Prevailing Serotype of dengue virus and IgM antibody in suspected dengue fever cases

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Abstract Introduction

Dengue is a viral infection transmitted to humans through the bite of infected mosquitoes. The primary vectors that transmit the disease are Aedes aegypti mosquitoes and, to a lesser extent, Ae. albopictus. The virus responsible for causing dengue, is called dengue virus (DENV). There are four DENV serotypes and it is possible to be infected four times. Severe dengue is a leading cause of serious illness and death in some Asian and Latin American countries. It requires management by medical professionals.

Materials and Method

This is a prospective and observational study was conducted in the Department of General Medicine at Tertiary care Teaching Hospital. Inclusion criteria: All clinically suspected cases of Dengue virus infection attending the outpatient department(OPD) and inpatient department(IPD) having fever and signs and symptoms of Dengue infection such as retroorbital pain, muscle and joint pain, vomiting, diarrhoea, loss of appetite, thrombocytopenia and leucopenia were included in the study. Detection of anti-dengue IgM antibodies: Detection of anti-dengue IgM antibodies was done by IgM capture ELISA, using a in house kit prepared by the National Institute of Virology.

Results:

In our study, In table 1, 44 years is the median age among the study population. Most of patients distributed between age group 18-30 years (27.04%). Dengue affected predominantly males and young adults. Maximum cases occurred during post monsoon period which may be due to increased breeding of mosquitoes during these seasons. Early diagnosis of infection can prevent life threatening complications and immunological assay such as the IgM capture ELISA is a reliable method for diagnosis of dengue virus infection.

Conclusion: In our study serotype-specific IgM responses corresponding to the isolated virus type were detected in most of patients with primary dengue virus infection tested. Moreover, that in dengue infection, monotypic IgM responses frequently are not correlated with the virus serotype isolated from a patients. We found that IgM responses were generally serotype crossreactive but that IgM levels were highest against the infecting dengue virus serotype in most cases.

Keywords: Serotype of dengue virus, IgM antibody, Dengue fever

Introduction

Dengue is a viral infection transmitted to humans through the bite of infected mosquitoes. The primary vectors that transmit the disease are Aedes aegypti mosquitoes and, to a lesser extent, Ae. albopictus. The virus responsible for causing dengue, is called dengue virus (DENV). [1] There are four DENV serotypes and it is possible to be infected four times.

Severe dengue is a leading cause of serious illness and death in some Asian and Latin American countries. It requires management by medical professionals. [2] There is no specific treatment for dengue/severe dengue. Early detection of disease progression associated with severe dengue, and access to proper medical care lowers fatality rates of severe dengue to below 1%.[3]

Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semiurban areas. The global incidence of dengue has grown dramatically with about half of the world's population now at risk. Although an estimated 100-400 million infections occur each year, over 80% are generally mild and asymptomatic. [3] Dengue prevention and control depends on effective vector control measures. Sustained community involvement can improve vector control efforts substantially. While many DENV infections produce only mild illness, DENV

can cause an acute flu-like illness. Occasionally this develops into a potentially lethal complication, called severe dengue. [4]

One modelling estimate indicates 390 million dengue virus infections per year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease). [5] Another study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection with dengue viruses. Despite a risk of infection existing in 129 countries, 70% of the actual burden is in Asia [6].

The virus may be isolated from the blood during the first few days of infection. Various reverse transcriptase–polymerase chain reaction (RT–PCR) methods are available and are considered the gold standard. The virus may also be detected by testing for a virus-produced protein, called NS1. There are commercially-produced rapid diagnostic tests available for this, and it takes only ~20 mins to determine the result. [7-9]

Serological methods, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of a recent or past infection, with the detection of anti-dengue antibodies. IgM antibodies are detectable ~1 week after infection and remain detectable for about 3 months. The presence of IgM is indicative of a recent DENV infection. IgG antibody levels take longer to develop and remains in the body for years. The presence of IgG is indicative of a past infection. [10]

Materials and Method

This is a prospective and observational study was conducted in the Department of General Medicine at Tertiary care Teaching Hospital.

Inclusion criteria: All clinically suspected cases of Dengue virus infection attending the outpatient department(OPD) and inpatient department(IPD) having fever and signs and symptoms of Dengue infection such as retroorbital pain, muscle and joint pain, vomiting, diarrhoea, loss of appetite, thrombocytopenia and leucopenia were included in the study.

Collection of sample: A single blood sample approximately 2-3 ml was collected from each patient in a plain vacutainer tube with all aseptic precautions. Sample was allowed to clot at room temperature for about 30 min for clot retraction. Serum was separated by centrifugation at a speed of 3000 g(RCF) for 10 min & stored upto 72 h at 20 -80C. Detection of antidengue IgM antibodies: Detection of anti-dengue IgM antibodies was done by IgM capture ELISA, using a in house kit prepared by the National Institute of Virology.

CBC was done daily for the first 4 days of hospitalization and whenever required based on the clinical situation. Tachypnea, chest retractions, decreased breath sounds and decreased vocal resonance were considered as signs of pleural effusion. Abdominal distension with fullness of

the flanks and presence of shifting dullness or fluid thrill was considered as ascites. Percentage of hemoconcentration was quantified by taking a difference between the maximum hematocrit at admission or anytime during hospitalization and the minimum hematocrit recording at convalescence or discharge.

