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Is dengue virus neurotropic?

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Abstract

Dengue viral infection is on the rise in tropical urban and semi-urban parts of the world. WHO fact sheet- N°117 updated in May 2015 estimates 390 million dengue infections per year and that 3900 million people, in 128 countries, are at risk. The WHO case definition of severe dengue includes organ impairment, but does not describe neurological manifestations as a separate entity. Earliest reports on neurological manifestations came from Indonesia 35 to 40 years back. Abundant literature is available on diverse neurological manifestations in dengue from India and other parts of the world. Encephalopathy, aseptic meningitis, papilledema, stroke, intracranial and sub- arachnoid haemorrhage (ICH/ SAH), Acute Disseminated Encephalomyelitis (ADEM), opsoclonus- myoclonus syndrome, myelitis cranial nerve palsies, polyneuropathies, Guillain-Barre syndrome (GBS), polymyositis, and hypokalemic paralysis are all reported. Long-term sequelae recorded are Parkinsonism, dystonias, cognitive changes, behavioural disorders, depression, amnesia, dementia and psychosis. The neurological manifestations in dengue can be grouped into 3 categories – (I) those related to the neuro-myotropic effects of the virus (II) those related to the systemic complications of dengue infection like vascular leakage, hemoconcentration, haemorrhage and dyselectrolytemia; and (III) Post- infectious manifestations like ADEM, encephalomyelitis, myelitis, optic neuritis, GBS and mono and polyneuropathies. Dengue virus belongs to the *flaviviridae* family, several members of which have proven neurotropism and propensity for the sub- cortical nucleii. CNS invasion, either through neurotropism or vascular leakage is supported by inconsistent evidence including animal experiments. Presence of virus and IgM- dengue in the CSF of some encephalopathy cases suggests capability, but not invariability of CNS invasion. Glycosylation of the envelope protein, increased inflammatory cytokines disrupting the blood brain barrier (BBB) allowing free virus access to the CNS and non-specific cellular virus attachment factors are all implicated. Some authors recommend a high index of suspicion for dengue in CNS infections occurring in endemic countries. Three tetravalent live-attenuated vaccines are under development in phase II and phase III clinical trials, and 3 other vaccine candidates (based on subunit, DNA and purified inactivated virus platforms) are at earlier stages of clinical development. Their immunogenicity may be implicated in neurological manifestations in the future.

Keywords: Dengue, Neurological Manifestations, Neurotropism, Systemic complications,ost – infectious complications.

INTRODUCTION

Dengue infection is widespread throughout the tropics in urban and semi- urban localities. The variations in risk are influenced by rainfall, temperature and unplanned urbanization. The virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *A. albopictus*. There are 4 distinct, but closely related, serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) with multiple subtypes being increasingly recognized. Recovery from infection by one provides lifelong immunity. However, cross-immunity to other serotypes is partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue [1]. Dengue virus belongs to the *flaviviridae* family, several members of which like Japanese encephalitis and West Nile Fever have proven neurotropism.

This year, the numbers of dengue cases and deaths in Delhi and surrounding areas have exceeded all previous figures. It is perhaps due to increased transmission combined with better diagnostics and reportage. WHO fact sheet- N°117 updated in May 2015, records that the incidence of dengue has grown dramatically around the world in recent decades and acknowledges that the actual numbers of dengue cases are underreported and many maybe misclassified [1]. One recent estimate indicates 390 million dengue infections per year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically with any severity of disease [2]. Another study, on the prevalence of dengue, estimates that 3900 million people, in 128 countries, are at risk of infection with dengue viruses [3]. The WHO case definition of severe dengue includes organ impairment, but does not recognize neurological manifestations as a separate entity [1]. However abundant literature is available on diverse neurological manifestations in dengue from India and other parts of the world.

Most literature has collective data over a few years without year-wise segregation, despite the well known cyclic nature of dengue serotypes. We undertook a student ICMR project in 2011, on neurological manifestations in adult dengue in-patients and compared data with previous and subsequent years [4].

