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# Prevalence of Dengue fever in Western Part of Uttar Pradesh: Serological investigation and Comparative Analysis of ELISA testing for IgM antibodies and NS1Ag

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## ABSTRACT

**Background**: Dengue cases in India are increasing in epidemic proportions. An early and accurate diagnosis of dengue in the acute phase of illness is important for initiation of therapy as well as for early enhancement of epidemic control measures. The serum of the patients presenting with signs and symptoms of Dengue are subjected to Dengue IgM Capture ELISA and $NS_1$Ag detection. NS-1 Ag appears in serum/plasma before the onset of IgM & IgG antibodies and its detection in suspected dengue patient can be used as an early marker for the disease. The study was undertaken to detect prevalence of dengue by ELISA testing of either NS1 antigen and/or IgM among the study population; to compare IgM capture ELISA with NS1 antigen detection for diagnosis of dengue.

**Materials and Methods**: A total of 9900 blood samples were collected aseptically from clinically suspected cases of dengue from August 2022 to July 2024. The Serum from suspected cases of Dengue was separated and subjected to ELISA for dengue ($NS_1$Ag and IgM Ab). All the data and variables relevant to patients such as gender and demographic profile were recorded and statistically analyzed. All were subjected for detection of NS1 antigen or IgM by ELISA, 1328 were selected based on their clinical severity of illness (fever, rash, bleeding manifestation, arthralgia) for further study of IgM ELISA

**Results**: A total of 881(8.9%) patients were found seropositive either by NS1 Ag and/or IgM Ab ELISA. The proportion of male was higher than female among all seropositive cases, with the ratio of (M: F) 1.54:1 ($P < 0.001$). The positivity of $NS_1$Ag ($P = 0.0002$) and IgM ($P = 0.0001$) were significantly associated with male gender.

**Conclusion**: Dengue NS1 antigen is used for rapid and accurate diagnosis in acute phase of illness. Therefore, early diagnosis of dengue could be mainly by NS1 antigen detection whereas IgM ELISA is a better tool during the later stage of infection.

Every suspected case of dengue must be screened for NS1 Ag and IgM Ab to increase the diagnostic precision and combat the morbidity and mortality, despite being males more affected in contrast to females due to socio-cultural differences.

**Keywords**: Dengue fever, IgM ELISA, Acute dengue infection, NS1 Ag, IgM Ab.

## INTRODUCTION

Dengue virus, a member of flavivirus is transmitted from person to person by the bite of *Aedes aegypti* mosquitoes. The etiological agent has four different serotypes (DENV-1 to DENV-4). Humans getting infection by one of the serotype confers immunity for life against the causative serotype but not against others[1]. The dengue viral serotype causing disease outbreaks has varied over time, along with the occurrence of severe dengue fever[2].

Dengue, the most prevalent mosquito-borne disease globally, is considered the most predominant human arbovirus in tropical and subtropical countries[3]. There are around 100–400 million infections per year and nearly half the world's population is at risk[4]. There is high mortality and morbidity caused by dengue infection.

The clinical features of Dengue by any of the serotype causes a spectrum of clinical features which ranges from asymptomatic infection, undifferentiated fever and classical dengue fever (DF) to life threatening materialization like dengue haemorrhagic fever (DHF) to dengue shock syndrome (DSS).

A rapid, efficient and accurate diagnosis of dengue in the acute phase of illness is important for early detection of severe cases, case confirmation and to rule out other differential diagnosis which in turn helps for initiation of therapy as well as for early enhancement of epidemic control measures especially in low endemic areas. However methods like virus isolation, RNA detection by PCR, need well trained staff and expensive set up which is not feasible in peripheral hospital setting. The isolation of virus in cell cultures is expensive and the results are usually available after 6 to 10 days. The service is only offered by some laboratories with the appropriate infrastructure for cell culture or mosquito colonies. Though the nucleic acid detection tests including the RT-PCR and other PCR-based techniques give results within 24-48 hours but they are also not available in all laboratories and these techniques also requires expertise and specialized equipments.

