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SEROLOGICAL MARKERS PREVALENCE AND TREND OF PROBABLE DENGUE INFECTION AT A TERTIARY CARE HOSPITAL IN BANGALORE

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ABSTRACT: BACKGROUND: Dengue is an important arthropod-borne viral disease of public health significance affecting tropical and subtropical regions of the world including Indian subcontinent. As there is no immunoprophylactic or specific antiviral therapy available, an epidemiological surveillance that provides reliable estimate of the disease is essential to implement effective vector control measures. **AIMS AND OBJECTIVES:** This study was conducted to understand the serological markers distribution, change in platelet count of probable dengue infection in view of its seasonal variation and demographic variables. **METHODS:** Blood samples were collected from 4015 patients with dengue fever-like febrile illnesses from January 2012 to December 2012.These samples were tested for dengue specific Ns1 Antigen, IgM and IgG antibodies using rapid immunochromatographic assay from SD diagnostics. Platelet counts were determined by using automated SysmexXT1800i-cellcounter analyser. The seropositivity results were correlated with 2010 and 2011 year's data.
RESULTS: Of the 4015 samples, 1385 (34.5%) were positive for dengue fever with peak incidence from June to October. Dengue seropositivity was 34.3% and 14.8% in 2010 and 2011 respectively. Male-to-female ratio was 1.7:1 among positives. Younger age group with age < 18 Years (42%) had higher seropositivity compared to adults with age > 18 years (33%) with p value of 0.00003. NS1 antigen was positive in 59% of cases. IgM and IgG antibodies were detected in 47.87% & 53.71 % cases respectively. Platelet counts of 822 positive patients were analysed. 590 (72.77%) patients had platelet counts < 1 lakh/ml and 232(28.22%) had platelet counts > 1lakh/ml. **CONCLUSION:** The probable dengue infection was higher in 2012 than in previous years. The observation of the present study emphasizes the need for continuous seroepidemiological surveillance for the timely formulation and implementation of effective dengue control programme during monsoon and post monsoon seasons.
KEYWORDS: Dengue, Rapid immunochromatographic test, Thrombocytopenia, Seasonal variation

INTRODUCTION: Dengue fever, dengue haemorrhagic fever, and dengue shock syndrome (DF/DHF/DSS) are tropical diseases that cause significant humanitarian and economic hardship. It is estimated that more than 2.5 billion people are at risk of infection and more than 100 countries have endemic dengue virus transmission.[1] Although dengue has a global distribution, Southeast Asian region together with Western Pacific region bears nearly 75% of current global disease burden. The Southeast Asian region is currently experiencing an upsurge in reported cases of dengue in a number of countries including Bangladesh, India, Sri Lanka and Thailand.[2] The first outbreak of dengue fever in India was recorded in 1812. In spite of preventive measures taken by the respective governments since then, recurrent outbreaks have occurred, and over the last 10 to 15 years DF has been the major cause of hospitalization and mortality after acute respiratory and diarrhoeal

infections among children. Rapid growth of the population and sudden climatic changes has contributed to the increase in cases of DF/DHF in India.[3]

The case fatality rate in patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) is found to be around 5%. [2] Hence early and rapid laboratory diagnosis of dengue is crucial and essential to provide an accurate diagnosis of acute dengue virus infection so that appropriate treatment and patient management can reduce the mortality rate to less than 1%. At present, the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation; detection of viral genomic sequence by a nucleic acid amplification technology assay (RT-PCR), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test[4]. Viral isolation by cell culture and subsequent detection by immunofluorescence, though the gold standard, cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure and time consumption. The requirement of a highly trained staff, the need of sophisticated equipment as well as the cost factor associated with molecular methods has limited its application as a routine diagnostic assay. The MAC-ELISA, which is a commonly used assay, has a low sensitivity in the first four days of illness. The requirement of paired sera at acute and convalescent phase, which improves the accuracy of the diagnosis, further delays it [5]. Simple and rapid immunochromatographic tests (ICT) with the detection of NS1 antigen, IgM/IgG antibodies provide opportunities for point-of-care diagnosis especially in many dengue endemic settings, where laboratory diagnostic resources are limited.[1, 6]

