillance along with mosquito-burden studies should be undertaken.

Conclusion

The current prevalence and burden of JE in Pakistan remains uncertain due to significant cross-reactivity of JE IgM with other flaviviruses. Laboratory diagnostics for flavivirus encephalitis are complex and need confirmation by more sophisticated procedures such as PRNT. Further testing with these laboratory methods is planned.

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References

1. Waughn, DW, Barrett A, Solomon T. Flaviviruses (Yellow fever, Dengue, Dengue Hemorrhagic fever, Japanese encephalitis, West Nile encephalitis, St Louis encephalitis, Tick-borne encephalitis. In: Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7 th Edition. 2010 Churchill Livingstone Philadelphia PA. pp 2133-2146
2. Japanese encephalitis. In: Atlas of Human Infectious Diseases, First Edition. Heiman F.L. Wertheim, Peter Horby and John P. Woodall. 2012 Blackwell publishing. Pp 229
3. Campbell GL, Hills SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 2011; 89:766–74E.
4. Centers for Disease Control and Prevention (CDC). Chapter 3. Japanese Encephalitis. In: The Yellow Book—CDC Health Information for International Travelers 2012. Available at: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3- infectious-diseases- related-to travel- japanese-encephalitis.htm
5. ReliefWeb report. http://reliefweb.int/map/pakistan/pakistan-baseline-japanese-encephalitis- risk-non- indigenous-personnel- jan-2010
6. Sugamata M, Ahmed A, Miura T *et al*. Seroepidemiological study of infection with West Nile virus in Karachi, Pakistan, in 1983 and 1985. *J Med Virol* 1988 Nov; 26(3):243-7
7. Igarashi A, Tanaka M, Morita K *et al*. Detection of West Nile and Japanese encephalitis viral genome sequences in cerebrospinal fluid from acute encephalitis cases in Karachi, Pakistan. *Microbiol Immunol* 1994; 38(10):827-30
8. Hayes CG, Baqar S, Ahmed T, Chowdhry MA, Reisen WK. West Nile virus in Pakistan. 1. Sero-epidemiological studies in Punjab Province. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1982 Jan 1;76(4):431-6.
9. Khan E, Barr KL, Farooqi JQ, Prakoso D, Abbas A, Khan ZY, Ashi S, Imtiaz K, Aziz Z, Malik F, Lednicky JA. Human west nile virus disease outbreak in Pakistan, 2015–2016. Frontiers in public health. 2018 Feb 27; 6:20.
10. Fulmali PV, Sapkal GN, Athawale S, Gore MM, Mishra AC, Bondre VP. Introduction of Japanese encephalitis virus genotype I, India. *Emerging Infectious Diseases* 2011 Feb; 17(2):319.
11. Solomon T, Thao TT, Lewthwaite P, Ooi MH, Kneen R, Dung NM, White N. A cohort study to assess the new WHO Japanese encephalitis surveillance standards. Bulletin of the World Health Organization. 2008; 86:178-86
12. Wong SJ, Boyle RH, Demarest VL, Woodmansee AN, Kramer LD, Li H, Drebot M, Koski RA, Fikrig E, Martin DA, Shi PY. Immunoassay targeting nonstructural protein 5 to differentiate West Nile virus infection from dengue and St. Louis encephalitis virus infections and from flavivirus vaccination. *J Clin Microbio* 2003 Sep 1;41(9):4217-23.