During primary infection a Non Structural Protein (NS-1) levels are raised and can be detected in early clinical phase of the disease from day 1-9, NS-1 Ag appears in serum/plasma before the onset of IgM and IgG antibodies and its detection in suspected dengue patient can be used as an early marker for the disease. Testing of specific IgM antibody in Dengue by ELISA forms the basis for diagnosis in the current era. Detection of IgM and IgG antibodies is a useful tool to distinguish between primary and secondary infection. Primary infection is characterized by the presence of significant or rising levels of IgM antibodies in the period 3–5 days after onset of infection, and can persist for3–5 months. Anti-dengue IgG levels are comparatively low during primary infection, but secondary infection often results in the appearance of high levels of IgG before the IgM response. IgG levels rise quickly, peak about2 weeks after the onset of symptoms, and then decline slowly over 3–6 months. Anti-dengue IgM levels are comparatively low during a secondary infection[5].

The present study aims to understand the gender based prevalence of dengue infection in Western Uttar Pradesh. In addition the objective was to examine serum samples for anti-dengue IgM and NS1 antigen positivity among the study population, to compare IgM capture ELISA with NS1 antigen detection in the Western part of Uttar Pradesh to provide basic epidemiological data on the situation of dengue infections in area.

## MATERIALS AND METHODS

**Study Design**: The present retrospective study was conducted in tertiary care dengue sentinel surveillance hospital in department of Microbiology from August 2022 to July 2024. Blood samples from clinically suspected cases of dengue fever from both, out patients department (OPD) and Inpatients department (IPD) were collected in plain vial in Department of Microbiology, SMMH Medical College, Saharanpur. Patients of all age groups who had clinical symptoms and signs of acute dengue like illness were included in the study. The duplicate patient has been excluded.

Serum was aseptically separated after centrifugation from the all samples, and was immediately subjected for detection of Non Structural Protein 1 antigen ($NS_1Ag$) $_{and}$/or IgM Antibody Capture (MAC) ELISA as per the manufacturer's instructions. Dengue IgM capture ELISA was performed as per manufacturer guidelines using kits from National Institute of Virology (NIV) Pune.

For NS1Ag ELISA, Microwell strips are coated with monoclonal anti-Dengue NS1Ag antibodies in which a total of 50 µL sample dilution buffer was added to each well followed by 100 µL of samples or controls in the corresponding wells. The plate was incubated at 37°C for 60 minutes. It was then washed to remove any unwanted and unbound material and blot dried. Further, 100 µL of monoclonal antibody-HRP conjugate was added to each well and plate again incubated for 30 minutes at 37°C followed by washing and drying. Additionally, 100 µL of substrate was introduced, and the plate was incubated for another 15 minutes in dark at room temperature. Finally, 100 µL of stop solution was added, and absorbance was read at 450 nm with 630nm.

For IgM ELISA, About 50 µL of positive and negative controls, diluted serum samples (1:100) were added to corresponding wells and are incubated at 37°C for 60 min. DEN antigen is added in the well which binds to captured human IgM in the sample. During the step of washing unbound antigen was removed. In subsequent step, Anti DEN Monoclonal antibodies added followed by Avid in-HRP is added. Subsequently, chromogenic substrate was added, the reaction was stopped by 1N $H_2SO_4$ solution was added, and absorbance was read at 450 nm. The result of Dengue IgM capture ELISA was interpreted as per the manufacturer's literature.

Data analysis: The data were analyzed using Chi-square test to compare the association between the categorical variables like gender and age. All the relevant variables were analyzed by descriptive statistics.

## RESULTS

Of the Total 9900 Samples Screened, 881 (8.90%) Patients Were Found Seropositive For Dengue Either By NS1 And/or IgM ELISA. The Observed Mean Age ± Standard Deviation Of All Patients Was 28.59 ± 12.52 Years. Out Of the 9900 Blood Samples Screened, 235 (2.3%) were positive for Dengue NS1 Ag with a two tailed test was applied and p-value of $< 0.001$ (Z-TEST for comparing the mean score of two samples) was applied, among which majority were males 128(54.5%) (Table 1), while 672(6.8%) were positive for dengue IgM among which majority were males 87 (12.9%) between age group 21-30 years (table 2).