In the absence of vaccines and specific treatment for the DF, prevention and control of the disease mainly depends upon effective vector control measures based on the epidemiological surveillance that provides reliable estimate of the disease[7]. Present study was designed to determine the frequency of ICT seropositive cases in and around Bangalore and to provide information on epidemiology of the disease which is essential to plan necessary measures to control and prevent dengue infection. [8]

MATERIALS AND METHODS:
Study design & Study site: This descriptive study was conducted over a period of twelve months (January 2012 to December 2012) at KIMS Hospital and Research Centre a 1000-bedded hospital in Bangalore, India. A total of 4015 blood samples from patients of all age groups (2417 from males and 1598 females) clinically suspected of dengue fever attending the paediatric, medicine, and fever clinics of the hospital were collected and screened for dengue infection. The clinical basis for suspecting the patients as having dengue virus infection was based on WHO definitions [9, 10]. Blood samples from patients confirmed with other causes of fever were excluded from the study. All the samples were collected after obtaining the informed consent from the patients. Briefly, 2 to 3 ml of blood was collected from each patient by nursing personnel, male orderlies or physicians using strict aseptic precautions and serum was obtained using standard methods.

Seroassays: Serum samples were subjected for NS1 antigen testing and anti-dengue IgM and IgG antibody testing by the solid phase immunochromatographic (ICT) assay, a commercial dengue virus rapid test kit manufactured by Standard Diagnostics, Inc., Korea, as per WHO criteria for serological diagnosis using rapid ICT assay with differential detection of Ns1 Antigen, IgM and IgG antibodies.

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The results were graded as reactive (visible bands in test and control regions) and non reactive (visible band only in control region) as per the manufacturer's instructions[11].

The dengue seropositivity was correlated with patient's platelet count. Platelet counts were analysed in Sysmex XT1800i-cell counter analyser.

The serological markers prevalence of the study was further compared with seroprevalence of probable dengue at our hospital during the years 2010 and 2011.

Statistical Analysis: The Chi square test was used to find out the P values of the results. P value < 0.05 was considered significant [12].

RESULTS: A total of 4015 serum samples (2417 from males and 1598 from female) from patients clinically suspected of dengue fever were screened for dengue serological markers at KIMSH & RC, Bangalore from Jan 2012 to Dec 2012 using SD rapid ICT. Of these 1390 were from out patients and 2625 were from inpatients. 1385 samples were found positive for dengue serological markers.

Analysis of month wise seropositivity of probable dengue fever showed the peak incidence from June to October. Similar seasonal trends were observed in previous years (2010 and 2011) on retrospective analysis (Figure 1).

Out of 1385 positive cases, 862 (35.7%- 862/2417) were male patients and 523 (32.7%- 523/1598) were female patients. The seropositivity was high in younger age group with age < 18 Years (41.86%- 309/738) compared to adults with age > 18 years (33%-1076/3277) with p value of 0.00003 (Table1)

Probable dengue infection was diagnosed by detecting NS1Ag, IgM and IgG antibodies. In total, NS1 antigen was detected in 59% of cases. IgM and IgG antibodies were detected in 47.87% & 53.71 % cases respectively. Individually, NS1 Ag was detected in 32.6% cases, IgM antibodies in 5% and IgG antibodies in 15% cases. (Table 2)

Seropositivity was correlated with platelet counts. Platelet counts were available in 822 patients and not available in 563 patients (Outpatient samples). Out of 822 patients, 590 (72.77%) patients had platelet counts < 1 lakh/ml and 232 (28.22%) had platelet counts > 1lakh/ml (Table 3).

DISCUSSION: Outbreaks of dengue fever are reported almost every year in India. The trend for the increased incidence of dengue infection among young adults has been observed in previous studies and has important implications for control and prevention. It has been earlier established that in many of the Asian communities, lower disease incidence in women may be a statistical artefact and hence well-designed and targeted studies are required to confine both biological and social factors that confer disease patterns in a community [3, 7]. In order to provide timely information for the management of the patients, and early public health control of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days after manifestation of clinical symptoms [4].

