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A Study on Seroprevalence of Dengue Infection in a Tertiary Care Centre and Role of Rapid NS1 Antigen Test in Early Diagnosis

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Abstract: ***Introduction: Dengue virus infection is one of the most important vector-borne human arboviral infection and has emerged as a notable public health problem in recent decades. AIMS: To study the trend of seroprevalence of dengue infection and to highlight the usefulness of rapid NS1 antigen test in early diagnosis of dengue. Materials and Method: A retrospective study was done in the Department of Microbiology of a tertiary care centre during the study period of October to December in the year 2017, 2018 and 2019. The serum samples were subjected to immuno-chromatographic Dengue NS1 antigen, IgM / IgG Antibodies rapid kit test and Dengue IgM Mac ELISA test. Results: In October to December 2019, 1138 samples of suspected dengue cases were sent to our lab for detection by rapid kit tests. Out of which 161 (14.1%) samples were NS1 positiv, 38 (3.3%) were only IgM positive and 4 (0.4%) were IgG positive. We received 107 samples for Dengue IgM MAC Elisa of which 28 (26.2%) were positive and 25 (23.4%) were equivocal. In October to December 2018, 285 samples were received for detection by rapid kit tests .Out of which 16 (5.6%) samples were NS1 positive, 11 (3.9%) were only IgM positive and 17 (6.6%) were IgG positive ;and 158 samples for IgMMAC Elisa of which 26 (16.5%) were positive and 23 (14.6%) were equivocal. In October to December 2017,out of a total of 165 samples for detection by rapid kit tests ; 18 (10.9%) samples were NS1 positive, 19 (11.5%) were only IgM positive and 5 (3%) were IgG positive; and 261 samples for IgM MAC Elisa of which 21 (0.1%) were positive and 21 (0.1%) were equivocal. Conclusion: This study reported an increasing trend in seroprevalence of Dengue virus which affected many people in recent years and also highlights that the availability of commercial dengue NS1 antigen test kits has provided an additional laboratory diagnostic tool for early detection of Dengue.***

Keywords: Dengue, Seroprevalence, rapid, NS1, IgM, ELISA

List of abbreviations
DENV = dengue virus
DF = Dengue fever
DHF = Dengue haemorrhagic fever
DSS = Dengue shock syndrome
NS = Non - structural proteins

1. Introduction

Dengue virus infection is one of the most important vector-borne human arboviral infection.[1] Denguearboviruses are transmitted by the mosquitoes : Aedesaegypti and Aedesalbopictus.[2]This viral infection may be asymptomatic or may give rise to undifferentiated fever with or without other associated clinical manifestations, namely, Dengue fever(DF), Dengue haemorrhagic fever(DHF),or Dengue shock syndrome(DSS).[3]ClassicDengue fever is presented by a rapid onset of high grade fever, headache, retro-orbital pain, diffuse myalgia, weakness, vomiting, sore throat, an altered taste sensation, and a centrifugal maculo-papular rash.[4]

DENV infection has emerged as a notable public health problem in terms of the mortality and morbidity associated with it in recent decades.[5]According to World Health Organization (WHO), Dengue represents a pandemic threat and is the fastest spreading tropical disease.[6]Appropriate clinical management can reduce the mortality to less than 1% and can save the lives of DHF and DSS patients.[7]Hence early and rapid laboratory diagnosis of Dengue is crucial.

Dengue virus belongs to family flaviviridae and consists of 10 proteins; 3 structural and 7 non-structural.[8]Non-structural protein 1 or NS1 is a highly conserved glycoprotein, which is essential for virus replication. NS1 protein is associated with intracellular organelles and can be transported via cellular secretion pathway to the infected cell surface during the acute phase of DENV infection. NS1 protein was also found to be released from infected mammalian cells and may be found circulating in the sera of patients.[9] The NS1 antigen can be detected on days 0-9 after the onset of symptoms and is found together with endothelium, free or soluble in the serum of patients.[10]There is no cross-reaction of Dengue NS1 protein with those of other related flaviviruses as it was not found in patients with Japanese encephalitis or yellow fever virus infections.[17] Thus,

detection of NS1 has been a promising test to diagnose Dengue in its early febrile stage.[11]

