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Seroprevalence of Dengue by Detection of NS1, IgM in Tertiary Care Hospital, RIMS, Ranchi

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ABSTRACT

Introduction: Dengue virus being most significant among vector borne diseases is leading to increased morbidity and mortality in India. Global prevalence of dengue has been increasing over the last decade. So, it is important to screen all dengue fever like cases.
Objective: The study was conducted to know seroprevalence of Dengue virus by detecting NS1, IgM in clinically suspected dengue fever cases.
Material and Method: Test was done in 315 blood samples of clinically suspected dengue fever cases, in various departments of RIMS, Ranchi. The study was done from November 2016 to August 2017. NS1 antigen and IgM antibody detection both were done by ELISA (NS1 by Qualisa Microwell Enzyme Immunoassay and IgM by NIV Dengue IgM Captured ELISA KIT).
Result and Discussion: Out of 315 blood samples, 84 samples (26.7%) were serologically positive for dengue infection. Among the positive cases 72.6% were males and 27.4% were females. Males are more affected than females, because males are more exposed during their outdoor visit for livelihood. Age group between 21-30 years were most affected. Most of the cases were reported in monsoon and post-monsoon season.
Conclusion: This study highlights the need of continuous surveillance by both NS1 antigen and IgM antibody by ELISA method for early detection of impending outbreak to initiate timely prevention and control measures.

Keywords: *Dengue, NS1, IgM*

INTRODUCTION

Dengue is a mosquito borne disease and transmitted mainly by *Aedes aegypti* and *Aedes albopictus mosquito*.[1] Wide range of symptoms are seen in infection ranging from mild non-specific fever to more severe and lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)2. It is caused by virus *Flaviviridae*. There are four serotypes of the virus referred to as DV-1, DV-2, DV-3 and DV-4. It is a positive-stranded encapsulated RNA virus with three structural protein genes. Genes encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins.1

According to classification of WHO 2009 dengue fever is classified into two groups: uncomplicated and severe[3], but still the 1997 WHO classification is widely used[4]. Dengue is classified into undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF) according to the 1997 classification. [3] There are four main characteristic manifestations of dengue illness, *(i)* continuous high fever lasting 2-7 days; *(ii)* thrombocytopaenia (platelet count $<100\times10^9$/l); *(iii)* haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; and *(iv)* evidence of plasma leakage manifested by haemoconcentration (an increase in haematocrit 20% above average for age, sex and population), ascites and pleural effusion and, *etc*. [1]

In routine laboratories Dengue IgM and IgG ELISA kits are widely used for diagnosis of dengue infection. Variations are there in detection limit during acute phase of the disease. Usually it takes 4–5 and 1–14 days respectively after the onset of symptoms for anti-DENV IgM and IgG antibodies to become detectable. But, it depends whether the patient has primary or secondary infection.[5]The gold standard for diagnosis of acute cases is isolation of virus in cell culture or in infant mouse. This requires specialized laboratories which takes more than a week for the test to be completed. In most of the situation it becomes impractical. Early diagnosis during febrile phase can be detection of viral RNA by RT-PCR. The procedure is cumbersome, in case of RT-PCR with difficult interpretation lack of immediate result thus the procedure becomes difficult.[6] In laboratory, during the late incubation period, or initial phase of dengue virus infection, for disease confirmation detection is done by viral isolation in cell culture and/or molecular investigations, or immunohistochemistry or immunofluorescence.[7]

For detection of dengue virus in the early stages of the disease there is need for rapid, sensitive methods.6 Right at the beginning of the feverish period and before the appearance of dengue IgM and/or IgG, non-structural antigen non-structural protein 1 (NS1) develops.[8]

During the first few days of illness, ELISA assays is used in combination of NS1 and IgM detection in samples. It was recommended to increase overall dengue diagnostic sensitivity.[9]

Platelet therapy is used in standard clinical practice for dengue patients with severe thrombocytopenia.[10] Platelet count is not done as introductory screening, in many cases. This results in delays in starting platelet therapy. In New Delhi, during the 2010 spurt in the incidence of dengue, a simultaneous screening for NS1, IgM and IgG and platelet enumeration was launched at the Sant Parmanand Hospital.[11]

In state of Jharkhand, RIMS is one of the best tertiary care institutes. Department of Microbiology, in RIMS, Ranchi caters in diagnosis wide range of vector-borne diseases and other infectious diseases. The study aims to know seroprevalence of Dengue virus by detecting NS1, IgM in clinically suspected dengue fever cases.

