ORIGINAL RESEARCH

Distribution of dengue in and around Bangalore Rural to analyze the serological and molecular tests in early infection

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Received: 02Sept, 2023 Accepted: 25Sept, 2023

Abstract
Early diagnosis and management help to reduce the morbidity and mortality rates of severe forms of dengue infection and decrease the risk of wider outbreaks as well. Efficient and accurate diagnosis of dengue is of great importance for clinical care, surveillance activity, outbreak control, academic research, clinical trials and vaccine development. All clinically suspected cases of dengue during the study period were included in this study. This study was conducted using cross-sectional study design. 71 cases observed in 0-15 years age group, 75 cases observed in 75 years age group, 43 cases observed in 31-45 years age group, 21 cases were observed in 46-60 years age group and 6 case observed in >60 years age group. Decreased platelet count observed in 204 (94.4%) out of 216 cases, 153 (70.83%) decreased platelet count observedin first week (1-5 days) of illness and 51 (23.61%) cases decreased platelet count observedin second week (6-11 days) of illness.

Key words: Dengue infection, serological, molecular tests, immunochromatographic test, Enzyme linked Immunosorbent assay

Introduction

Dengue infection is a common arboviral disease prevalent worldwide. World Health Organization has reported that 3.9 billion people are at risk of infection with dengue viruses and around 390 million infections occur per year, of which 96 million manifests clinically.Around 5, 00,000 clinical patients progress to severe illness and require hospitalization.[1]

Dengue is a major public health problem in India. The number of dengue cases has shown an increase in recent years due to rapid urbanization and deficient water management leading to proliferation of mosquito breeding sites. In 2022, about 2,33,251 cases were reported with 303 deaths. The highest numbers of cases were reported from west Bengal followed by Uttar Pradesh, Bihar, Rajasthan, and Punjab.[2]

Dengue is an acute, life threatening viral infection caused by an arbovirus belonging to genus Flavivirus and family Flaviviridae. There are four antigenically distinct serotypes of the dengue viruses (DENV-1-4) causing human infections. The virus is transmitted through the bite of infected female Aedes mosquitoes. [2]

Dengue virus infections manifest with wide spectrum of clinical features ranging in severity from asymptomatic or mild undifferentiated fever to complicated dengue hemorrhagic fever and dengue

shock syndrome. Patients present with sudden onset of fever, headache, severe myalgia, arthralgia, retro orbital pain and macula-papular rash. Small proportion of cases progress to severe disease characterized by plasma leakage, severe thrombocytopenia, haemoconcentration, hemorrhagic shock and multiorgan failure leading to death.Therefore the diagnosis has to be based on high degree of clinical suspicion supported by positive serology and molecular test. [7]

Early diagnosis and management help to reduce the morbidity and mortality rates of severe forms of dengue infection and as well decrease the risk of wider outbreaks. [5]Efficient and accurate diagnosis of dengue is of great importance for clinical care, surveillance activity, outbreak control, academic research, clinical trials and vaccine development. Current diagnostic modalities available are serological test like IgM capture ELISA and detection of NS1 antigen by ELISA, molecular test like detection of RNA of the virus with reverse transcriptase PCR (RT-PCR) and viral isolation. [5,6]

Materials and Methods

This study was done at Department of MicrobiologySSIMS&RC Nelamangalaand this study was cross-sectional design.All clinically suspected cases of dengue were from in and around Bangalore Rural are included. Ethical clearance obtained from the institutional ethical committee. After obtaining informed written consent details of the patient were recorded.

Inclusion criteria: All patients clinically suspected of having dengue were included in this study

Exclusion criteria: In case with proven etiology of disease like pneumonia, otitis, malaria, typhoid or any other infection was excluded.

Sample Collection and Processing

- All patients who are clinically suspected to be suffering from dengue infection were included in the study after obtaining informed written consent. Detailed history regarding clinical features was recorded.
- After this, 5ml of venous blood was collected from the patient in vaccutainers.
- The blood samples collected will be transported to the Microbiology laboratory immediately.[6]

Blood collected in tubes was centrifuged at 3000rpm for 10 minutes to separate serum from blood. Serum samples were subjected to both ICT (J.Mithra PVT LTD) and ELISA NSI antigen ELISA, Dengue IgM antibody ELISA and Dengue IgG antibody ELISA (J.Mithra PVT LTD).The serum samples positive by any one of the above parameters were further processed by molecular assays for the detection of the dengue virus specific RNA. Viral RNA was extracted from 200µl of serum sample using XactSpin viral NA Extraction kit. Real time Reverse Transcriptase PCR was performed using NeoDx Dengue detection PCR kit.

Manufactures instructions were followed strictly while performing the test. Values were calculated and results were interpreted as per manufacture's guidelines.

Results

During the study period, a total of 1461 samples were collected from clinically suspected cases of dengue in and around Bangalore rural. Out of 1461 suspected cases, 216 are seropositive. [Table1].

