**Original Research Article**

**Dengue IgM antibody sero-status assessment: a current experience in a teaching institution**

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**ABSTRACT**

**Background:** In 2015, Delhi city experienced the worst outbreak of dengue infection since 1996 and our laboratory received a very high number of cases for IgM ELISA testing following clinicians’ referrals. The present study intends to correlate IgM antibody response with duration of fever, platelets counts and ‘with age and gender distribution’ of such cases attending OPDs and subsequently admitted to the medicine and pediatric wards in our tertiary care hospital, New Delhi, albeit, with some useful conclusions and suggestions.

**Methods:** A hospital based observational study was conducted enrolling a total of 500 cases, clinically suspected of DF/DHF/DSS that either reported directly or were referred to Safdarjung Hospital from region in and around Delhi and NCR and subsequently admitted to Emergency Medicine and Paediatrics wards. A total of 500 cases and 50 controls were tested for IgM antibody by ‘IgM antibody capture ELISA (MAC ELISA).

**Results:** Of total 500 serum samples tested, 169 i.e. 33.8% were found to be sero positive. Seropositivity was more among the males 99/169 (58.57%) than the females 70/169 (41.43%). The highest numbers of cases were seen in the age group 41-60 years (46.15%). Amongst seropositive cases, 146 (86.39%) cases had fever of >5 days duration while remaining 23 (13.60%) presented with fever of <5 days. Low platelet count between 20,000-IL cells/mm³ was a feature in all the seropositive cases and statistically it was found significant (p-value 0.001).

**Conclusions:** Detection of IgM specific antibodies by MAC ELISA in cases with high index of clinical suspicion continues to be an effective tool providing a supportive criterion to clinical diagnosis, especially during outbreak periods.

**Keywords:** Dengue fever, ELISA, IgM, Platelet count

**INTRODUCTION**

The number of dengue cases in Delhi in the year 2015 amounted to more than 15,000 making it the worst outbreak of the vector-borne diseases in the national capital since 1996.¹ In 2015, a higher number of cases than usual were referred by clinicians to our laboratory for IgM ELISA sero testing. It is well documented that dengue infection often presents as asymptomatic or acute mild undifferentiated fever.² It is usually diagnosed clinically and is best supported by laboratory evidence for ELISA based IgM antibody detection on paired sera.³,⁴ IgM levels rise quickly and appear to peak about 2 weeks after the onset of symptoms and then gradually decline to undetectable levels over 2-3 months.⁵ However, detection of IgM antibody by ELISA test in suspected dengue cases with duration of fever >05 days but up to 10 days places clinical diagnosis on firm footing and hence better clinical care.⁶ It also provides a satisfactory evidence for epidemiological record purposes.⁷,⁸ Therefore, the present
A retrospective study aims at analyses of the IgM ELISA serology status and tends to correlate it with - the gender distribution, age groups affected, variations in duration of fever and platelet counts, and, is expected to provide a presumptive, yet very definitive, evidence about the prevalence and distribution of dengue cases in our hospital with the attached teaching institution, with some useful conclusions and suggestions, expectedly valid for real world situation about dengue infection in Delhi in current times.

**METHODS**

The study was conducted in a tertiary care hospital in between a time of August 2014 to April 2016. A total of 500 cases, clinically suspected of DF/DHF/DSS who either reported directly or were referred to Safdarjung hospital for treatment from region in and around Delhi and NCR, formed the study group.

Patients in the age group 10 to 60 years with acute febrile illness, both male and female were recruited for the study. Fifty age and gender matched patients with febrile illness other than dengue formed the control group. Patients with conditions viz. Malaria, typhoid, UTL, and any other acute febrile illness were excluded from the study period. Demographic characteristics of patient viz. age, sex, residential address, were recorded with preformatted questionnaire to complete the records. Sera from all the 500 cases and the 50-control tested for IgM antibody by IgM antibody capture ELISA (MAC ELISA) as per manufacturer’s instructions. Laboratory investigations of routine nature were done on all blood samples to rule out other common important conditions viz. rapid qualitative immunoassay for the detection of P. falciparum specific histidine rich protein and Pan-malaria specific Parasite lactate dehydrogenase (pLDH) to rule out other species including P. vivax (RMAT).² Peripheral blood smear examination for malaria detection was also undertaken.

Widal test for typhoid; urine routine and microscopic examination (RE/ME) and culture for urinary tract infection were also performed. In addition to these tests, IgM ELISA for chikungunya was also done to exclude the mixed chikungunya and dengue infections. Data was be entered in to Microsoft excel sheet analysed by SPSS software version 21.0. A value of p <0.05 was considered statistically significant.

**RESULTS**

Of 500 serum samples tested, 169 (33.8%) were found to be sero positive for presence of IgM antibody by ELISA. Males were more affected (59% approx.) and age group of ‘41-60’ appeared more vulnerable. Fever of duration - more than 5 days but not more than 10 days - was more prevalent (86%) than of duration less than 5 days (14%). Platelet count of 20-60 thousand to ‘more than 60 thousand but not more than 1 lac’ was the predominant finding. All the values were significant as justified by their p values (Table 1).

