



“Laboratory Parameters in Clinically Suspected Dengue Cases in Tertiary Care Teaching Hospital in North Maharashtra”

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ABSTRACT

Background: Dengue an arbovirus infection, has significantly increased in past decade causing increased mortality & morbidity in temperate countries including India. Earlier diagnosis helps in prompt treatment resulting in decrease in mortality & prevention of complications. In present study we have evaluated laboratory parameters for diagnosis along with different demographic profile for prevention of disease.

Methods: All clinically suspected patients tested for NS1Ag & IgM by ELISA along with platelet count & Peripheral blood smear. Individual results were used in comparative analysis according to demographic (gender, age) and laboratory (platelet counts, NS1 Ag & IgM) profiles

Result: Total serologically confirmed cases were 583(21.12%) . Lower platelet counts was the most important factors in predicting dengue infection. Furthermore, all demographic and laboratory profiles presented a conservative temporal pattern throughout this long-lasting outbreak.

Conclusion: As consistency throughout the epidemic facilitated defining the conservation pattern throughout the early stages, this was useful for improving management during the remaining period. Also NS1 Ag is early & reliable test.

Keywords: Demographic Profile, Dengue Fever, NS1 Ag, IgM ELISA

Introduction

Dengue an important emerging disease of tropical & sub tropical region is a Vector born disease is caused by Dengue Virus belonging to the family Flaviviridae & genus Flavivirus. Globally dengue is most important mosquito born viral disease presenting with varied clinical symptoms ranging from flue like fever to deadlier DHF & Shock. ⁽¹⁾

Epidemiologically, globally 50 million people are infected with dengue virus every year while 2 & half billions are at risk of infection². Similarly in India with population of more than 1 billion, it is estimated that India has largest number of dengue cases, with about 33 millions apparent & another 100 million asymptomatic infections occurring annually.³ The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964.^{4/5/6}

The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi ⁷ and Lucknow ⁸and then it spread to all over the country⁹ India has documented infections from all four serotypes.

According to WHO , the incidence of dengue globally has shot up 30 folds in last 50 yrs. The cumulative dengue diseases burden has attained an unprecedented proportion in recent times with a sharp increase in the size of

population at risk. Dengue disease presents highly complex pathophysiological, economic & ecological problem¹⁰

Currently neither effective antiviral agents to treat dengue infection nor an effective vaccine are available. So main approach for prevention, control & management of dengue lays with vector control & early diagnosis of disease.

In current study, we have studied different parameters like seasonal variations, gender, age along with laboratory parameters in relation with clinical symptoms which will help us to determine burden of disease in this region & preventive measures to be taken.

Materials and Methods

Study was conducted at SBH GMC, Dhule after the Ethical committee approval. Study caters to both urban population and referred rural cases during the period Jan 2016-Dec 2016 .Blood samples were collected in the plain & EDTA bulb from patients clinically diagnosed as dengue fever .The serum was separated. Cold chain was maintained during transportation of the serum samples whenever required. Data were extracted from patient’s notes collect to socio-demographic factors, clinical characteristics of presenting illness, probable diagnosis, management & outcome of disease. In laboratory investigations Platelet count, Peripheral Blood Smear for Malarial Parasite was done for all cases .Investigations like NS1 antigen ELISA, IgM antibody ELISA were done on all serum samples.

Cases with Positive NS1 antigen , IgM Dengue Antibody or both were recognized as Dengue fever.(NS1 antigen ELISA(Dengue NS1Ag MICROLISA)&IgM antibody ELISA kits(NIV DEN IgM Capture ELISA Kit) were standardized and supplied by NIV Pune. Ig G antibody is not reliable marker as it is a cross reacting antibody to other flaviviruses also hence not considered in this study. ¹¹

Result

Clinically suspected dengue patients from Jan. 2016 to Dec 2016 were included in study. Total 2755 samples from patients were collected & tested as per protocol. Patients tested positive for malaria were excluded from study. Of these 583 were confirmed dengue cases & remaining 2172 were considered as dengue negative & appropriate control. Among the dengue positive cases, 67.93% (396/583) were male & 32.07% (187/583) were female. (Table 1)

A substantial number of patients belonged to the age group of 16-30 yrs i.e. 325(55.75%) followed by age group 1 month -15 yrs. i.e. 156 (26.7%). Youngest case

was diagnosed at age of 4 months with total 13 cases were detected in less than 1 yr age group.