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Simultaneously we conducted extensive literature review. The strength of our study was that we had year-wise segregation of data for 2009, 2010 and 2011. The weaknesses of our study were that we had only included adult in-patients; not recorded history of previous dengue infection; or time from onset of fever to development of neurological complications. The last would have differentiated viraemic from immunological causes. Inability to identify the serotype and unavailability of dengue PCR or immune-histochemistry for confirmation of the virus in CSF and tissue were institutional limitations. For similar reasons exclusion of other pathogens that cause similar clinical picture like Japanese Encephalitis (JE), herpes simplex virus (HSV), Ebstein- Barr virus (EBV), Varicella Zoster Virus (VZV) polio virus, tick borne encephalitis, leptospirosis, etc; was also not done although co-infections is always a possibility. CSF was examined sparingly due to thrombocytopenia. One fatal case had IgM Dengue positive in the CSF. We found an overall incidence of neurological manifestations of 2.4 and 2.6 % amongst all hospitalized adult dengue cases in 2009 and 2010 respectively. There were no cases in 2011 [4].

MATERIAL AND METHODS

Ours is a tertiary care hospital in Delhi catering to a population of almost 10 lakhs, residing across the National Capital Region of Delhi. Institutional and ethical clearance was obtained. A systematic review of available retrospective institutional data for 2009 & 2010 and prospective collection of data for 2011 between the months of July to December (peak months for occurrence), was undertaken along with extensive literature review. Proportion of dengue cases with neurological manifestations in each year was compared for any statistically significant trend.

Inclusion Criteria: All confirmed dengue in- patient cases in adults and adolescents above 12 years age were included. Diagnosis was based on (a) Clinical case definition i.e. high continuous fever lasting 2–7 days, hemorrhagic tendency and thrombocytopenia < 100,000 cells/µl. (b) Positive IgM- dengue around 5th day of fever. (c) NS1 Antigen positivity where clinical suspicion was high despite negative serology. (d) Only objective evidence of neurological involvement e.g. diffuse/ focal signs, seizures altered sensorium, neuromyopathy and CSF or neuro-imaging abnormalities, in confirmed cases were considered for inclusion.

Exclusion Criteria: (a) Cases that fit the clinical case definition but were negative for both IgM- dengue and NS1 antigen; (b) Those showing IgG-dengue positivity with an absent or weak IgM response (indicating a past infection); (c) Paediatric cases because they came under a different registry; (d) OPD cases which would have been impossible to follow up; and (e) those without objective evidence of neurological manifestations (only headache, bodyache and vomiting) were excluded from the study.

Additional evaluation: Patients with objective neurological manifestations underwent additional neuro-diagnostic evaluation with relevant investigations like cerebrospinal fluid (CSF) study, computerised tomogram (CT) magnetic resonance imaging (MRI) electroencephalography (EEG), electromyography (EMG) and nerve conduction velocity (NCV) studies, which was part of their routine clinical care. No additional interventions were made solely for this study.

Statistical Method: All data was reviewed along with our institutional biostatistician. Mean with SD was calculated for quantitative data and frequencies with proportions were used for categorical data. Z test for two independent proportions was used for statistical association. P value less than 0.05 was considered as statistically significant.

RESULTS

In 2009, a total of 487 adult in-patients were treated for dengue of which 13 (2.6%) developed neurological manifestations. In 2010, the year of the Commonwealth Games, out of 1481 confirmed adult cases 41 (2.7%) showed neurological involvement. In the same year, amongst 2 cases of encephalitis, one died and this patient's CSF tested positive for IgM dengue. The patient who survived made a gradual clinical recovery. We noted occurrences of acute transverse myelitis, encephalitis, polymyositis, intracranial haemorrhage, hypokalemic paralysis and hyperventilation syndrome in 2009 and 2010. There was no statistically significant increase in neurological manifestations, either individually or collectively in these two years. The cases of intracranial bleeding could well have been hemorrhagic complications. In 2011 total number of confirmed adult dengue admissions was 367, but none showed any neurological manifestation.