MATERIAL AND METHODS

This retrospective study was conducted in the department of Microbiology (Virology) RIMS, Ranchi. Study period was from November 2016 to August 2017. Total 315 blood samples of clinically suspected cases of dengue fever were collected from both outdoor and hospitalised patient from RIMS, Ranchi. Patient's details were taken from data recorded in Department of Microbiology, RIMS, Ranchi. After standard separation of serum, samples were analysed for the detection of NS1 antigen and IgM antibodies. Both the tests were done by ELISA method (NS1 by Qualisa Microwell Enzyme Immunoassay) and (IgM by NIV Dengue IgM captured ELISA Kit. Manufacturer's instructions were strictly followed for performing the test and interpreting the results.

RESULT

A total of 315 blood samples were tested from suspected dengue fever cases. Of these 84 samples (26.7%) were serologically positive to have dengue infection. Out of 84 samples 14 (16.70%) were NS1 positive and 59 (70.20%) were positive for IgM. Among them 11(13.10%) were positive for both NS1 and IgM. Among the positive dengue infection patients, male patients were 61 (72.6%) and female patients were 23 (27.4%). Male dominance was seen among positive patients.

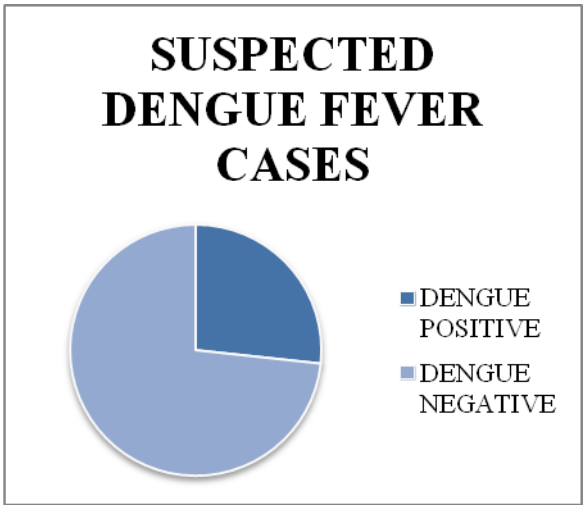

Fig.1 Positive cases among suspected Dengue fever cases

Table 1: NS1 antigen in Dengue samples

NS1 Antigen	No. of patients	Percentage %
Only NS1	14	16.70%
NS1 + IgM	11	13.10%
NS1 negative	59	70.20%

Table 2 : IgM antibodies in Dengue samples

IgM antibody	No. of patients	Percentage%
Only IgM	59	70.20%
IgM with NS1	11	13.10%
IgM negative	14	16.70%