Virus isolation, molecular diagnosis using reverse transcriptase polymerase chain reaction (PCR) and serological methods have been used for laboratory confirmation of dengue infection. Viral isolation takes several days and is not available in many endemic countries. Although PCR is a useful tool for identification and dengue strain characterisation, its widespread use for diagnosis of dengue infection is limited due to high cost, especially in developing countries. In the clinical setting,

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diagnosis of dengue infection is primarily based on serology by detecting dengue specific antibodies. Serological tests that have been available for the detection of dengue specific antibodies include hemagglutination inhibition (HAI) assay, enzyme linked immunosorbent assay (ELISA), dot blot assay, dip stick and rapid ICT assay. To distinguish between primary and secondary dengue infection HAI, capture IgM and IgG ELISA, and rapid ICT have been employed. The rapid ICT is a commercial kit that incorporates 4 recombinant proteins from DEN 1- to- DEN 4. This assay detects both IgM and IgG antibodies in 15 minutes and helps in differentiation of primary and secondary dengue infections[8]. The use of rapid ICTs for dengue has become increasingly popular, particularly in developing countries, due to their simplicity, affordability and their suitability for use at or near the point of care[13].

With rapid ICT assay, 34.5% (1385) of 4015 serum samples were serologically positive for dengue parameters at our hospital. The dengue seroprevalence rate in North India and central India On analyzing the year-wise distribution of dengue cases, 34.35 % (815/2372) seropositivity was noted in 2010 and 14.76 % (259/1754) in 2011 with an increased incidence of 34.5% during 2012. Similar observations were reported by Sultana N, Garg A and Sharma Y in their studies [2, 14, 15]. This may be partially attributed to the rapid unplanned urbanization with unchecked construction activities and poor sanitation facilities contributing fertile breeding grounds for mosquitoes; it is also true that an increase in the alertness among medical fraternity following the initial epidemic and the availability of diagnostic tools in the hospital have contributed to the increased detection of cases [14].

Month wise data analysis reported a gradual increase in cases from June with a peak in September and decreased seroprevalence by the end of December. Similar seasonal variation was observed during 2010 and 2011. The seasonal disparities of dengue occurrence with monsoon and post monsoon seasons are analogous to the findings of Ukey P, Kidwai AA, Garg A and Sharma Y [6, 8, 14, 15]. The periodicity of dengue occurrence could be because of climate and environmental aspects that play a critical role in the distribution and prevalence of both the dengue virus and its vectors (A. aegypti and A. albopictus). This seasonal outbreak of disease transmission emphasizes the need for appropriate vector control measures to be implemented into full swing during water stagnation periods after the initial bouts of rainfall, at the end of monsoon and post monsoon months to reduce the case incidence. [3, 14, 15]

Demographic characteristics like age distribution and gender differences are important for the successful planning of public health programmes and effective control of communicable diseases [8]. In the present study, males had higher prevalence of probable dengue infection than females. The male-to-female ratio was 1.7:1 which is in accordance with other studies [8, 14]. The lower infection rates in females of Asian community might be attributed to lower reporting rate and the fact that they remained stationed at home and are less exposed to this vector born infection [8].

Several international studies have reported dengue as main paediatric public health problem because true endemicity of dengue is reached when the adult infection declines and only the new entrants into the population, that is, the children, are affected more by the disease [14]. Children and adolescence have been reported to be the predominant group in Southeast Asia including some parts of India. In our study, dengue seropositivity was high in younger age group with age < 18 Years (42%-309/738) compared to adults with age > 18 years (33%-1076/3277) with p value of <0.001 (p=0.0000301). Similar observations have been reported by Ukey P, Kidwai AA and

Sharma Y [6, 8, 15]. The shift in the age preponderance can be partly explained by the accumulation of multitypic immunity in the adult population. It is suggested that over a period of time, as the length of time of co-circulation of multiple serotypes of dengue in a particular geographic area increases, adults have a lower probability of remaining susceptible to infection. This results in the young population to become completely susceptible individuals to primary and secondary infection. Therefore, monotypically immune individuals are more likely to be from younger age groups [15]. In absence of specific treatment for dengue fever, management is mainly supportive, further there are no vaccines currently available in market thus early diagnosis and vector control is the only method by which dengue can be controlled[14].

