ORIGINAL RESEARCH

Correlation of IgM seropositivity in dengue NS1Ag positive samples of patient from rural population

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Abstract
Introduction: Dengue, also known as "break bone fever," is a viral infection transmitted by Aedes aegypti followed by Aedes albopictus. It is more prevalent in tropical and subtropical climates. While many individuals infected with dengue may not experience any symptoms, those who do may commonly exhibit high fever, headache, body aches, nausea, and rash. In severe cases, dengue can be fatal, with some individuals developing dengue haemorrhagic fever characterized by bleeding, low blood platelet levels, and blood plasma leakage, or dengue shock syndrome, which involves dangerously low blood pressure.
Aim: To detect Dengue IgM with IgM capture ELISA in NS1 positive samples of patients attending a tertiary care hospital.
Material and Methods: This study was conducted as a prospective cohort study, conducting within the study period June 2022 to December 2022 involving patients admitted to GBCM and Subharti hospital, Dehradun. A Comparative study to detect Dengue IgM with IgM capture ELISA in NS1 positive samples of patients attending a tertiary care hospital among 498 patients.
Results: Among 258 female patients, 24 were NS1-positive, 36 were IgM-positive, and 46 were IgG-positive and in male patients (240), 59 were NS1 positive, 40 were IgM positive, and 39 were IgG positive. In July 2022, a higher correlation coefficient of 0.422029 (p-value: 0.000570) was observed, indicating a stronger association between NS1 antigen and IgM seropositivity. Thrombocytopenia with most cases showing platelet counts ranging from 50,000 to 100,000 platelets/cu mm.
Conclusion: This study enhances our understanding of the correlation between IgM seropositivity and NS1 antigen positivity in dengue cases within rural populations. The findings have significant implications for early detection, diagnosis, and management of dengue infection, emphasizing the value of IgM as a diagnostic marker.
Keywords: Dengue Infection, ELISA, IgM, NSIAg

Introduction
Dengue, also known as "break bone fever," is a viral infection transmitted by Aedes aegypti followed by Aedes albopictus. It is more prevalent in tropical and subtropical climates. While many individuals infected with dengue may not experience any symptoms, those who do may commonly exhibit high fever, headache, body aches, nausea, and rash. Most cases resolve

within 1-2 weeks, but severe dengue can occur, requiring hospital care. In severe cases, dengue can be fatal, with some individuals developing dengue haemorrhagic fever characterized by bleeding, low blood platelet levels, and blood plasma leakage, or dengue shock syndrome, which involves dangerously low blood pressure[1-2].

Dengue haemorrhagic fever was first recognized during a 1950s epidemic in the Philippines and Thailand. It has since become a leading cause of death among children in Asian and Latin American countries. Unlike Ebola virus infection, which affects the liver and leads to a depletion of coagulation proteins, dengue fever causes haemorrhages by depleting platelets within the circulation[3].

In India, until 2012, Dengue 1 and 3 were the dominant strains. However, in recent years, Dengue 2 has become more prevalent, while Dengue 4, previously considered the least infectious, has emerged in South India[4]. Scientists believe that if the second serotype is similar to the first, antibodies generated after the initial infection can bind to the new serotype and immune cells called macrophages. This proximity enables the newcomer serotype to infect macrophages, leading to more severe infection.

The global incidence of dengue has increased significantly, with a 30-fold upsurge between 1960 and 2010. This increase can be attributed to factors such as population growth, global warming, unplanned urbanization, ineffective mosquito control measures, frequent air travel, and limited access to healthcare facilities [5-7].

The first reported case of dengue-like illness in India dates back to 1780 in Madras. The first virologically confirmed epidemic of dengue fever in India occurred in Calcutta and the Eastern Coast in 1963-1964 [8].

While most cases of dengue present with mild symptoms, a small percentage of cases develop severe life-threatening conditions known as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). These severe forms of the disease are characterized by a sudden onset of fever and non-specific signs and symptoms. The critical stage of DHF occurs 24 hours before or after the temperature falls to or below normal. During this time, haemorrhagic manifestations usually occur, and signs of circulatory failure may emerge. Laboratory tests show thrombocytopenia and evidence of vascular leak syndrome. Hypovolemia, shock, and death can occur in cases of DSS [9-10].

Primary infection with one serotype of dengue provides lasting immunity against that serotype. However, secondary infection with a different serotype increases the risk of developing DHF. Due to the increasing incidence of dengue infections and complications like DHF and DSS, this study aims to correlate IgM seropositivity in Dengue NS1Ag samples from patients in rural populations, aiming to improve the diagnosis and treatment of dengue infection.