DISCUSSION

Neurological manifestations in dengue were recognized almost forty years ago. Sanguansermsermi et al (1976) [5], Sumarmo, et al (1978) [6], and Sumarmo, et al (1983) [7] published the earliest reports. Sumarmo in his second publication described 30 virologically confirmed fatal cases in Jakarta during 1975 -78, without year- wise segregation. All 4 serotypes were isolated, but DEN 3 was responsible for 21 (70%) deaths. Amongst fatalities 70% had encephalopathy (convulsions and somnolence); 3 had spastic tetraparesis and 2 died of viral encephalitis. That far back, he had suggested that this was an entity distinct from DHF or DSS since hemoconcentration and thrombocytopenia were not dominant co- findings.

Review of Indian literature revealed several articles on all complications in dengue. Some included neurological manifestations as a subset [8, 9, 10, 11, 12], while others reported on neurological findings alone [13, 14, 15]. Common neurological complications recorded were encephalitis, aseptic meningitis, polyneuropathies, Guillain-Barre syndrome (GBS), polymyositis, myelitis, stroke and intracranial haemorrhage (ICH). Long-term sequelae were Parkinsonism, dystonias and cognitive changes. In the past 3 decades numerous publications from endemic countries like Thailand, Indonesia, Vietnam, Srilanka, Brazil, Mexico and India were found. These are probably due to increased awareness, better diagnostics, and a higher index of suspicion [14, 15, 16, 17, 18, 19, 20, 21]. Overall incidence of neurological manifestations in hospitalized dengue cases as per several national and international reports varies between 0.5% and 6.2% [17, 19, 22, 23, 24, 25, 26, 27].

In 2013, amongst 74 ELISA positive dengue cases, the highest recorded incidence (11.11%) of neurological manifestations was reported from Eastern India. Additional unilateral VII and VI cranial nerve palsies were reported in 3 cases. Other infective causes like HSV, JE, VZV, polio and EBV were not excluded. This might account for the unusually high incidence [11].

A report from South-India recorded all complications of dengue in paediatric ICU admissions during the study-period (2001 to Jan 2003). Encephalopathy was the commonest neurological complication and presented mainly with seizures. Also noted were Acute Disseminated Encephalomyelitis (ADEM), meningitis and subarachnoid haemorrhage (SAH). In all, 24 out of 858 patients had neurological manifestations, bringing the incidence to 0.027%, which was the lowest in literature. Year-wise segregation was not available. Like Sumarmo's findings [7] only a fifth of neurological cases were in shock. There were 9 deaths. Hepatic encephalopathy and cardiac arrest- sequelae were included as neurological manifestations in this study [12].

At a tertiary centre in North India, 26 cases had neurological complications amongst consecutive IgM-ELISA confirmed cases during July 2008 to Sep 2010. Here, we neither found a year-wise segregation, nor the total number of confirmed cases. HSV, JE, VZV and EBV had been excluded, which was strength of this study. A high incidence of brachial neuritis (10 cases) was recorded. Other complications were encephalopathy (4 cases), hypokalemic paralysis (3 cases), acute myositis (2 cases), GBS (3 cases), opsoclonus- myoclonus syndrome (2 cases), and ADEM (2 cases). New features like- neck rigidity, pyramidal signs, papilledema, myoclonus, behavioural disorders and depression were also mentioned. The main post- infectious sequelae were amnesia, dementia and psychosis [13].

Mishra UK and Kalita J (2006) correlated clinical, radiological and neuro-physiological changes in dengue patients with neurological manifestations. Consecutive IgM seropositive cases admitted to a neurology-ward during 2003 -2005 were prospectively evaluated in detail. Additional testing included coagulation profile, serum creatine kinase (CK), CSF, CT/MRI, EEG, NCV studies and needle EMG. Out of 17 patients, 11 presented with encephalopathy and 6 with acute motor weakness. Amongst the patients with encephalopathy; seizures (3 cases), myoclonus (1 case), CSF pleocytosis (8 cases), EEG slowing (8 cases); and MRI involvement of globus pallidus and thoracic spinal cord (1 each) were recorded. In the pure motor weakness group, CK was elevated in 5 cases while EMG and muscle biopsy were consistent with myositis (1 each). Those with pure motor weakness improved completely but amongst the encephalopathy group- 3 died, 2 had partial recovery, one had poor recovery and 5 showed complete recoveries at the end of one month. Gadolinium contrast MRI showed break in the blood-brain barrier (BBB) which probably accounted for inconsistent CSF presence of antigen and antibody in the encephalopathy group. They concluded that the encephalopathy group had more severe illnesses and worse outcomes compared to the pure motor weakness group [14].