Table 1: Sero-positives in ICT and ELISA

Sl. No.	Test	Number of positives	NS1	IgM	IgG	NS1,IgM	IgM, IgG
1.	ICT	168	116	35	2	10	5
2.	ELISA	216	150	41	3	15	7

Table 2:Sero-positives ELISA in comparison to ICT

True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	Positivepredictivevalue (%)	Negative predictive value(%)
216	0	1245	48	81.81%	100%	100%	96.28%

The sensitivity and specificity of ELISA is 81.81% and 100% respectively. PPV is 100% and NPV is 96.28%

Table 3: Year wise distribution of suspected dengue cases, seropositive cases and seronegative case

Year	Total number of suspected cases	Total number of sero-positive cases	Total number of sero-Negative cases
2021	248	46 (18.54%)	202 (81.45%)
2022	805	112 (13.91%)	693 (86.08%)
2023	408	58 (14.21%)	350 (85.78%)
Total	1461	216 (14.78%)	1245 (85.21%)

The percentage of seropositive cases in the year 2021 18.54%, 2022 was 13.91% and 2023 was 14.78% till September as shown in Table 3

Table 4: The age distribution of the study population

Age group (Years)	No. of cases	Percentage
0-15	496	33.94%
16-30	548	37.50%
31-45	280	19.16%
46-60	119	8.14%
>60	18	1.23%

The study population taken in all age groups. Representation of different age group were as follows 0-15 years33.94%, 16-30 years 37.50%, 31-45 years 19.16%, 46-60 years 8.14% and >60 years 1.23% as show in Table-4

Table 5: Age and sex wise distribution of sero-positive dengue cases

Age group (Years)	No. of cases	Percentage
0-15	71	32.87%
16-30	75	34.72%
31-45	43	19.90%
46-60	21	9.72%
>60	6	2.7%
Sex		
Male	142	65.74%
Female	74	34.25%

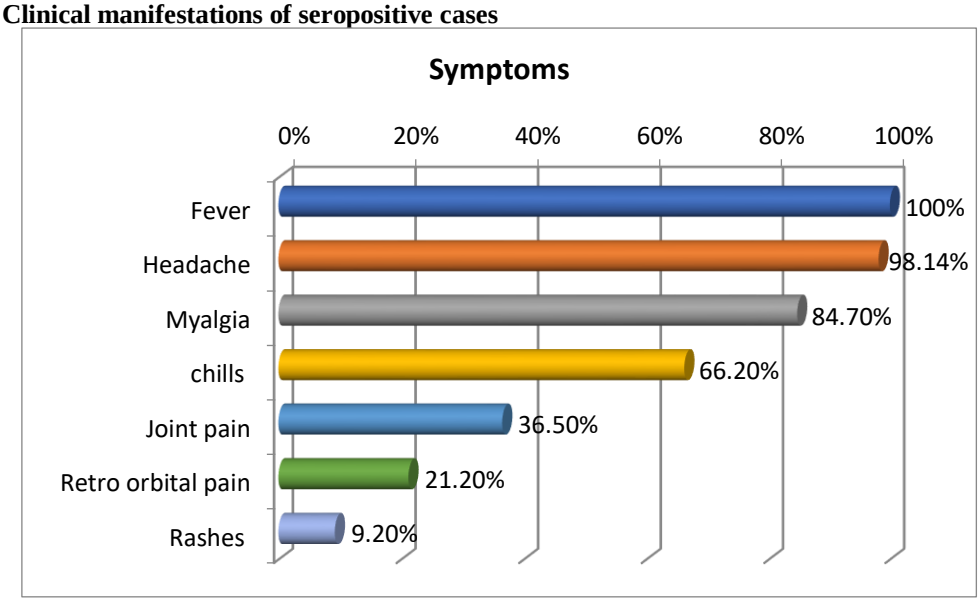
Out of 216 sero-positive cases 142 were males and 74 were females as shown in Table-5.

Seventy one cases observed in 0-15 years age group, 75 cases observed in 75 years age group, 43 cases observed in 31-45 years age group, 21 cases were observed in 46-60 years age group and 6 case observed in >60 years age group. Table-5.

Month wise distribution of sero-positive cases

Seasonal trend in each year showed that there were very less number of positive cases from January to May, the infection start spreading in June, reaching its peak in august, September slowly declined by December. Majority of cases found in July, August and September. However the maximum cases were recorded during the monsoon and post monsoon periods.

Graph:1 Clinical manifestations of seropositive cases



The fever is the most common clinical presentation (100%) whereas rash is seen in only 9.2% of the cases.Graph-1

Table6: Laboratory parameters

Investigation	Normal Range	Number of Cases Decreased	%	Normal	%
Platelet count	1.5 to 4 lakhs/cumm	204	94.4%	12	5.5%
1STWeek (1-5 Days)	1.5 to 4 lakhs/cumm	153	70.83%	8	3.7%
6-11 Days	1.5 to 4 lakhs/cumm	51	23.61%	4	1.8%

Out of 216 cases decreased platelet count showed in 204 (94.4%). 153 (70.83%) decreased platelet count showedin first week (1-5 days) of illness and 51 (23.61%) cases decreased platelet count showedin second week (6-11 days) of illness.