Material and Methods

This study was conducted as a prospective cohort study, conducting within the study period June 2022 to December 2022 involving patients admitted to GBCM and Subharti hospital, Dehradun. A comparative study to detect Dengue IgM with IgM capture ELISA in NS1 positive samples of patients attending a tertiary care hospital. Before, starting the study ethical clearance was taken from ethical Board. Written informed consent was obtained from all participants after providing detailed information about the study.

Inclusion criteria for the study patient be the residents of rural areas of age group, Admitted to GBCM and Subharti hospital, Dehradun., Clinical suspicion of dengue infection based on symptoms (fever, headache, myalgia, rash, etc. and Patients attending both IPD/OPD with history of fever $\geq$ 72 hours was included. Exclusion criteria involve repeat sample or Haemolysed sample.

Sample Size Determination

The sample size was calculated based on the expected correlation coefficient between IgM seropositivity and NS1Ag, the desired power of the study, and the significance level. Previous studies suggest a correlation of 0.7 between these markers [11]. With an alpha level of 0.05 and power of 80%, a minimum sample size of 100 patients were targeted.

Method

1. **Rapid Dengue Duo Cassette Method**[12]: The cassette consists of a square well for the addition of buffer solution, a circular well for the serum sample, including NS1, and a lateral flow membrane with colloidal gold complexes containing recombinant dengue 1-4 antigens and a control.

2. **Dengue IgM Capture ELISA**[13]: Dengue IgM CAPTURE ELISA is an enzyme-linked immunosorbent assay designed to detect the presence of dengue-specific IgM antibodies in patient samples.

Interpretation of results: It is based on the comparison of the patient's OD values with the cutoff value. The cutoff value is determined based on the absorbance readings of the negative control samples.

Sample Collection

Blood samples were collected from the selected patients to obtain serum for laboratory analysis. Trained healthcare professionals performed venipuncture using sterile techniques to collect the blood samples. A total of 498 patients were included in the study, and blood samples were collected within the first five days of symptom onset. The collected blood samples were then processed to separate the serum using centrifugation. The serum samples were stored in appropriately labeled vials and maintained at a temperature of -80°C to preserve the integrity of the samples until further analysis.

Data Collection

Relevant data were collected from the study participants to provide comprehensive information for analysis. In addition to the laboratory results for IgM seropositivity and Dengue NS1Ag, various clinical data were recorded. These included the patients' age, gender, occupation, residential area, travel history, duration of symptoms, presence of warning signs (such as severe abdominal pain or bleeding), and comorbidities (if any). Data on the patients' medical history, including any previous dengue infection episodes, were also documented. These additional data points help in understanding the characteristics of the study population and enable a more detailed analysis of the correlation between IgM seropositivity and Dengue NS1Ag.

Statistical Analysis

Statistical analysis was performed to examine the relationship between IgM seropositivity and Dengue NS1Ag. The collected data, including laboratory results and clinical information, were analyzed using appropriate statistical techniques. The correlation between IgM seropositivity and Dengue NS1Ag was assessed using common correlation coefficients, such as the Pearson correlation coefficient or Spearman's rank correlation coefficient. These coefficients provide measures of the strength and direction of the correlation. Additionally, hypothesis testing was conducted to determine the significance of the observed correlation. Statistical software packages such as SPSS or R were utilized for data analysis, ensuring accurate and reliable statistical outcomes.