The accurate laboratory diagnosis of DENV infection is now possible with Dengue NS1 Ag detection tests, taking into account the timing of serum collection after the onset of fever. The development of a single immunochromatographic assay combining NS1 antigen and dengue virus (DENV) IgM antibody detection would provide clinicians with a rapid test for dengue diagnosis sensitive during both the acute and early convalescence phases, between days 0 and 8 after the onset of fever. During the acute phase of the disease, the presence of DENV IgM antibody alone suggests primary infection, and the concomitant detection of DENV IgM and IgG antibodies is suggestive of secondary infection. The absence of DENV IgG antibody in serum samples collected between days 0 and eight makes it possible to classify the case as primary DENV infection. It has also been shown that, during the IgG antibody response, NS1-specific IgG antibodies start to appear on day 8 of primary infection and are still present during the first few days of DENV secondary infection [16].

In our study, NS1 Ag positivity along with other parameters was 59 % and only NS1Ag positivity was observed in 32.6% cases. Other studies have reported 30 to 83% NS1 positivity [4, 5, 13, 17]. NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness. NS1 Ag levels varies from 0.04 - 2 µg/ml in acute-phase serum samples, to only 0 .04µg/ml or even less in convalescent phase serum. This is the reason for its higher detection rate in acute phase sera [18].

Anti-dengue IgM and IgG antibodies were found in 47.87% and 53.71% of positive cases in the current study. Others have reported 6 to 41% IgM seropositivity and 42 to 51.68% of IgG seropositivity, with increased seropositivity of up to 93% in convalescent phase sera[2, 8, 14, 18]. Different immunological response patterns have been observed during primary and secondary dengue infections [8, 13]. In the present study using ICT assay, probable primary (positive for NS1 Ag, IgM, NS1 and IgM) and probable secondary infection (positive for IgG, NS1 + IgG, NS1+ IgM+ IgG) was found in 641(46.28%) and 452 (32.63%) patients respectively. 292/1385(21.08%) samples positive for IgM+IgG could not be classified as primary or secondary infection as ELISA was not done to assess the ratio of IgM / IgG [9, 10]. The percentage of ICT reactive cases might have been higher if paired sera were analyzed. Similar observations have been reported by Kidwai AA[8]

A sudden drop in platelet count to below 100000/ml occurs by the end of the febrile phase before the onset of shock or subsidence of fever. The level of platelet count is correlated with severity of DHF. In addition there is impairment of platelet function. These changes are of short duration and return to normal during convalescence [19, 20]. We tried to find the association between seropositivity of dengue parameters and platelet counts. In the present study, platelet counts were analysed only in 822 patients (inpatients) as the rest of the positive samples were from outpatients.

Out of 822 patients, 590 (72.77%) patients had platelet counts < 1 lakh/ml and 232(28.22%) had platelet counts > 1lakh/ml. 66.23% of probable primary dengue, 79.10% of probable secondary dengue and 72.78% of IgM+IgG positive patients had platelet counts less than 1 lakh/ml (Table 3). Detection of IgM antibodies alone would have resulted in false negativity in 52.12% cases of whom 42.38% (306) cases had platelet counts less than 1 lakh/ml. Concurrently, if Ns1 Ag detection was not used, diagnosis would have been missed in 32.6% (452/1385) patients of whom 44.24% (200/452) had platelet counts less than 1 lakh/ml. Data from this study supports a growing body of evidence that a combination of NS1 and IgM/IgG detection provides increased diagnostic sensitivity without reducing specificity. These observations are comparable to the findings of Fry SR, Kulkarni RD, Ahmed S, Whitehorn J and Ferrar J. [12,16,20,21].