Table-7 Distribution and positivity of seropositive cases and sero-negative cases with ELISA and real time PCR

Seropositive cases=90			Seronegative cases=10	
Parameters	**ELISA**	**Real time**	**ELISA**	**Real time**
NS1	60	60	5	0
IGM	20	6	5	0
NS1+IGM	10	0		
Total	90 (100%)	66 (73%)	100%	100%

Ninety NS1 Ag positive samples (Positive both by ICT and NS1 ELISA) and IgM positive samples (Positive both by ICT and MAC ELISA) along with 10 sero-negative cases were subjected to RT-PCR (Real time Reverse Transcriptase polymerase chain reaction. Out of sero-positive 90 samples 66 (73%) were positive by Real time Reverse Transcriptase PCR assay. Table-7

Discussion

Dengue infection usually presents like any other viral illness but its clinical spectrum ranges from asymptomatic febrile illness to DHF or DSS which has high mortality rate. There is no prevention in the form of any vaccine, as there is no marker to predict the infection from dengue fever to severe dengue forms of the disease, correct diagnosis, early diagnosis, complication and serotype identification is greater importance. Hence, this study has given priority to various diagnostic tests in early diagnosis of dengue fever.

In a study by SathishJV*et al.* in Karnataka, 21.46% seropositivity was reported for dengue infection [3].Gupta.D*et al*. in central India, inurban 70.80% and rural 29.20% seropositivity was reported [4].Damodar T*et al*. reported seropositivity 29% in Mangalore, India[5]. Occurrence of dengue during our study period was found to be 14.78% in clinically suspected cases. The difference in our results from other studies because of epidemiological changes in dengue infection in different location.

In the present study the maximum seropositivity of 34.72% were in the age group of 16-30 years, followed by 0-15 years was observed. Similar result showed by Begum T*et al*. in south India,15-30 years 43% [6]. Damodar T*et al*. reported 54% patients were in the age group of 21-40 years followed by 19% who were 20 years or below [5]. People of all age group are susceptible but relatively greater prevalence is seen in adolescent age group.

It was observed that majority of cases were 142 (65.74%) males followed by 74 (34.25%) females, ratio of (2:1)showing male predominance. Almost similar finding 61% of males were affected observed in a study by Damodar T*et al*. in 2020 [5].In a study by Begum T*et al*. reported 104 were males while 53 were females, ratio of (2:1) [6]. Gupta D*et al*. reported male and female ratio of 2.2:1[4]. Male preponderance might be because of increased exposure at work places or outdoor activities.

In the present study seasonal trend in each year showed that there were very less number of positive cases from January to May, the infection start June reaching its peak in august, September slowly declined by December. The study conducted by SathishJV*et al.* showed positivity rate more in July and peaked at august (55.37%) [3]. RJ Girish Babu *et al*. reported dengue cases were more in monsoon season. The reason may be due to prime occupation of the people being agriculture and breeding of aedesaegypti in highest during pre and post monsoon period[6].

Thrombocytopenia was the common haematological finding noted in maximum number of cases94.4%. A study donebyGirish BRJ*et al*. showed that thrombocytopenia 77.2% of cases [7] and Meena*et al*. reports thrombocytopenia in 90% of cases which is approximately similar to our study.[8]

In our study fever is the most common clinical presentation (100%) whereas rash is seen in only 9.2% of the cases. This is in concordance with a study conducted by DeeptiGupta *et al*., showing fever in 100% cases and rash only in 10%[4].

In the present study the sensitivity and specificity of ELISA is 81.81% and 100% respectively. Gill MK*et al*. showed similar result as 79.71% of sensitivity and 100% of specificity.[9]

In my study maximum positivity of RT-PCR was observed in NS1 positive samples in the first week of fever (97.5%) which is due to high viremia. This is in concordanance with study conducted by Kumar D *et al*. reported that maximum positivity of RT-PCR was observed in NS1 positive samples.[10]

Conclusion

- To conclude, a comprehensive analysis of dengue suspected cases by several serological and molecular analysis together confirms that no single test can be individually used as a diagnostic marker.
- The clinical symptoms are not sufficiently distinctive to provide a lead, especially in the early infection.
- Reporting the number of days post-onset of fever is a valuable tool in deciding the best diagnostic test to be performed.
- Out of different diagnostic techniques used ICT, ELISA and RT-PCR assay proved to be highly sensitive in diagnosis of early dengue infection.
- After 5 days of illness IgM ELISA proved to be more sensitive in diagnosis of dengue infection compared to NS1 ELISA and RT-PCR
- Though real time PCR was found to be a sensitive assay for early diagnosis of dengue, but after 5 days of illness IgM ELISA proved to be more sensitive compared to NS1 ELISA and RT-PCR.

Acknowledgement

We are thankful to Sri Siddhartha Institute of Medical sciences and Research Centre, SSAHE for allowing us to conduct this study.

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