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Research Article

Detection of Anti-Dengue IgM Antibodies in Clinically Suspected Dengue Cases at A Tertiary Care Hospital

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Abstract: Background: Cyclic epidemics of dengue infection are increasing with time in India. The disease shows a wide spectrum of clinical manifestations ranging from mild self-limiting illness to severe fatal haemorrhagic condition. The present study was conducted to detect dengue infection in its peak season in Jamnagar, Gujarat using Dengue capture-ELISA. Materials and Methods: Serum samples from 1979 patients clinically suspected of having dengue infection visiting a tertiary care hospital during the period, from July 2017 to September 2018 were screened for the presence of Dengue IgM antibodies using Dengue capture-ELISA provided by NIV Pune. Results: Amongst 1979 clinically suspected cases of dengue virus infection, 387 were positive for anti-dengue IgM antibodies. Infection was predominant in the age group of 21-40 years (47.54%) and 67.18% of the male population was affected. Seasonal trend showed a peak level of infection in the month of October-December, 2017 (post-monsoon). Conclusion: Epidemiological surveillance of dengue infection is necessary to monitor the spread of dengue virus and for implementation of effective prevention and control strategies.

Keywords: Dengue virus, capture-ELISA, IgM, Haemorrhagic, Post-monsoon, Epidemiological surveillance.

INTRODUCTION

Dengue Virus (DV) is a single stranded positive sense RNA virus belongs to family Flaviviridae under genus flavivirus. Five serotypes of dengue virus (DENV-1, DENV-2, DENV-3, DENV-4) have been found; (5th serotype) DENV-5 was reported in October 2013 detected during screening of viral samples taken from a 37-year-old farmer admitted in Hospital Sarawak, State of Malaysia (Mustafa, M. S. *et al.*, 2015). Dengue is an acute febrile illness caused by transmission of this virus from human to human via bites of Aedes aegypti and less frequently Aedes albopictus mosquitoes (Gubler, D.J. 1998). They typically bite during early morning and in the evening, but may bite throughout the day and thus spreading of infection at any time of day. They prefer to breed in area of stagnant water such as flower vases, uncovered barrels, buckets and discarded tires, but the most dangerous areas are wet shower floor and toilet tank as they allow the mosquitoes to breed in the residence.

Dengue viral infection in human causes a wide spectrum of illness from asymptomatic or mild febrile illness, i.e. Dengue Fever (DF), which may evolve to severe disease form like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (World Health Organization 2009). The characteristic symptoms of dengue are sudden onset of fever, severe headache, retro-orbital pain, muscles, joint and bone pain (the alternative name for dengue, "break bone fever" comes from associated muscle and joint pain), macular or maculo-papular rash and minor hemorrhagic manifestation, including petechiae, ecchymosis, purpura, epistaxis, bleeding gums, hematuria or positive tourniquet test result (chikungunya cases reported, 2006; Rothman, A.L., 2004).

The dengue virus genome is about 11,000 base of positive-sense single stranded RNA (ssRNA) that coded for 3 structural proteins (capsid protein C, membrane protein M, envelope protein E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5), it also included short noncoding region on

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both the 5' and 3' end (Centers for Disease Control and Prevention. 2016).

MATERIALS AND METHODS:

STUDY DESIGN

A retrospective study was conducted at Microbiology Department of M. P. Shah Govt. Medical College and Guru Gobindsingh Hospital Jamnagar, Gujarat (India) from July 2017 to September 2018. Total 1979 blood samples were received from different wards of Guru Gobindsingh Tertiary Care Hospital from suspected cases of dengue fever and tested for anti-dengue IgM antibody by IgM-capture ELISA which was supplied by NIV Pune.

Sample collection and storage:

Patients suspected of dengue fever were examined by hospital clinicians at either outpatient services or for inpatients, when attending the emergency unit or upon admission to a ward. A single blood sample (approximately 2-3 ml) was collected from each patient suspected of dengue virus infection at the time of admission into hospital. Specimen collection and separation of serum were performed using strict aseptic precautions and following standard microbiological methods. Serum samples for ELISA test were prepared and stored at 2-8°C until tested.

