A STUDY ON SEROPREVALENCE OF DENGUE IGM ANTIBODY IN SUSPECTED CASE OF DENGUE FEVER IN AND AROUND JAMNAGAR, GUJARAT (INDIA)

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ABSTRACT

BACKGROUND

Dengue virus belongs to family Flaviviridae, it spread by the bite of infected Aedes mosquitoes. It breeds in fresh water. It causes a wide spectrum of illness from mild illness to severe fatal Dengue Haemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS).

The aim of the study is to find out seroprevalence of dengue IgM in and around Jamnagar by presence of virus specific dengue IgM antibody from serum samples taken from suspected dengue patients.

MATERIALS AND METHODS

This is retrospective study conducted at tertiary care hospital, Jamnagar. Patient with history of dengue fever more than 5 days were included in the study. Serum samples were screened for dengue IgM by ELISA method. Study was conducted in our institute from July 2016 to June 2017. Total 1202 serum samples of suspected patient of dengue IgM by ELISA method.

RESULTS

Out of 1202 samples, total 254 (21.13%) were positive for dengue IgM Ab among this- 177 (69.69%) male and 77 (30.31%) female and 78 (30.71%) were 0-20 of age, 131 (51.57%) were 20-40 of age and 45 (17.72%) were 40 of age. 175 (82.28%) positive cases between October to December in post-monsoon season.

CONCLUSION

Study shows that prevalence of dengue fever is more in post-monsoon season, more common in middle age group and in male than female, so early diagnosis is very important to reduce mortality rate.

KEYWORDS

Dengue, Seroprevalence, Jamnagar, ELISA, IgM, P-Value, Post Monsoon.

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BACKGROUND

Dengue Virus (DV) is a single stranded positive 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.[1] 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.[2] They typically bite during early morning and in the evening, but may bite throughout the day and thus spread infection at any time of day.

They prefer to breed in area of stagnant water such as flower vases, uncovered barrels, buckets and discarded tyres, 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 Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[3] 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 maculopapular rash and minor haemorrhagic manifestation, including petechiae, ecchymosis, purpura, epistaxis, bleeding gums, haematuria or positive tourniquet test result.[4,5] 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,

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NS4a, NS4b, NS5),[6] it also included short noncoding region on both the 5' and 3' end.[7]

Dengue become a global problem since Second World War and common in more than 110 countries. Each year, around 50 million of peoples are infected and approximately 10,000 to 20,000 peoples die.[2,8] Over the last 50 years, the incidence of dengue has risen 30 fold with increasing geographically expansion to new countries and in the present decade from urban to rural setting mainly in tropical and subtropical areas. An estimated 50 million dengue infection occur annually and approximately 2.5 billion peoples live in dengue endemic countries, some 1.8 billion (more than 70%) of peoples at risk for dengue worldwide live in member state of the World Health Organization (WHO), which had been nearly 75% of global burden due to dengue.[9]

In 2006, dengue outbreak in India, cases of dengue fever were reported first from New Delhi in early September and by end of September other states also started to report deaths. Approximately, 3613 confirmed cases of dengue fever were reported and over 50 peoples died in outbreak.[10] In this, 424 positive cases reported from Gujarat district.

MATERIALS AND METHODS

Study Design- This retrospective study was conducted from July 2016 to June 2017 at Shri. M. P. Shah Medical College, the principal tertiary care centre in Jamnagar District, Gujarat State. Sample received to tertiary care hospital Jamnagar from indoor patient who admitted in hospital, outdoor patients, community and primary health center of suspected cases of dengue fever more than 5 days. A total of 1202 samples were collected and processed for anti-dengue IgM by IgM-capture ELISA.

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. All cases of fever for which the individual showed two or more of the following dengue-like signs and symptoms were suspected as a dengue virus infection patient unless strong evidence of other infections were justified- retro-orbital pain; muscle and joint pain; loss of appetite; vomiting; diarrhoea; abdominal pain; metallic taste in mouth; leucopenia and thrombocytopaenia in complete blood cell count; haemorrhagic manifestations.