Table 1: Age and sex distribution of the NS1Ag positive cases

| Age Group | Male | Female | P- VALUE (Z- TEST DOUBLE SAMLE PRO-PORTION TEST) | Total (%) |
|---|---|---|---|---|
| 0-10 | 23 (17.96%) | 14 (13.08%) | P=.0003* P<.001 (SIG.) | 37(15.74%) |
| 11-20 | 28 (21.88 %) | 25 (23.36%) | P=.0051** P>.001(N.S.) | 53(22.55%) |
| 21-30 | 18 (14.06%) | 17 (15.89%) | P=.0084** P>.001(N.S.) | 35(14.89%) |
| 31-40 | 24 (18.75 %) | 17 (15.89%) | P=.0001* P<.001 (SIG.) | 41(17.44%) |
| 41-50 | 15 (11.72 %) | 16 (14.95%) | P=.0008* P<.001 (SIG.) | 31(13.19%) |
| >50 | 20 (15.63 %) | 18 (16.82%) | P=.0089** P>.001(N.S.) | 38(16.17%) |
| Total | 128(54.50 %) | 107(45.50%) | ------- | 235(100) |

*shows a significant difference b/w gender for different age groups at 0.001 level of significance (P<.001).
**shows no significant difference b/w gender for different age groups at 0.001 level of significance (P>.001).

**Figure 1: The bar diagram of age and sex distribution of the $NS_1$Ag positive cases**

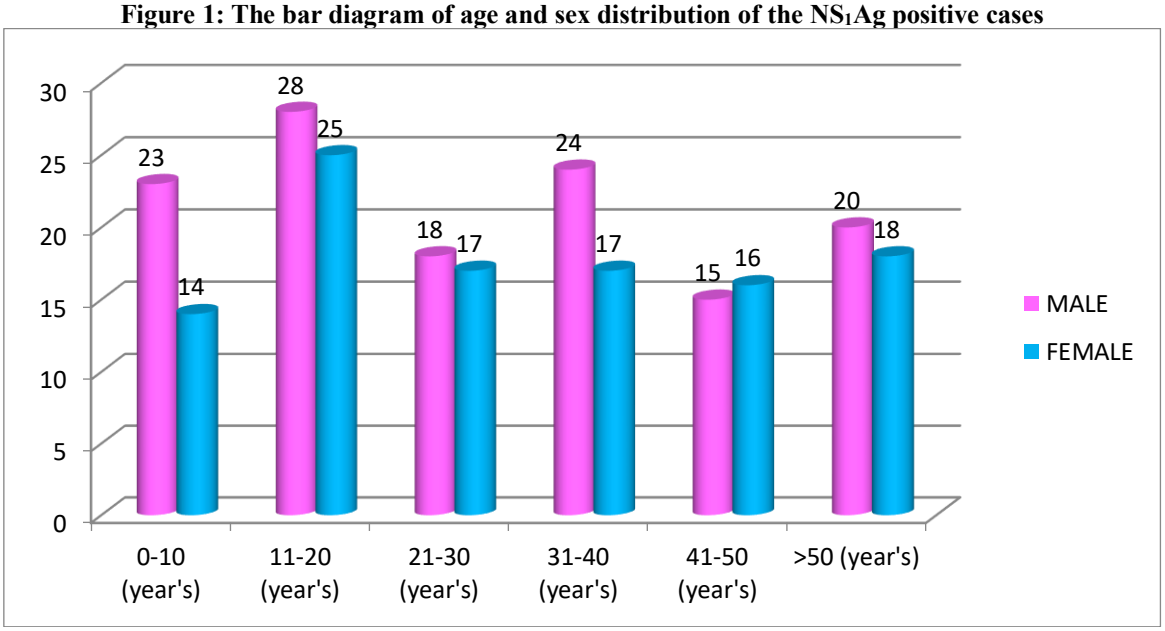
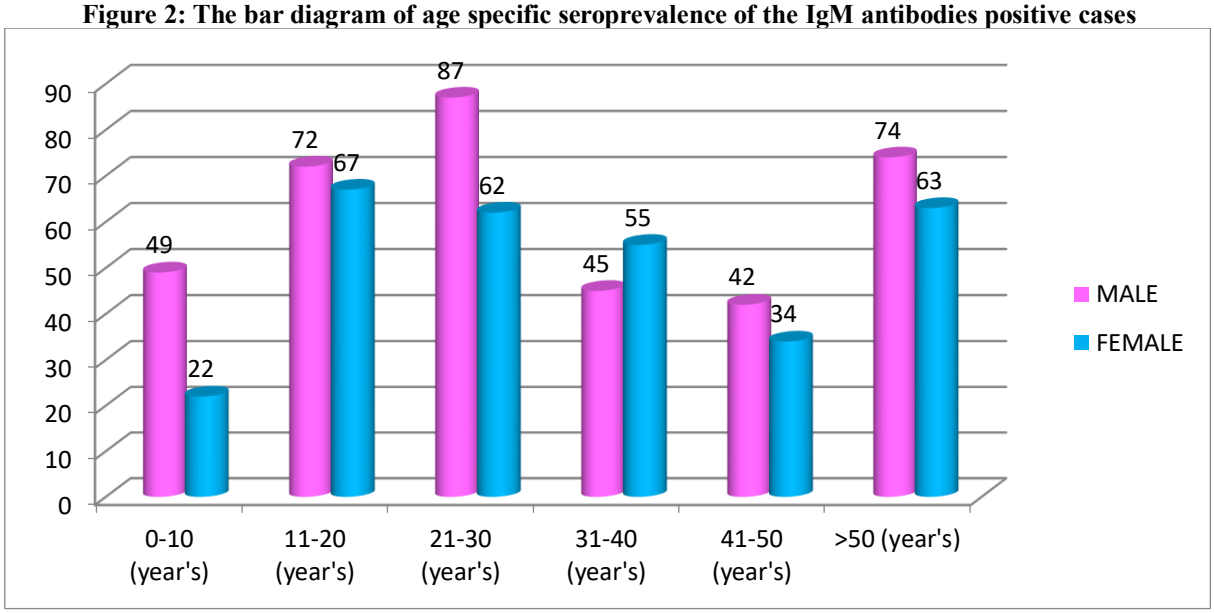