During Study period highest number of cases were noted in the month of September 210 (36%) followed by October 171(29.34%) & November 93(15.95) respectively. Sporadic cases were noted throughout year.(Figure 1)

Out of 2755 serum samples tested, 583 (21.16%) were positive for either one or both serological marker. Of these 583 sample 378(64.8%) were exclusively positive for NS1 antigen while 167(28.6%) sample showed IgM antibody titer positivity. 38 serum samples were positive for both serological marker i.e. NS1 antigen & IgM antibody (Table 2)

Among 583 sero-positive cases thrombocytopenia (Platelets <1 Lac) was evident in 299 (51.29%) while out of 2172 sero-negative cases thrombocytopenia was seen in 159(7.3%) Out of the 378 NS1 positive cases, 224(59.26%) had platelet count less than 100,000/ml while out of 167 IgM positive samples, 83(49.7%) had thrombocytopenia. (Table 3 & 4)

Table:1 Age, Sex & Month wise distribution of cases.

Characteristic	No. of patients	Percentage
Age Group		
1 month-15 yrs	156	26.7
16-30 yrs	325	55.75
31-40 yrs	58	10
41-50 yrs	22	3.7
51-60 yrs	16	2.7
61-75yrs	6	1
Sex		
Male	396	67.93
Female	187	32.07
Month		
August	50	8.57
September	210	36
October	171	29.34
November	93	15.95
Other	59	10.13

Table 2: Serological marker wise distribution of cases.

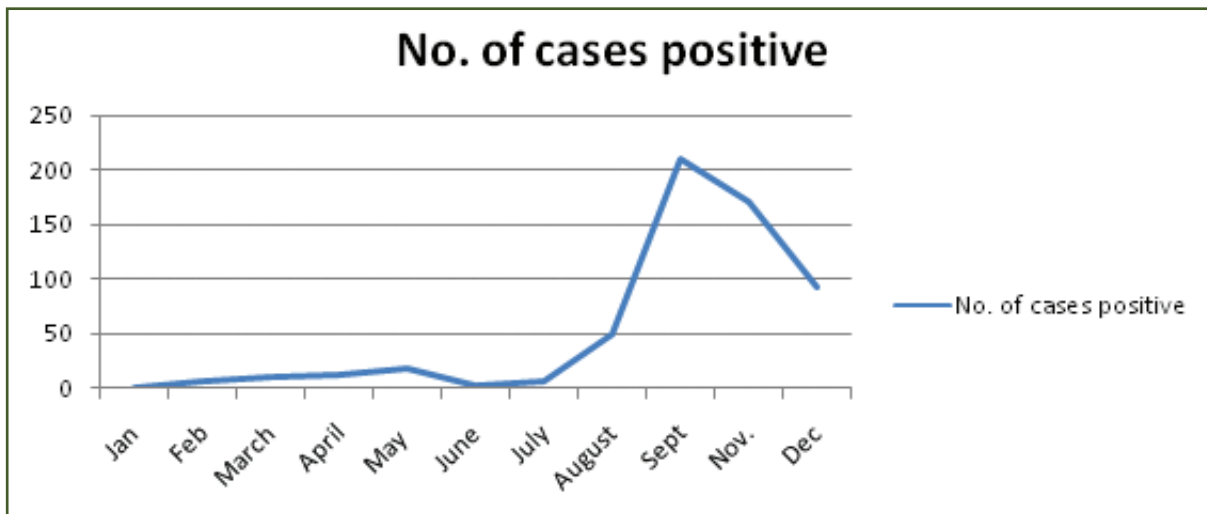
Dengue specific marker	Total number of positive serum sample	Percentage
NS 1antigen only	378	64.8
IgM Only	167	28.6
NS1+ IgM	38	6.6

Table: 3 Comparison of Dengue marker & Platelet count.

Dengue specific marker	Total positive serum sample	Platelet counts (<1,00,000)	Percentage (%)
NS 1 Antigen only	378	224	59.26
IgM Only	1867	83	49.7
NS1+ IgM	38	12	31.5
Total	583	299	54.7

Table: 4 Study of dengue cases with platelet count (n=583).