Detection of anti-dengue IgM by capture ELISA:

Serum samples were screened for dengue IgM antibody by μcapture dengue IgM Enzyme-Linked Immunosorbent Assay (ELISA) kit was used (supplied by the National Institute of Virology, Pune, under the National Vector-Borne Disease Control Program). The presumptive diagnosis by NIV dengue MAC-ELISA maybe confirmed by a confirmatory test as per WHO guidelines (Dengue Guidelines for Diagnosis 2009). Manufacturers' instructions were strictly followed for performing the test and interpreting the results. Optical Density (O.D.) was measured at 450 nm using ELISA reader method at Department of Microbiology of M. P. Govt. Shah Medical College Jamnagar, used and test results were interpreted either positive or negative according to manufacturers' instructions. The sensitivity and specificity of detection quoted by the manufacturer were 98.53% and 98.84%, respectively. This diagnostic kit provided qualitative detection of IgM antibodies specific to dengue virus in human serum, dependent on the following principle. IgM antibodies in patients' serum are captured by antihuman IgM (μ chain specific) coated on to the solid surface (wells). In the next step, dengue antigen is added, which binds to capture human IgM in the sample. Unbound antigen is removed during the washing step. In the subsequent step, biotinylated flavivirus anti-DEN monoclonal antibodies are added followed by avidin-HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added. The reaction is stopped by 1N H_2SO_4. The intensity of colour/optical density is measured at 450 nm. The test was standardized and reported by NIV in 1984 (Gadkari, D.A., Shaikh, B.H. 1984). The performance of the test was evaluated by Christian Medical College (CMC), Vellore, in 2002 (Sathish, N. *et al.*, 2002).

Interpretation of Results:

1. If OD, value of sample tested is less than OD of negative control by a factor 2.0, the sample should be considered as negative for dengue IgM.
2. If OD value of sample tested exceeds OD of negative control by a factor 3.0, the sample should be considered as positive for dengue IgM.

RESULTS AND DISCUSSION

Out of the 1979 cases tested, 387 (19.56%) were positive for Dengue IgM Antibody.

Table-1: Sero-prevalence of Dengue IgM Antibody

Sample Tested	Positive Sample	Sero-prevalence (%)
1979	387	19.56%

Out of these 387 positive samples, males were 260 (67.18%) and females were 127 (32.82%) (Table-2 & Figure-1).The chi-square statistic is 4.3875 and P-value is 0.0362 this show male to female ratio was statistically significant (P-value <0.05)

Table-2: Sex wise Sero-positivity of Dengue IgM Antibodies

	Total Samples	Positive Samples (%)	Chi-square	P value
Male	1218	260 (67.18%)	4.3875	<0.05
Female	761	127 (32.82%)		
	1979	387 (100%)		

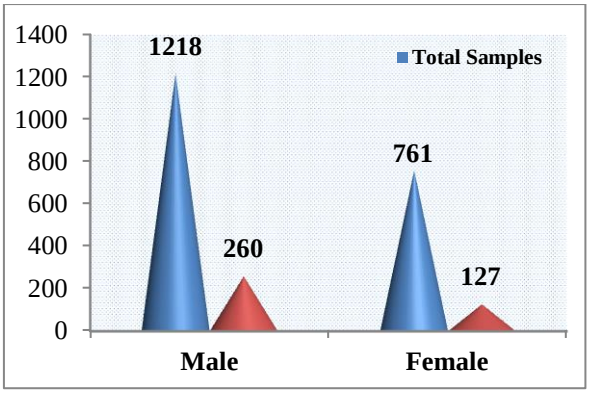

Fig.--1: Sex wise Sero-positivity of Dengue IgM Antibodies

Among the total positive case, 139 (35.92%) were between 0 to 20 years of age groups, 184 (47.54%) were between 21-40 years of age years of age groups, 64 (16.54%) were from>40 years of age groups.