Table 2: Age specific seroprevalence of the IgM antibodies positive cases

| Age Group | Male | Female | P- VALUE (Z- TEST DOUBLE SAMLE PRO-PORTION TEST) | Total (%) |
|---|---|---|---|---|
| 0-10 | 49(13.35%) | 22 (7.26%) | P=.0003* P<.001 (SIG.) | 71(10.56%) |
| 11-20 | 72(19.61%) | 67 (22.11%) | P=.0013* P<.001 (SIG.) | 139(20.68%) |
| 21-30 | 87(23.71%) | 62 (20.46%) | P=.0002* P<.001 (SIG.) | 149(22.17%) |
| 31-40 | 45(12.26%) | 55 (18.15%) | P=.0006* P<.001 (SIG.) | 100(14.88%) |
| 41-50 | 42(11.44%) | 34 (11.22%) | P=.1965** P>.001(N.S.) | 76(11.31%) |
| >50 | 74(20.16%) | 63 (20.79%) | P=.1160** P>.001(N.S.) | 137(20.39%) |
| Total | 367(54.60%) | 303(45.10%) | ------- | 672(100) |

*shows a significant difference b/w gender for different age groups at .001 level of significance (P<.001).
**shows no significant difference b/w gender for different age groups at .001 level of significance (P>.001).

**Figure 2: The bar diagram of age specific seroprevalence of the IgM antibodies positive cases**




As comparison of IgM ELISA & NS1Ag was not possible for all samples in our resource constraint laboratory, 1328 samples were selected, for comparison of the tests in which 1090 samples were found to be negative by both tests. (Table 3)

Table 3: Comparison of NS1Ag and IgM among seropositive dengue fever cases (n=1328)

| Test Name | | IgM (MAC ELISA) | | Total (%) | P- VALUE ( BY CHI-SQUARE TEST STATISTICS) |
|---|---|---|---|---|---|
| ELISA | | Positive (%) | Negative (%) | | |
| NS1 | Positive | 26(1.90%) | 95(7.20%) | 121(9.10%) | **P=.0847** P>.001 (NO SIGNIFICANT ASSOCIATION)** |
| | Negative | 117(8.80%) | 1090(82.10%) | 1207(90.90%) | |
| Total | | 143(10.80%) | 1185(89.20%) | 1328(100.00%) | |

NS1: Non structural protein 1; IgM (MAC): IgM antibody capture; ELISA: Enzyme linked Immunosorbent assay