**DISCUSSION**

In the present study, of 500 sera tested, 169 i.e. 33.8% samples were found to be seropositive for the presence of IgM antibody and 361 (66.2%) were seronegative as shown in Table 1. It is comparable with studies done by other workers from India viz. a study by Chakravarti A, et al in Delhi in a tertiary care hospital covering a period of 7 years i.e., 2002-2008 reported 30.15% positivity in 7846 sample.¹³ Similarly, a study result at AIIMS, Delhi, reported in 2006 based on 1820 samples (received over a period from 2003-2005) by Gupta E, et al, reported

<p>| Table 1: IgM seropositivity status correlation with age, gender, duration of fever and platelet count in 169 seropositive cases. |
|-----------------------------------------------|----------------|-----------------|----------------|----------------|----------------|
| IgM Seropositive | Gender M/ F | Age Group | Fever duration | Platelet count |</p>
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<td>169 (33.80%)</td>
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<tr>
<td>M =99 (58.57%)</td>
<td></td>
<td>A=5-20</td>
<td>X=146/169 (86.39%)</td>
<td>P1=20 k</td>
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<td>F=70 (41.43%)</td>
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<td>B=21-40</td>
<td>Y=5 days</td>
<td>P2=20-60 k</td>
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<td></td>
<td></td>
<td>C=41-60</td>
<td>(86%)</td>
<td>P3=60 k - 1 lac</td>
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<tr>
<td>0.0045</td>
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<td>P4=1 lac</td>
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<td>0.0255</td>
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44.56% Seropositivity. The dengue virus infection outbreaks are currently the most important arthropod borne viral disease of public health concern globally and, in particular, in tropical and subtropical countries including India. Current resurgences to the extent of 30-fold rise have been attributed to a combination of factors viz. population growth, increasing industrialization, global warming, rapidly expanding haphazard urbanization, lack of effective mosquito control measures and insufficient public health services including increasing migration to capital city of Delhi and NCR. More than 100 countries including India have been affected and it is estimated that approximately 390 million dengue infections occur annually. It is also true that cumulative dengue disease burden is growing out of proportion due to increase in the size of human population at risk and indeed nearly 1.37 million billion people i.e. about 15% of world population reside in India. A study done by Kumar R et al on the "Global Distribution and Burden of Dengue" published in Nature (2013) using a map based approach to model how many dengue deaths were occurring in various parts of the world, estimated that India has largest number of dengue cases with about 33 million apparent and another 100 million asymptomatic infections occurring annually.

Sero-Surveillance for Dengue has been very limited in India; there are inherent gaps in epidemiological data making the assessment and/or quantification of dengue burden difficult. The problem gets further compounded by the observation that most of the data in Indian context is retrospective analyses based. As per the current scenario, appropriate surveillance, strengthening laboratory services, introducing quality assurance, reviewing case definitions and studying correlational complexities with the hope of addition to the current guidelines, recommendations, or suggestions have become mandate of the day.

From laboratory diagnosis view point, it is worthwhile to know that experience reveals ELISA test as the most preferred for serological diagnosis because the IgM antibody persists for 60-90 days and there is a reasonable amount of sensitivity. In present study, lack of seropositivity in 66.20% cases may be attributed to secondary infections in which IgM antibody is still mounted but at insufficient detectable levels. Since during secondary infection memory IgG antibody overwhelm IgM antibody and blocks the detection of IgM antibody. In addition, it is important to recall that since 1996, mixed dengue serotypes are involved and many of these patients infected with other serotypes may remain quantitatively serologically negative for IgM antibody competitive response to infection with current serotype circulating in community. Among the 169 IgM seropositive cases - 99 (58.57%) were males and 70 (41.43%) were females. By application of Chi Square test, it is significant with a p-value of 0.033. Three independent studies from India - Aggarwal et al, Ray et al, and Wali et al found nearly twice the number of male patients infected with dengue as compared to females with M: F ratio being 1.9:1, 1: 0.57, and 2.5:1 respectively. In present study, this ratio is 1.41:1. Although, some contrasting results, but very scarce, are reported in literature, for example, in a study by Anita Chakravarti et al, the population of females was higher (M:F::1:1) (p-value 0.001). Present study, on 500 patients, has demonstrated male predominance in the DF cases. These results are like quite a few studies from India revealing greater male predominance. This greater predominance in males has been attributed to the prolonged outdoor activities that increase chances of mosquito bite. Interestingly, according to Global Health Atlas Gunther J et al, Amedia PA, et al, the reports from Asia are a relative contrast to studies from South America which have found either equal proportion of males and females’ dengue cases or a greater proportion of female cases which may be explained and is attributed to the known global regional differences in epidemiological patterns.