A single blood sample (approximately 2-3 mL) was collected from each patient suspected of dengue virus infection at the time of admission to hospital. Specimen collection and separation of serum were performed using strict aseptic precautions and following standard microbiological methods. Serum samples for ELISA 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 μ-capture 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.[9] 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 Shri M. P. 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/H2O2) is added. The reaction is stopped by 1N H2SO4. The intensity of colour/optical density is measured at 450 nm. The test was standardised and reported by NIV in 1984.[11] The performance of the test was evaluated by Christian Medical College (CMC), Vellore, in 2002.[12]

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

In this study, total 1202 serum samples of suspected case dengue virus infection, collected and processed for dengue IgM antibody between July 2016 to June 2017. Total 254 found positive for dengue IgM, so seroprevalence of dengue IgM antibody was 21.13% (Table 1).

Total	Positive	Seroprevalence
1202	254	21.13%
Table 1		

Age and gender wise distribution of confirmed case of dengue IgM from 1202 suspected cases of dengue, 492 belonged to 0-20 years age group, 490 belonged to the 20-40 years age group, whereas the lowest number belong to the age group >40 years. Gender-wise, 63.06% of suspected cases were males (758/1202) and 36.94% were females (444/1202).

Age (In years)	Tested (n=1202)	Positive (n=254)	Chi-Square	P-Value
0-20	492	78 (30.71%)	11.382	<0.01
21-40	490	131 (51.57%)		
>40	220	45 (17.72%)		
Table 2				

Table 2 (Age wise Distribution)- Among the total positive case, 78 (30.71%) were between 0-20 years of age groups, 131 (51.57%) were between 21-40 years of age groups and 45 (17.72%) were from > 40 years of age groups. The Chi-square statistic is 11.382. P-value is 0.00337, this show age groups wise distribution of dengue IgM is statistically significant (P-value is <0.01).

Sex	Tested (n=1202)	Positive (n=254)	Chi-square	P-Value
Male	758	177(69.69%)	4.003	<0.05
Female	444	77(30.31%)		

Table 3

Table 3 (gender wise distribution)- among the total 254 positive case of dengue IgM, 177 (69.69%) male and 77(30.31%) female. The Chi-square statistic is 4.0033. P-value is 0.045, this show male-to-female ratio was statistically significant (P-value is <0.05).

Monthly distribution of positive cases.

Month	Total (n=1202)	Positive (n=254)
July - 2016	61	4 (1.57%)
August - 2016	110	15 (5.91%)
September - 2016	169	34 (13.39%)
October - 2016	267	82 (32.28%)
November - 2016	132	40 (15.75%)
December - 2016	213	53 (20.87%)
January - 2017	70	10 (3.94%)
February - 2017	34	0 (0.00%)
March - 2017	44	3 (1.18%)
April - 2017	24	5 (1.97%)
May - 2017	32	6 (2.36%)
June - 2017	46	2 (0 78%)
Total	**1202**	**254**

Table 4

Table 4 (Monthly distribution)- Monthly percentage detection of confirmed positive dengue cases between July 2016 to June 2017 n clinically suspected patients presenting at tertiary care hospital, Jamnagar, Gujarat State. Most of positive case (82 of 254) occurred in October 2016 followed by December 2016.

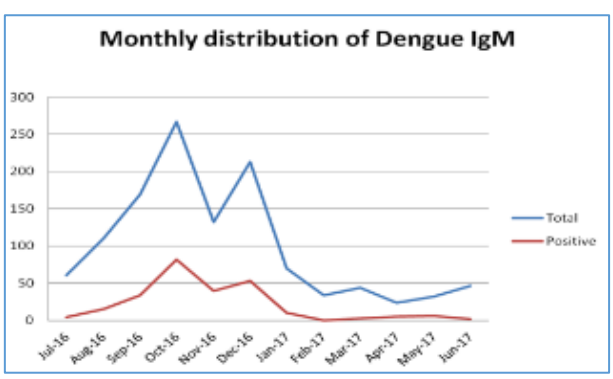

Figure 1

Figure 1- Monthly distribution of clinically suspected (blue) and confirmed positive (red) case of dengue IgM between July-2016 to June-2017 at tertiary care hospital, Jamnagar, Gujarat.