There are certain limitations in our study. Since our data analysis is based on laboratory diagnosed cases, they may represent only the tip of the iceberg in the overall pattern of dengue spread. In addition, ELISA and molecular methods of higher sensitivity and specificity were not used. Research on the circulating serotypes and their genotypes may be of help in addressing the probabilities of DSS/DHF incidence in future.

CONCLUSION: The study draws attention towards the younger age group which represents the endemicity observed in this part of India, where dengue has emerged in more recent years with large outbreaks occurring in monsoon and post monsoon seasons. This finding may be of help to the epidemiologists, paediatricians, and dengue prevention and control measure authorities for early diagnosis and to plan and implement various measures targeting the young population[15]. Rapid immunochromatograpic tests to detect NS1 antigen and IgM/IgG antibodies should be available at in absence of specific treatment and vaccines available for dengue fever, the need of the hour is long-term vector control strategy; so that the outbreaks can be prevented and this will simultaneously solve the problem of other mosquito borne diseases like Chikungunya, Japanese encephalitis, Malaria and Filariasis [14].

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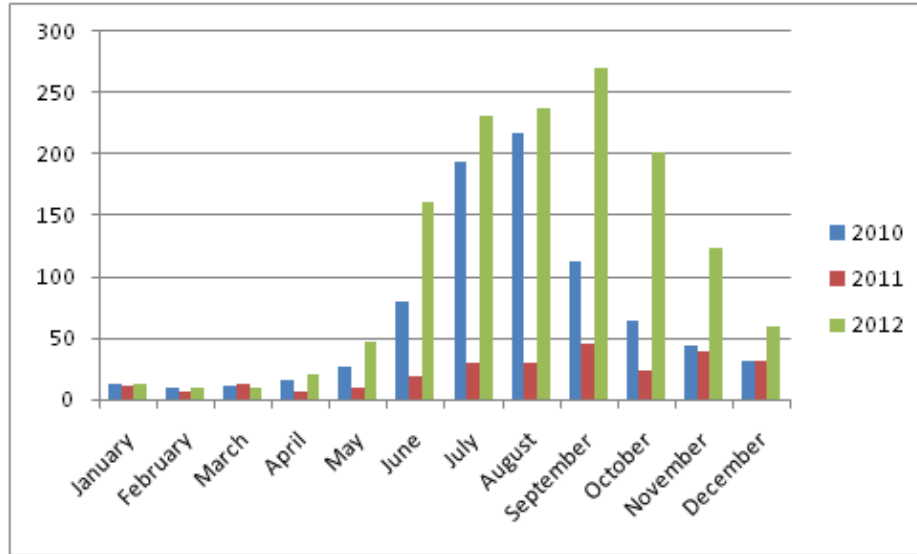21. Whitehorn J, Farrar J Dengue Br Med Bull. 2010;95:161-73

Fig 1: Seasonal distribution of probable dengue fever

AGE	Dengue positive	Dengue negative	Total cases screened
< 5 years	48	68	116
5-18 years	261	361	622
>18 years	1076	2201	3277
Total	1385	2630	4015
Table 1: Age wise distribution of probable dengue cases			

Parameters	Number of samples Positive (n=1385)	Percentage
Ns1 Ag Only	452	32.6%
IgM Ab Only	69	5%
IgG Ab only	208	15%
IgM & IgG	292	21.1%
NS1Ag & IgM	120	8.7%
Ns1Ag & IgG	62	4.5%
Ns1 Ag , IgM & IgG	182	13.1%
Table 2: Seropositivity of Dengue specific parameters		

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Parameters	Platelet count available(n)	Platelet count <1lakh/ml	Percentage (%)
Ns1 Ag Only	302	200	66
IgM Ab Only	28	18	64
IgG Ab only	112	79	70
IgM & IgG	169	123	73
NS1Ag & IgM	55	37	67
Ns1Ag & IgG	33	27	82
Ns1 Ag , IgM & IgG	123	106	86

Table 3: Correlation of Dengue parameters with platelet count

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