Platelet range	Dengue positive cases	Dengue negative cases
Platelet counts less than <1,00,000/cmm	299	159
Platelet counts more than >1,00,000/cmm	284	2013
Total	583	2172

**Fig. Line Diagram showing month wise break down of cases.**

Discussion

Dengue is an important emerging disease of the tropical & sub tropical regions today. India is one of the seven countries in the South East Asia, endemic for DF & Dengue Hemorrhagic Fever & may soon become a major niche for dengue infection.¹² In present study, maximum numbers of patients suffering from dengue were in age group of 16-30 yrs. This finding is in accordance with study done by Doke et al in Maharashtra¹³ Mohamed Murtuza Kauser et al¹⁴ from Central Karnataka, Kumar et al from Udupi Karnataka¹² Hemant Kumar et al from Surat¹⁵. Though maximum positive cases belonged to young adult group who are exposed to more outdoor activities, extreme age variations were observed. Youngest patient in present study was 4 months old while oldest was 72 yrs in age. The youngest patient reported by Jonathan G. Lim et al in a study done in 2000-04 was 4 months old¹⁶. M.J. Kulkarni et al reported 6 cases of newborn admitted for dengue¹⁷ evidently showing all ages are more or less susceptible for dengue infection.

Further majority of patients were male (396) while female constitute rest (187) thus male to female ratio is 2.1: 1. M.J. Kulkarni in 2010 found two third patients to be males¹⁷ In contrast to this, a study reported by CV Prathyusa et al from Andhra Pradesh state showed almost equal distribution of male and female ratio in 2012¹⁸. Equal sex distributions was reported by study of Jonathan G. Lin et al

from Malaysia¹⁶. Male to female ratio in a study by Mohan Kashikanti et al in 2013 found a ratio of 1.2:1¹⁹ Majority of patients (92.03%) were found to be males and females constituted 7.96% by a study done by Hemant Kumar et al¹⁵ A. Abrol et al reported male to female ratio of 1:1 from a study in Chandigarh²⁰

The present study shows that outbreak occurred from August to December. Dengue outbreak in India have generally occurred between August and November²¹ M.J. Kulkarni et al in their study of Dengue cases in a tertiary care center in Jaipur reported cases from September to November¹⁷ Mohan D.K. reported most of the cases during the month of June to September¹⁹ A study of 766 patients of Dengue in 2011-2013 in A.J. Institute of Medical Sciences and Research Center, Mangalore, Karnataka, reported cases throughout the year with peak admission in April followed by May and June.¹⁵

Before the advent of NS1 Ag testing, demonstration of dengue specific IgM /Ig G antibodies was mainstay for diagnosis of dengue infection since viral culture or detection of viral genome by molecular method is not feasible at grass root level. As antibodies begin to appear on 5th day of fever in primary & 3rd day in secondary infection, diagnosis is delayed due to lag period in this test. In contrast NS1 Ag is detectable in blood from day 1 of fever & can be seen up to 9 day even when viral RNA detection is negative by RT PCR.²²

Out of total 583 sero-positive cases, 378(64.8%) were positive for NS1 Ag, 167(28.6%) for IgM Antibody & 38(6.6%) for both. Similar studies by Santosh Tathe et al²³ shows NS1 Positivity in 88.23 % , IgM 68.75% & both NS1+ IgM 100% respectively. Also Sindhanai V et al documented 86.5% NS 1 positivity with 44.4.% positivity for IgM. ²⁴ While most studies shows more NS1 positivity compared to IgM, study by Mohite et al²⁵

This study also tried to find the association of thrombocytopenia with dengue parameter. Among 583 seropositive dengue cases thrombocytopenia was detected in 299(51.3%) while only 159(7.3%) cases of 2172 were thrombocytopenic, signaling importance of thrombocytopenia as precious clue for diagnosis.

Out of 378 NS1 positive cases, 224(59.26%) showed thrombocytopenia while 83(49.7%) of exclusively IgM positive cases were thrombocytopenic. This difference was statistically significant using Z test. This is in accordance with study by Tanvi Panwala et al²⁶, Kulkarni et al¹⁷. It also signifies importance of doing NS1 Ag detection test in all febrile patients with thrombocytopenia.

Conclusion

The present study shows that dengue infection is more common in young adult age group favoring males as this group more involved in outdoor activities, which exposes them to mosquitoes. Since monsoon in our region is not heavy & has intermittent showers, peak of case is seen during mid & end of monsoon. For all febrile patients with thrombocytopenia, Dengue NS1 Ag, IgM antibodies detection should be done as low platelet is important but not exclusive test for this infectious disease.

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