Though dengue IgM detection is a commonly performed test for diagnosis of Dengue, it has limitations due to cross-reactivity between other circulating flaviviruses. Dengue virus specific IgM antibodies appear as early as three days of Dengue viral fever and can persist for 30-60 days and IgG antibodies appear at about seventh day, peak at 2-3 wk and persist for life.[12]

In the present study, we report the trend of seroprevalence of dengue infection in Goa Medical College, a tertiary care centre over a period of 3 consecutive years 2017-2019 in post-monsoon season from October to December and highlight the usefulness of rapid NS1 antigen test in early diagnosis of DENV infection.

2. Materials and Method

A retrospective study was done in the Department of Microbiology of Goa Medical College, a tertiary care centre in Goa over a period of 3 consecutive years during the months of October, November and December in the year 2017, 2018 and 2019. The serum samples obtained from various out-patient departments and in-patient wards obtained during October to December in the above consecutive years were subjected to serological tests to assess the magnitude of dengue infection and the rising or falling trend of seroprevalence over a period. Trend was obtained by calculating the percentage positivity of various serological tests done during the above period in each year.

The samples were subjected to immuno-chromatographic (Dengucheck combo) rapid kit for detection of Dengue NS1 antigen and IgM/ IgG antibodies in human serum. Dengue check combo is a new generation rapid immuno-chromatographic test system for detection in very early stage and differential diagnosis of primary or secondary DENV infection. The procedure and interpretation of test results of the rapid assays were carried out according to the manufacturer's literature guidelines. Also samples sent during above period by clinicians specifically for detection by Dengue ELISA test were tested by Dengue IgM Mac ELISA test by NIV DEN IgM Capture ELISA kit. Elisa test was performed as per the manufacturer's instructions.

The NS1 positivity indicates an acute dengue infection.[13]The primary infection was defined by a visible IgMb and without a visible IgGband, whereas a secondary infection was defined by a positive IgG band with or without a positive IgM band. Specific antibody response to dengue virus enables serodiagnosis and differentiation between primary and secondary dengue infections and detection of potentially life threatening conditions such as DHF and DSS.

3. Results

In October to December 2019, we received a total of 1138 samples for detection by rapid kit tests.Out of which 161 (14.1%)samples were positive for NS1, 38 (3.3%) were only IgM positive and 4 (0.4%) were IgG positive. The positivemalesamples were 136 (67%) and female were 67 (33%) with male: female ratio of 2:1. Amongst the paediatric age group out of 244 samples 30 (12.3%) were positive. 107 samples were received for Dengue IgMMAC Elisa of which 28 (26.2%) were positive and 25 (23.4%) were equivocal.

For the study period in 2018, 285 samples were sent to our lab for detection by rapid kit tests .Out of which 16 (5.6%)samples were positive forNS1, 11 (3.9%) were only IgM positive and 17 (6.6%) were IgG positive. The positive male and female samples were 32 (72.7%), 12 (27.3%) respectively (M:F ratio=2.6:1). 59 samples were paediatric patients of which 3 (5.1%) samples were positive. Out of 158 samples for Dengue IgMMAC Elisa test 26 (16.5%) were positive and 23(14.6%) were equivocal.

In the year 2017 from October to December, out of 165 samples for detection by rapid kit tests; 18 (10.9%) samples wereNS1 positive, 19 (11.5%) were only IgM positive and 5 (3%) were IgG positive. The positive male and female samples were 31 (73.8%), 11 (26.2%) respectively (M: Fratio=2.8:1). 26 samples belonged to paediatric age group of which 1 (3.8%) sample was positive. Amongst 261 samples for Dengue IgMMAC Elisa test 21 (0.1%) were positive and 21 (0.1%) were equivocal.