**Figure 3: Pie diagram comparison of $NS_1$Ag (positive cases) and IgM among seropositive dengue fever cases**

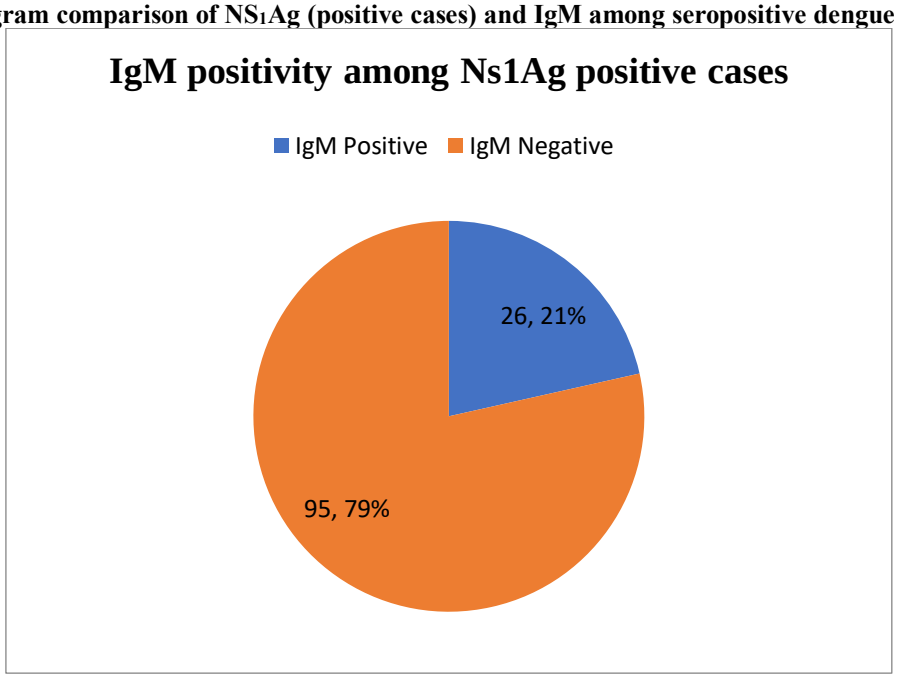
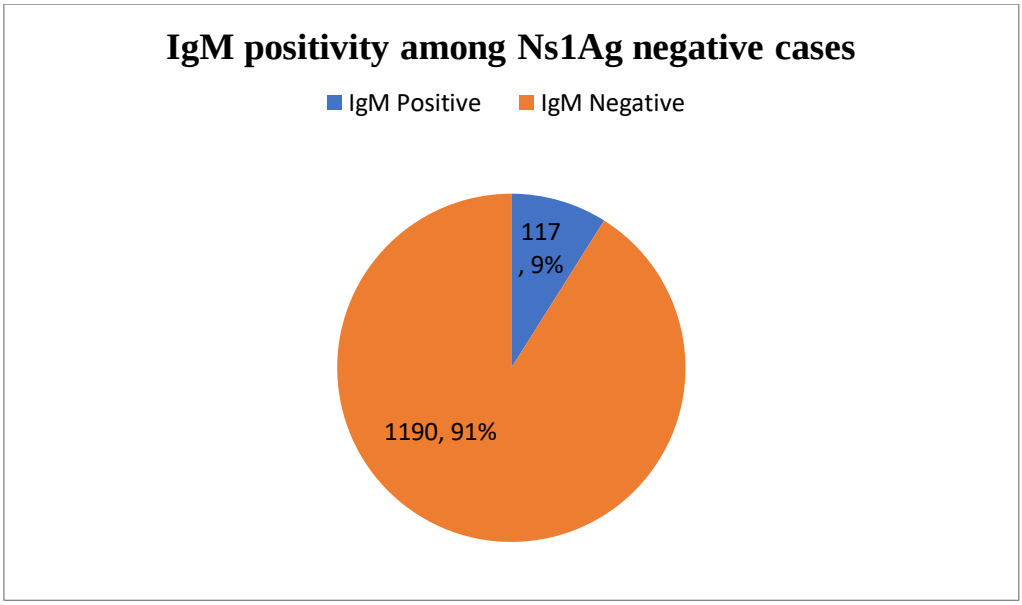


**Figure 4: Pie diagram comparison of $NS_1Ag$ (negative cases) and IgM among seropositive dengue fever cases**




**DISCUSSION**

Dengue fever and associated complications has become a major public health problem in India, especially the northern part of India, including the national capital and its adjoining states. It is even endemic to many parts of the country including, both urban and rural areas. At present to estimate the extent

The incidence reported in our study is 8.9% which is lower than another study from India estimated an annual force of infection of 11.9%[6], 29% found in kerala[7] and also less than that from Maharashtra, another state in India; which reported an overall incidence rate of primary dengue as 54.2 infections/1000 children years (95% CI 43.0–67.3), in the 5–15 year age group[8]. The age-specific seroprevalence reported from our study is 27.89 % are lower to that of other study reports a seroprevalence of 30.9% among 9–12 year old children and 24.6% among 5–8 year old children this can be classified as low to moderate (<50%)[7]

In our study male preponderance among all seropositive cases was statistically significant ($P < 0.001$). Among which majority were males 174 (19.2%) between the age group of 21-40 years is very less compared to other study conducted which showed 427(83.2%) between the age group of 21- 40yrs[9].