Seasonal Variation of Positive Dengue IgM Cases- In this study, out of 254 confirmed positive case for dengue IgM. Highest positive case (175 positive cases) was found in between October 2016 to December 2016 in post-monsoon season, followed by 53 positive case was found in July 2016 to September 2016 in monsoon.

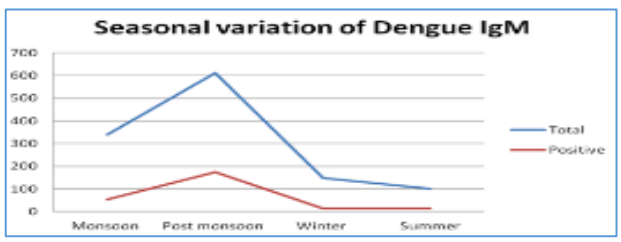

Figure 2

Figure 2- Seasonal distribution of clinically suspected (blue) and confirmed positive (red) case of dengue IgM between July-2016 to June-2017 at tertiary care hospital Jamnagar, Gujarat

DISCUSSION

Statistically comparison of present study with other study done. (1) In present study, seroprevalence was 21.13% is very much similar to Sanghmitra study (21.05%).[13] (2) In present study positive case from male and female were 177 (69.69%) and 77 (30.31%), respectively, which is very much similar to Nishant study (male and female positive case is 62.47% and 37.53% respectively).[14] (3) In present study, 131 (51.57%) positive cases for dengue IgM antibody were between 21-40 years of age group, which much similar to Nishant study (46.89%).[14] (4) In present study, 175 positive case (68.90%) found during post monsoon season, which much similar to Nishant study (79.64%)[14] (Table-5).

Study	Seroprevalence	Male	Female	Age Groups (21-40 years)	Post Monsoon season
Present Study	21.13%	69.69%	30.31%	51.57%	68.90%
Sanghmitra Padhi[13]	21.05%	61.83%	38.17%	44.50%	-
Nishant Hussain[14]	31.27%	62.47%	37.53%	46.89%	79.64%

Table 5

Dengue is an important emerging disease of the tropical and subtropical regions of the world. In common with other vector-borne diseases, dengue requires conducive predisposing conditions for endemicity and outbreaks. The countries of South East Asia share such common features as large human populations, rapid urbanisation, development activities and monsoon rains. Urban populations now

constitute the natural reservoir and travelers are the predominant factor for dissemination of viruses between countries. In this study, 21.13% of patients were serologically positive for dengue IgM antibody. This higher prevalence rate maybe due to its endemic nature in that country. The age wise distribution of seropositive cases shows that a statistically significant proportion of cases was in the 21-40 years age group as compared to other age groups ($P<0.01$). The prevalence of dengue-positive cases was also statistically significant towards males over females ($P<0.05$). This difference is probably due to a gender-related variance in lifestyle, namely the relatively higher exposure of males over females to an outdoor environment through farming and other occupations. In the present study, cases of dengue were reported all year round at a very low level in winter and summer months, so it was possible for dengue virus to be transmitted irrespective of prevailing climactic conditions. However, the preponderance of anti-dengue IgM antibody-positive cases was reported in post monsoon season. This was indicative of a cyclic increase in the occurrence of dengue fever consequent to the post monsoon season in October to December, which would trigger an explosion in the population of A. aegypti mosquito vector for dengue transmission.

CONCLUSION

Dengue is becoming an emerging problem in India. It demands early investigation and careful management. IgM capture ELISA was used in this study and our informed opinion remains a reliable and routine method for the diagnosis of dengue in our institute and similarly less-developed countries. National control programs should address this major public health issue in all its aspects including diagnosis, clinical management and continued surveillance. Moreover, capacity building should aim to provide the infrastructure to facilitate molecular laboratory confirmation of virus detection and to enable virus isolation.

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