Murthy JMK wrote an elegant review article in 2010. He grouped neurological manifestations of dengue into 3 categories based on case reports and institutional experiences from world literature. Category (I) included those related to the neuro-myotropic effects of the virus: encephalitis, meningitis, myositis, rhabdomyolysis and myelitis. Category (II) included those related to the systemic complications of dengue infection: encephalopathy, stroke (both hemorrhagic and ischemic), hypokalemic paralysis and papilledema; Category (III) cases mainly had post- infectious manifestations: ADEM, encephalomyelitis, myelitis, neuromyelitis optica, optic neuritis, GBS, Miller-Fisher syndrome, phrenic and long thoracic neuropathy, occulomotor palsy, maculopathy and fatigue syndrome [16].

N Gupta in ICMR's centenary review article (2012) comprehensively reviewed the work done by Indian scientists. In *Table-1* epidemiological studies identifying dengue virus in different epidemics from 1964 to 2010 were recorded, but the genotype during the 2010 Delhi outbreak was not found. In *Table-2* atypical clinical presentations of dengue infection with relevant references were recorded. *Tables-3, 4 and 5* related to the pathophysiologic changes studied in human and animal experiments and *Table-6* listed candidate dengue vaccines [17].

Solomon and Dung in their historic study published in the Lancet (2000), investigated all patients with suspected CNS infections admitted to a referral hospital in southern Vietnam during 1995 by viral culture, PCR, and antibody measurement in serum and CSF, for dengue and other viruses. They found that out of 378 patients, 16 (4·2%) were infected with dengue. Five additional dengue positive patients with neurologic abnormalities were studied subsequently in whom no other CNS infection was identified. Out of a total of 21 cases, 7 were primary dengue and 13 secondary and one was unclassified. Dengue viruses were detected by PCR in 10 patients and 3 had dengue antibody in the CSF. Twelve of the total 21 cases had no characteristic features of dengue on admission. The most frequent neurological findings were reduced consciousness and convulsions. Encephalitis was seen in 9 cases. No patient died, but six had neurological sequelae at discharge. Phylogenetic analysis implicated DEN 2 strain. They recommended that in dengue endemic areas all patients with encephalopathy should be investigated for this infection, whether or not they have other features of the disease [21].

One West-Indian group- Jackson, Mullings and Bennet in 2008, studied 401 suspected viral CNS infections in an endemic zone. They found a frequency of dengue in 13.5% (54/401) of all CNS infections. Fifty three cases were confirmed by hemagglutination inhibition assay (HI) and IgM ELISA. The virus was isolated in only one case. Neurological features among dengue positive cases included encephalitis in 51.8% (24/54), meningitis in 33.3% (18/54), seizures in 11.1% (6/54) and acute flaccid paralysis in 3.7% (2/54) of cases. They recommended a high index of suspicion of dengue in CNS infections in endemic countries. [22].

Soares and Faria from Brazil (2006), studied neurological and CSF findings of 13 patients with dengue. Seven had encephalitis, two had myelitis and four had GBS. No alteration in CSF was found from 57% of those with encephalitis. Patients with GBS and myelitis showed a CSF–blood barrier dysfunction. The CSF differences may be related to the location of the lesion and multiple mechanisms of the disease in the CNS [23].