Irrespective of gender differences, there were a total of 35/169 (20.71%) seropositive cases in the age group 10-20 years but the highest number of cases were seen in the age group 41-60 years i.e. 78/169 (46.15%), followed by 56/169 (33.13%) in the age group 21-40 years. The observation is significant with p-value 0.025. In a study by Dayaraj Cecilia, that involved analysis of more than 1820 samples from year 2003-2005, maximum number of cases belonged to 21-30 years of age group. Similarly another study involving testing of 5106 samples revealed highest seropositivity in the age group 21-30 years (6 years study). Another study by Ekta Gupta et al from Department of Microbiology (AIIMS), New Delhi has shown age group 21-30 years as the most affected. According to Sreejith M.G. and Peter George, a study on DF shows maximum number of cases in the age group 20-29 years. However, for the age group affected, a well-known observation is that dengue worldwide affects humans of all age groups.

DF is no longer a childhood disease and shift in modal age is well documented in literature as is also seen in our study. Since the early 1980s, several studies in both Latin America and South East Asia have reported a higher escalation of DF and DHF with older ages. It has been reported so in Nicaragua and Brazil also. Present study clearly reveals age shift indicating epidemiological changes in DENV infection including the capital city of Delhi. It is equally important to understand that all age groups during outbreaks are vulnerable and such a trend has clear cut implications demanding rigorous and well planned prevention and control measures.

About dengue fever duration, in the present study, 354/500 (70.80%) cases reported fever of more than 5 days’ duration and 146/500 (20.20%) reported fever of less than 5 days duration. Presence of fever in all 500
cases is suggestive of classical DF presentation that invariably as an acute febrile illness. It is also an inclusion criterion in our study. Among the 169 seropositive cases, irrespective of gender, 146 (86.39%) cases presented with fever of ‘more than 5 days’ duration but up to only 10 days’ while rest of 23 (13.60%) presented with ‘fever of less than 5 days’ (an important inclusion criterion). It is well known that IgM antibody response comes to detectable level usually after 3rd day of infection and peaks about 5th day.30,31 According to a WHO document, infections with DENV, the 1st class of antibody to be produced is IgM and can be detected from day 3rd of illness, and by day 10 at least 99% of individuals mount IgM detectable levels, and indeed high seropositivity in acute illness agrees with acute phase.30 Sreejith MG et al in a study published in International Journal Of Recent Trends Of Science and Technology have shown evidence for increasing sensitivity of IgM antibody during the course of illness that reaches to 80-100% after 5 days of illness.27

About platelet counts, in present study, 46.74% seropositive cases had count between 20,000-60,000 cells/mm³ and in 53.25%, it was between 60,000-1,00,000 cells/mm³. None of seropositive cases had platelet count less than 20,000 cells/mm³ and none had platelet count more than 1,00,000 cells/mm³. The platelet count between 20,000-1,00,000 cells/mm³ in present study was a feature in all seropositive cases and statistically also significant (p-value 0.001). Among the laboratory parameters that significantly contribute to the diagnosis of DF, thrombocytopenia is the most characteristic feature associated with DF.27 It is interesting to note that in several studies, the platelet count less than 1 lakh/mm³ has been observed in 16-55% patients.28 In a study by Sreejith and Peter George in IgM antibody seropositive dengue cases, thrombocytopenia was present in 50 out of 55 cases (91%).27 Similarly, a study by Balvinder S Arora et al documents platelet count less than 1,00,000cells/mm³ in 94.6% cases.15

MAC ELISA is frequently run as non-quantitative, single dilution test and positive results are reported to support the clinical diagnosis but despite this limitation of being non-quantitative, it is believed that the detection of dengue specific IgM is a useful diagnostic and surveillance tool and has sensitivity and specificity of 90% and 98% respectively especially when assays are taken 5 days or more after the onset of fever.29,30 Dengue specific IgM is expressed earlier than dengue specific IgG.31 In one study in Puerto Rico, by day 5 of illness nearly 80% patients with dengue infection that was subsequently confirmed by haemagglutination inhibition test (HA) on paired sera or by viral isolation, had detectable IgM in acute phase serum.32 Nearly all patients (93%) developed detectable IgM 6-10 days after the onset of fever and 99% of patients tested between 10-20 days after the onset of fever had detectable IgM and this observation is well documented in journal of virology methods therefore, despite these limitations we feel our study is very useful, and of significant relevance.20,26,33

Globally, many of the dengue virus infection affected countries, some of poorest, and India, a developing country, clearly need control measures that are realistic for their existing infrastructure. Present results imply a need for better policies and operational strategies. Such operational strategies need be ‘evaluated and reevaluated’ on ‘periodic basis’ to introduce modifications as per the ongoing situations on the spread of the outbreak. From the laboratory diagnosis view point, the present study clearly indicates need for continuation of cost effective ELISA testing as a routine procedure.

On 18th May 2002, the ‘WHO General Assembly’ confirmed DF as a matter of international public health priority to strengthen dengue control and research 1 and with this study it is felt we have contributed our bit to the vast arrays of all round national and international efforts, ad continuum and in progress, at controlling the burden of dengue illness in India.

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Ethical approval: The study was approved by the institutional ethics committee

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