The results in our study are supported by above studies, where males are more affected compared to females[9].Goswami et al. have found a statistically significant association between the presence of NS1 antigens and IgM antibodies among male gender[9]. Brown et al. have reported a significantly higher sensitivity of IgM antibody ELISA in male gender[10] similar to our study. A few international studies that have examined male and female dengue incidence have reported a significant association with male gender[11]. Similar results have also been obtained in various other studies across the world. A contrasting result of an Indian study suggested that seropositivity and hemorrhagic findings were reported with greater propensity in females[12].

Amongst these, total of 121 cases were detected positive for dengue by NS1, 143 by IgM and 26 (21%) cases were detected by both NS1 and IgM ELISA [Table 3]. It was also observed that 95 (79%) cases were detected positive by NS1Ag ELISA where IgM ELISA was negative. It might be due to the fact that the sample may have been collected within a week of the illness when IgM antibodies against the virus were not formed. IgM antibodies were detected in 117 cases only, and these cases might have reported after 1 week of illness.

The NS1 ELISA being the most potent tool, it was able to detect 95 cases more where IgM was negative. IgM was detected in 117 (9%) cases more amongst the cases that were negative for $NS_1Ag$. The NS1 protein is highly sensitive; it becomes detectable from the onset of dengue fever among both primary and secondary infection. At the onset of infection, it is presented in higher concentration in the patient's blood samples[13]. It reliable viral markers for diagnosis initially (from day 1) fever, but the sensitivity declines after 4–5 days of onset and finally antigen titer becoming undetectable[14]. The present study has been carried out at this tertiary care hospital, and some cases might have reported later in the illness. Thus, to compare the diagnostic accuracy and efficacy, we used NS1 and IgM ELISA simultaneously in 1328 samples.

The IgM ELISA is able to detect up to 50% of cases more when used alone, and it is a highly sensitive and specific test. The inclusion of NS1 ELISA in the test panel minimizes the chance of missing any case[15]. In the present study 25.3%

samples were found to be positive by NS1Ag detection with a highly significant p-value (<0.001). For long time, detection of dengue specific antibody has been main stay of diagnosis of dengue infection. The role NS1 Ag for early detection of dengue infection is currently being evaluated by many investigators without requirement of paired sera [16].
High sensitivity and specificity were obtained after combining IgM and NS1 ELISA results were 98.48 % AND 95.62 % similar to other study conducted (96.3% and 96.4%)[2].
Drawback of this study is Serological cross reactions are common within the flavivirus i.e. Dengue Serotypes 1, 2, 3, Japanese encephalitis, West Nile Encephalitis, etc are not excluded from this study. Also absence of Dengue NS1 does not indicate that an individual is absolutely free of Dengue infection. Thus reactive samples should be retested with confirmatory tests like PCR but PCR test is expensive so it is not a feasible method and not available in every medical facilities.
Clinical signs, symptoms and epidemiology of Dengue in the geographical region and IgM results of the immunocompromised were considered critical for the interpretation of the results. As with all diagnostic tests, a definitive clinical diagnosis should not be based on single result of a single test, but should only be made by the physician after all clinical and laboratory finding have been evaluated.
Thus, the combined results of both assays (NS1 and IgM ELISA) show that the overall accuracy and diagnostic ability to identify dengue fever may help increase the overall diagnostic sensitivity.

## CONCLUSION

NS1Ag is an effective method in comparison to MAC ELISA for diagnosis of dengue virus infection, especially within first five days of illness. The morbidity and mortality of dengue fever can be reduced by early diagnosis and symptomatic management. However, from sixth day onwards combination of MAC ELISA and NS1Ag assay would increase the sensitivity of diagnosis and after 10 days IgM ELISA should be the method of choice for diagnosis. Thus, we conclude here that despite the male preponderance, every single case must be investigated for dengue serologically for NS1 Ag and IgM ELISA, irrespective of the gender which can improve the diagnosis of dengue without the requirement of paired sera.

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Conflicts of interest
There are no conflicts of interest

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