Table 1: Result of samples of suspected dengue cases received for detection of Dengue by rapid kit tests

Study period	Total samples	Ns1 positive samples	Only IgM positive	IgG positive with/ without IgM
Oct-Dec 2019	1138	161 (14.1%)	38 (3.3%)	4 (0.4%)
Oct-Dec 2018	285	16 (5.6%)	11(3.9%)	17 (6.6%)
Oct-Dec 2017	165	18 (10.9%)	19 (11.5%)	5 (3%)

Table 2: Result of samples of suspected dengue cases received for detection of Dengue by Dengue IgM MAC Elisa

Study period	Total samples	Positive	Equivocal
Oct - Dec 2019	107	28 (26.2%)	25 (23.4%)
Oct - Dec 2018	158	26 (16.5%)	23 (14.6%)
Oct- Dec 2017	261	21 (0.1%)	21 (0.1%)

4. Discussion

Laboratory diagnosis by serological tests is very crucial along with clinical correlation to confirm the diagnosis of Dengue infection.There is a considerable increase in number of suspected Dengue cases and the samples receivedin our tertiary care centre for detection by rapid kit tests. We received 1138 samples during study period of October-December in 2019 compared to 285 samples in 2018 and165 samples in 2017. Dengue has been held to be a disease of high population density tropical urban areas. However, increasing reports of Dengue cases and outbreaks were reported from rural areas of western india.[14] Also, poor sanitation facilities and rapid unplanned urbanization with heavy construction activities contribute to fertile breeding grounds for the mosquitoes.

There is increase in the seroprevalence in 2019 compared to 2018 -17ie. 14.1 % NS1 positive cases indicating acute infection compared to 5.6% and 10.9% in previous years. The trend of distribution of cases and the burden of Dengue

cases during the study period (2017 -2019) is similar to neighbouring states Maharashtra and Karnataka as per the NVBDCP data of *dengue cases and deaths in the country since 2015* showing increase in dengue cases in 2019 compared to previous years.[15]

Dengue IgM MAC Elisa test showed increasing trend of seroprevalence from 2017 to 2019 during the study period. However, there was a decrease in samples received for Dengue IgM MAC Elisa test owing to increased sensitivity and availability of rapid tests like NS1 antigen detection rapid kit in recent years. Samples collected up to day 3 after the onset of symptoms showed more sensitivity byNS1 antigen assay as was showed by a study of Dussart et al.[16] Also there is no cross-reaction of dengue NS1 protein with those of other related flaviviruses as the Dengue NS1 antigen was not found in patients with Japanese encephalitis virus or yellow fever virus infections.[17]The incidence of NS1 positivity i.e. acute infection by Dengue virus has increased considerably in 2019 to14.1% from 5.6% in 2018.

Amongst the paediatric age group the trend of seroprevalence showed that infection rate has increased to 12.3% in 2019 compared to 5.1% and 3.8% in 2018 and 2017 respectively. In some parts of the world, Dengue is mainly a paediatric health problem.[19]

The male female ratio showed male preponderance with ratio around 2:1 in all the three consecutive years and this finding is in concordance with that of an earlier study.[18]Themale preponderance indicates more transmission of Dengue at work sites.However, the study findings are the representation of patients who visited our tertiary care centre during the study period rather the truly infected population.

The study presents the occurrence of Dengue during October –December. It was observed that dengue transmission occurred round the year with highest incidence in the post-monsoon period i.e. to December with a peak incidence around September –October.[18]An increase in Dengue cases in post monsoon months is due to the presence of stagnant water after rainfall which favors the breeding of mosquito vector. Hence, vector control measures should be implemented during the monsoon and post monsoon months.

5. Conclusion

This study reported an increasing trend in seroprevalence of Dengue virus which affected health of many people in recent years; thismay be a warning sign of the future epidemics; hence there is a need to develop vaccine that can protect against all serotypes. The availability of commercial Dengue NS1 antigen test kits has provided an additional laboratory diagnostic tool for early detection of DENV. Such tests may be used in laboratories that have limited resources, lack viral culture, or RT-PCR facilities. Also since most cases were reported during post monsoon period, continued and coordinated efforts coupled with public awareness should be made to control the transmitting vectors to prevent Dengue.

6. Future Scope

Our study provides prevalence based on the patient load in our tertiary care centre alone, hence a more coordinated study including data from all the hospitals and health centres of the state will provide the actual prevalence of the truly infected population.

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Conflict of interest- nil

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