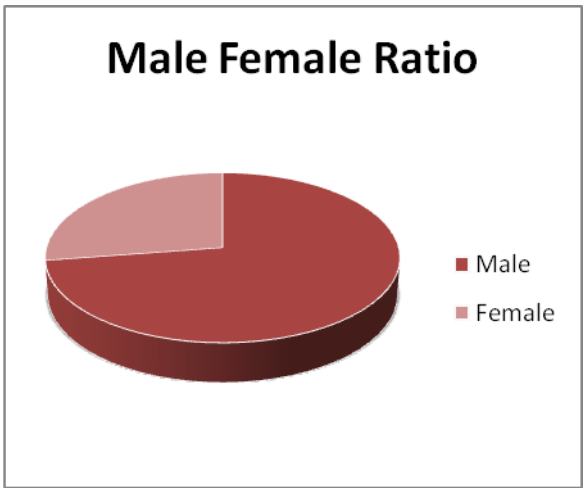

Fig.2 Male Female Ratio

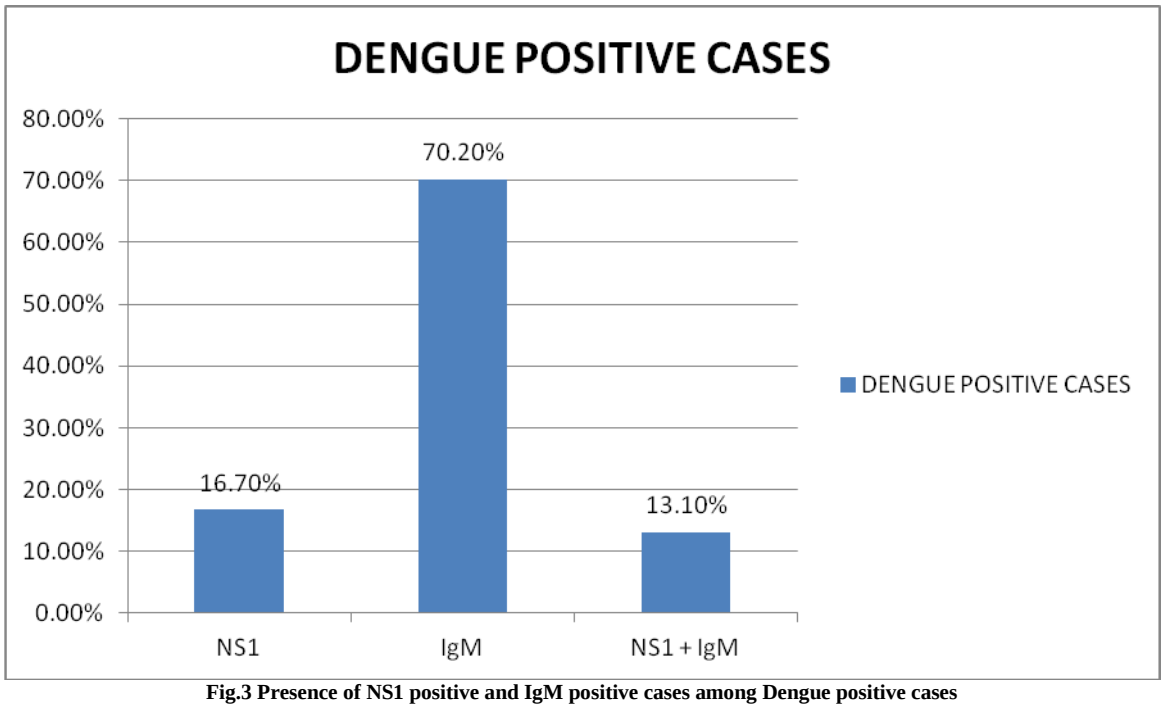

Fig.3 Presence of NS1 positive and IgM positive cases among Dengue positive cases

Age group between 10 – 65 years were affected. The age group affected the most was 21-30 years. Most of the cases were reported in monsoon and post-monsoon season.

DISCUSSION

Patients with NS1 positive were 14.73%. There were 62.10% of positive cases which showed only IgM antibodies and 13.10% cases showed both NS1 and IgM. Patients positive with only NS1 indicated initial stage of dengue infection. Thus, only 14.73% cases came during the initial stage of disease. After initial stage 13.10% cases (i.e. NS1 + IgM) came for tests. Maximum number of patients came when only IgM antibodies was left in blood tests of patients, which indicated late arrival of maximum patients for tests. It was

followed by appearance of only IgM antibodies. Present study reveals significant role of dengue serology in early diagnosis of dengue. Implying that the combination of these serological tests would increase the rate of detection of dengue fever. We found that the age group affected the most was 21-30 years. Study showed male predominance in Dengue infection cases (72.6%).

Study highlights continuous surveillance by dengue serology for early detection of impeding outbreak, to initiate timely prevention and control method. Due to cross reactivity with other flaviviruses IgM detection is not always conclusive[12]. Dengue RT-PCR use in most laboratories is currently difficult, because of the cumbersome procedure, prolonged time and difficult interpretation.[13–15]

For all dengue serotypes NS1 protein is highly conserved. It is circulating in high levels during the first few days of illness. No cross-reaction of dengue NS1 protein with those of other related flaviviruses is seen.[16] The detection of plasma NS1 would be associated with plasma viremia levels. NS1 is the product of infected cells. It is reported in Srivastava *et al* that in patients who were NS1-positive, viremia levels were significantly higher when compared to those who were NS1-negative.[17] In another study in dengue-2 virus the level of NS1 in plasma was correlated with viremia levels. Within 72 h of onset of illness, the level was significantly higher in patients with dengue hemorrhagic fever when compared with patients of dengue fever [18].The nested RTPCR developed by Lanciotti *et al*[19], and it's modification by Harris *et al* is the most widely used PCR based method for detecting DENV nucleic acid in the serum.[20] This test is having high sensitivity and specificity, and that reaction set-up to result time that is much shorter as in recent literature [21–23].Due to formation of immune complexes there can be decreased levels of NS1 after 4 days of illness thus monoclonal antibodies does not assess the target epitopes. [24,25]

CONCLUSION

Controlling dengue infection is becoming challenging because it requires effective vector control. Dengue NS1 has gained considerable interest as biomarker for early diagnosis of dengue illness. ELISA based NS1 and IgM antibody detection is more efficient and popular due to its simplicity, high specificity and great sensitivity.

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Conflict of Interest: None

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Ethical Approval: Approved

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