Possible mechanisms for Dengue encephalopathy have been attributed to metabolic derangements like hypokalemia, hyponatremia, hepatic encephalopathy, cerebral hypoperfusion, intracranial haemorrhages, cerebral edema and vascular leakage of IgM dengue and the dengue virus into CSF; yet, a subset remains where direct invasion or immunological mechanisms are implicated. Viral CNS invasion, either through neurotropism or vascular leakage have been postulated and confirmed by investigations including autopsy and at times replicated in animal experiments. In these reports, the discovery of virus and IgM-dengue antibody in the CSF of some but not all encephalopathy cases suggests capability, but not invariability of CNS invasion [18, 19, 20, 21].

Varatharaj A, from Oxford (UK), in his review article gave an overview of the clinical spectrum of dengue infection, and examined supporting evidence of dengue encephalitis using 4 main studies where other causes of (HSV, JE, VZV, EBV and tick-borne encephalitis) were excluded. Two of these 4 studies are listed here [14, 21]. He had relied on viral isolation by PCR or NS1 antigen testing on serum and CSF in the first 5 days of fever and IgM Dengue in serum and CSF during the immune phase, preferably on paired sera; and immune-histochemistry on autopsy samples for dengue. He discussed which class of test to use depending on timing and recommended using PCR or NS1 antigen testing in the patient with fever for fewer than five days, and IgM ELISA in patients beyond five days. He further discussed the non- specificity of CT or MRI imaging techniques which are similar in most encephalitides. Breakdown of the blood-brain barrier may be visualized as signal enhancement on MRI with gadolinium contrast. This finding is extrapolated later in the article to postulate vascular leakage as a mechanism for the presence of dengue virus and or IgM dengue in the CSF of some, but not all cases, with neurological manifestations [24].

Overall incidence of neurological manifestations in dengue is varied depending upon the diagnostic criteria used; patient population selected; and exclusion of other infections that mimic the clinical picture [25, 26, 27, 28]. Some series included ICH, hepatic encephalopathy and post cardiac- resuscitation sequelae as neurologic manifestations, while others did not. The tools used for diagnosis – paired/ unpaired serological testing or antigen detection and timing of testing; all influenced diagnostic outcomes as is expected. Different methods of

PCR, with their cut off values; body fluid or tissue tested; and use of immune-histochemistry also influenced results. This was seen as a wide variation in incidence between 0.02 % to 11.11% amongst Indian literature [11, 12, 13, 14, 15]. Apart from exceptional groups, the average incidence in world literature ranged from 0.5% to 6.2%′ depending on the population selected and the year of study [20, 21, 22, 23].

Neurotropism of flaviviruses (JE and West Nile Fever) is established. Neal JW has in a recent publication (Sep 2014) deliberated on what makes flaviviruses neurotropic. This review describes the multiple pathways responsible for the neuroinvasive properties of flaviviruses which have a propensity for sub-cortical nuclei (substantia nigra & thalamus), anterior horn cells and neocortex. Glycosylation of the envelope protein; increased inflammatory cytokines disrupting the blood brain barrier (BBB) allowing infected leucocytes and free virus access to the CNS (Trojan-horse effect); Non-specific cellular virus attachment factors (complex carbohydrate molecules like Glycosaminoglycans, Heparan sulphate, Semaphorin 7A, Low Density Lipid receptors; etc); are all implicated [29].

In order to establish neurotropism of dengue viruses unequivocally the virus or antigen must be isolated from nerve tissue consistently. This is not possible in most clinical settings. Larger longitudinal studies with the depth of research discussed in reference numbers [7, 14, 17, 20, 21, 22, 23, 28] conducted over several successive years is required to identify if there exists a cyclic pattern of neurotropism and association with a particular serotype if any. Such studies should include viral isolation, antigen and antibody- testing from CSF and immuno-histochemistry on nerve tissue, wherever possible.

Of concern is the rapid and major progress made in developing a vaccine against dengue/severe dengue. Three tetravalent live-attenuated vaccines are under development in phase II and phase III clinical trials, and 3 other vaccine candidates (based on subunit, DNA and purified inactivated virus platforms) are at earlier stages of clinical development [1]. Their immunogenicity may be implicated in neurological manifestations in the future.

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