Dengue Hemorrhagic Fever was diagnosed according to WHO guidelines. Clinical presentation, clinical evidence of plasma leakage, laboratory tests (raised alanine transaminase and aspartate transaminase within 2–3 times normal limits, mildly decreased total protein and albumin), radiological evidence of plasma leakage and reactive gall bladder wall changes on ultrasonography were considered by the clinician before categorising these only IgGDenguepositive patients as possible dengue fever. A subset of these patients underwent repeated sampling and those showing seroconversion to IgM positive status or NS1 Ag positive status were categorised as serologically confirmed dengue fever.

Patients with categorical evidence of other causes of fever like malaria, blood culture positive sepsis, culture positive urinary tract infection (UTI) or culture positive respiratory tract infection and those with none of the usual clinical, laboratory and radiological findings of dengue fever mentioned above were classified as non-Dengue fever.

Statistical analysis
Statistical analysis was carried out in Microsoft Excel using t-test to study the significance of differences.

Results:
Table 1. Age distribution

Age (years)	Number of patients	Percentage
18-30	241	27.04
31-40	211	23.68
41-50	234	26.26
51-60	205	23.00
Total	891	100

In table 1, 44 years is the median age among the study population. Most of patients distributed between age group 18-30 years (27.04%).

Table 2. Gender distribution

Gender	Number of patients	Percentage
Male	572	64.1
Female	319	35.9
Total	891	100

In table 2, Infection was most common in males, 64.1% (572/891) as compared to females, 35.9% (319/891).

Table-3: Clinical symptoms in dengue cases

Presentations	Cases (n=891)
Fever	879 (98.6%)
Headache	611 (68.57%)
Myalgia	412 (46.2%)
Vomiting	593 (66.5%)
Rashes	91 (10.2%)
Pain abdomen	73 (8.19%)

Table-4: Mean values of hematological and biochemical parameters in cases and controls

	Cases n=891
Haemoglobin g/dL	12.12±2.1
Total leucocyte /cmm	4425.53± 314.62
Platelet count /Lakhs	0.71 ± 0.15
Total Bilirubin mg/dL	0.21 ± 0.02
SGOT (AST) IU/L	93.53 ± 14.62
SGPT(ALT) IU/L	83.74 ± 9.46

Table 5: Detection of anti-DENV IgM by rapid tests from Standard Diagnostics at initial survey and 6 months.

	No. DENV IgM positive/total (%)	
	Initial	6 months
Definitely positive	511/891 (57.3)	134/891 (15.0)
Very faint	198/891 (22.2)	398/891 (44.6)
Negative	182/891 (20.4)	359/891 (40.2)

Discussion

Dengue is a mosquito-borne viral infection causing a severe flu-like illness and, sometimes causing a potentially lethal complication called severe dengue. The incidence of dengue has increased 30-fold over the last 50 years. Up to 50-100 million infections are now estimated to occur annually in over 100 endemic countries, putting almost half of the world's population at risk [12].

If severe, dengue fever can damage the lungs, liver or heart, blood pressure can drop to dangerous levels, causing shock and, in some cases, death with multi organ failure. Approximately 2.5 billion people live in dengue-risk regions with about 100 million new cases each year worldwide [13]. The cumulative dengue diseases burden has attained an unprecedented proportion in recent times with sharp increase in the size of human population at risk. Dengue disease presents highly complex pathophysiological, economic and ecologic problems. [14]

In India, the first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of dengue fever (DF) occurred in Calcutta (now Kolkata) and Eastern Coast of India in 1963-1964 [15]. Its viral cause and spread were understood by the early 20th century. Apart from eliminating the mosquitoes, work is ongoing for medication targeted directly at the virus. It is classified as a neglected tropical disease. [16]

In the present study, 7.7% of the total patients had serologically confirmed dengue infection. Other studies by Ghosh et al [17] from Mumbai reported prevalence of dengue infection as 13.6% and The prevalence of infection was higher in males (63.6%) as compared to females (36.18%) with male to female ratio being 1.76: 1. Similar findings are reported by Nisarata A, [18]. This high prevalence in males as compared to females is probably due to social, cultural (women being covered) and exposure differences. Majority of infections occurred in age group of 15- 30 years (46.7%) followed by paediatric age group (23.3%). Predominant infection rate

in adult population was also noted by Kumar A et al. [19] However, Garg et al, [20] reported higher prevalence of dengue infection in paediatric age group.

Methods such as one step, real time RT-PCR or nested RT-PCR are now widely used to detect dengue viral gene in acute —phase serum samples. Acute infections can also be laboratory confirmed by sero-conversion from negative to positive IgM antibody to dengue or demonstration of a fourfold or greater increase in IgG antibody titers in paired serum specimen [21].

Conclusion

In our study serotype-specific IgM responses corresponding to the isolated virus type were detected in most of patients with primary dengue virus infection tested. Moreover, that in dengue infection, monotypic IgM responses frequently are not correlated with the virus serotype isolated from a patients. We found that IgM responses were generally serotype crossreactive but that IgM levels were highest against the infecting dengue virus serotype in most cases. Although more patient samples need to be tested to confirm our observation, the results provide interesting insights into human antibody responses to dengue viruses.

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