Table-3: Age-group wise Sero-positivity of Dengue IgM Antibodies

Age(Years)	Total Samples	Positive Samples (%)
0-20	820	139 (35.92%)
21-40	812	184 (47.54%)
>40	347	64 (16.54%)
	1979	387

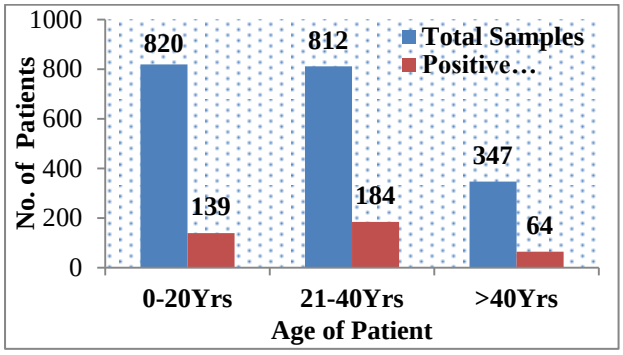

Fig.-2: Age-group wise Sero-positivity of DengueIgM Antibodies

Seasonal Variation of Positive Dengue IgM antibody Cases:

In this study, out of 387 confirmed positive cases for dengue IgM antibody. Highest positive case, 229 positive cases was found in between October 2017 to December 2017 in post-monsoon season, followed by 82 positive cases was found in July 2017 to September 2017 and 48 positive cases was found in July 2018 to September 2018 in monsoon season (Table-4).

Table-4: Month wise distribution of Dengue IgM Antibodies

Month	No. of cases (n=1979)	Positive cases (n=387)
July-17	85	17
August-17	142	12
September-17	247	53
October-17	310	92
November-17	386	108
December-17	156	29
January-18	82	9
February-18	74	4
March-18	41	2
April-18	34	4
May-18	35	3
June-18	49	6
July-18	71	4
August-18	119	14
September-18	148	30

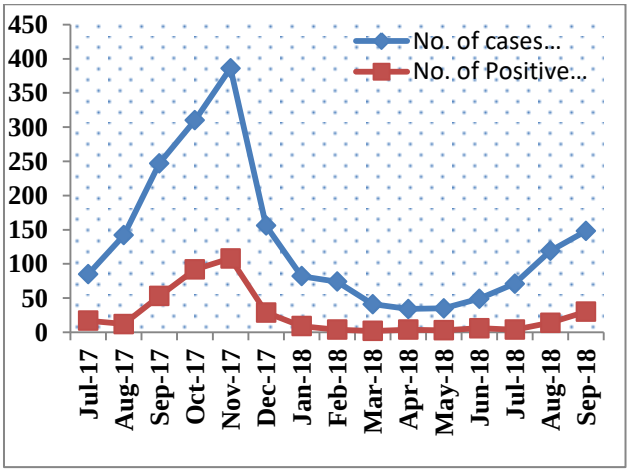

Fig.-3: Month wise distribution of Dengue IgM Antibody cases

DISCUSSION

Statistically comparison of present study with other study done. In present study, seroprevalence was 19.56% (387/1979) is very much similar to Smita sood *et al.*, (2013) study show 18.99% (412/2169), Neeti Mishra *et al.*, (2017) study show 18.6% (147/789) and Gamit SC *et al.*, (2017) study shows 21.13% (254/1202) sero-prevalence.

In present study positive case from male and female were 260 (67.18%) and 127 (32.82%), respectively, which is very much similar to Ingale H *et al.*, (2017) study, male and female positive case is 63.81% and 36.19% respectively and Gamit SC *et al.*, (2017) study, male and female positive case is 69.69% and 30.31% respectively.

In present study, 47.54% (184/387) positive cases for dengue IgM antibody were between 21-40 years of age group, which much similar to Nishant *et al.*, (2015) study, 46.89% (797/1700).

In present study, 59.17% (229/387) positive cases were found during post monsoon season, which much similar to Nair A. B. *et al.,* (2016) study show 56% (14/25).

CONCLUSION

Dengue fever is an acute febrile arbo-viral disease affecting the tropical and subtropical regions of the world. Dengue is endemic to the Indian sub-continent and it is associated with explosive urban epidemics. Dengue is a notifiable disease and has become a major public health problem in India. It is important to study the exact prevalence of dengue.

The accurate early and efficient diagnosis of the disease is important for clinical care, surveillance, pathogenesis studies and vaccine research. As just based on clinical presentation we cannot diagnose Dengue infection, efficient laboratory diagnosis is an important tool to support Epidemiological Surveillance Programs.

There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates. Dengue is usually a short lasting and self-limiting disease. However, severe infections can be lethal, especially if it is a secondary infection. Public awareness and control of vector are important factors to be taken into consideration in order to control dengue.

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