Results

The study included a total of 498 individuals from a rural population, with 240 males and 258 females, providing a balanced representation of both sexes. The mean age of the study population was 35.12 years, ranging from 3 to 84 years, reflecting the inclusion of individuals across different age groups. Gender distribution analysis revealed that out of 258 female patients, 24 were NS1-positive, 36 were IgM-positive, and 46 were IgG-positive. Among the male patients (240), 59 were NS1 positive, 40 were IgM positive, and 39 were IgG positive. Among the study participants, 156 patients were from the outer department, with 19 NS1-positive, 21 IgM-positive, and 33 IgG-positive cases. The inner department included 342 patients, with 64 NS1-positive, 55 IgM-positive, and 52 IgG-positive cases showed in showed in Graph 1.
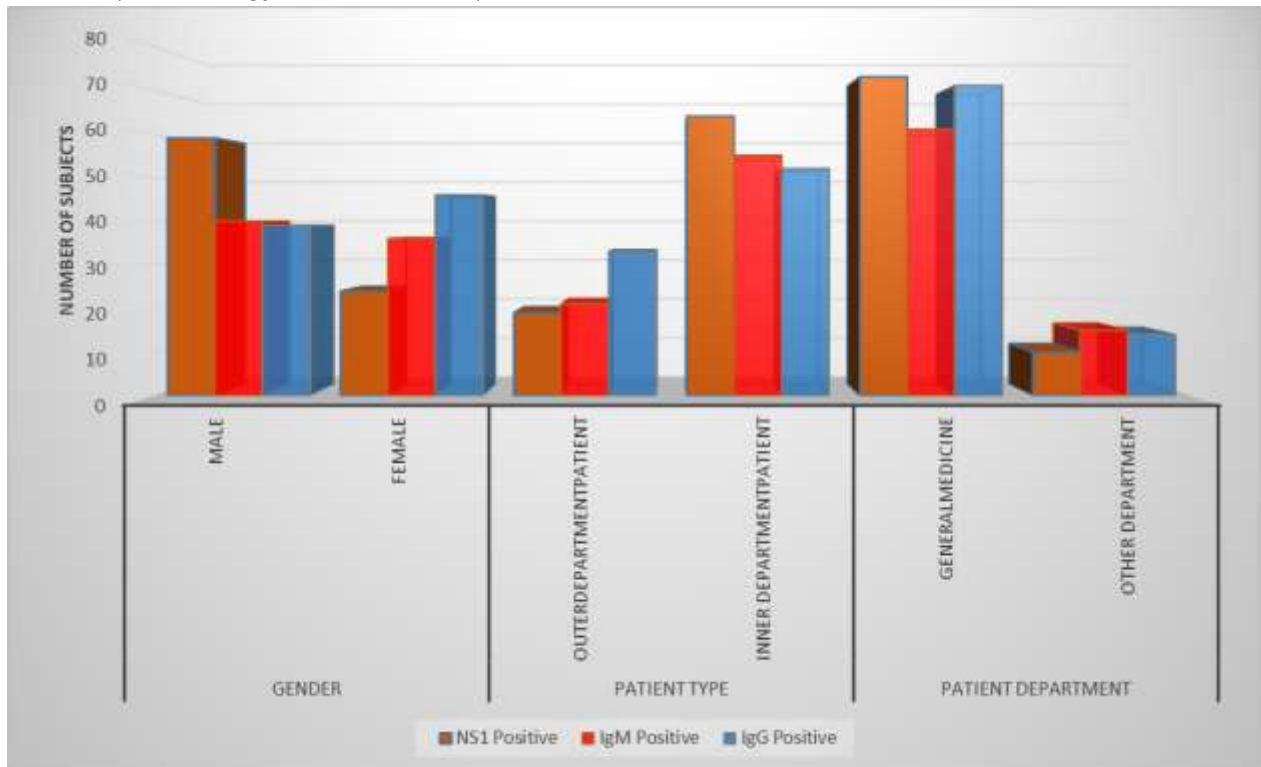
Graph 2 showed the Distribution Of IgM, Ns1 Antigen and IgG Status by Month in which month of august 2022 showed the maximum IgG positive patient (n=41). The IgM Positive results seen high in the month of September 2022 (n=17) and Ns1 Positive results shows in the month of October 2022 (m=35).

Table 1 showed the correlation analysis between NS1 antigen and IgM seropositivity revealed a significant positive correlation coefficient of 0.2597079, indicating a moderate association between the two variables. The p-value associated with the correlation coefficient was 0.004, demonstrating high statistical significance and indicating that the observed correlation is unlikely to be due to chance.

Table 2 showed the monthly correlation analysis revealed that June 2022 exhibited a significant correlation coefficient of 0.278582 (p-value: 0.043391), suggesting a potential phase change from NS1 to IgM during this month. In July 2022, a higher correlation coefficient of 0.42 (p-value: 0.005) was observed, indicating a stronger association between NS1 antigen and IgM seropositivity. However, in August 2022, the correlation coefficient was only 0.004437 (p-value: 0.959731), indicating a lack of significant correlation between NS1 antigen and IgM seropositivity during this month. September 2022 showed a correlation coefficient of 0.215 (p-value: 0.038), suggesting a moderate association between NS1 and IgM seropositivity. The correlation coefficient for October 2022 was 0.184 (p-value: 0.103), indicating no significant correlation between NS1 antigen and IgM seropositivity during this month. November 2022 exhibited the highest correlation coefficient of 0.556 (p-value: 0.001), indicating a robust phase change from NS1 to IgM during this month.

Table 3 showed Thrombocytopenia, characterized by low platelet count, was observed in dengue cases, with most cases showing platelet counts ranging from 50,000 to 100,000 platelets/cu mm.

Graph 1: DISTRIBUTION OF IgM, NS1 ANTIGEN AND IgG STATUS BY GENDER, PATIENT TYPE & DEPARTMENT

Graph 2: DISTRIBUTION OF IgM, NS1 ANTIGEN AND IgG STATUS BY MONTH OF ADMISSION
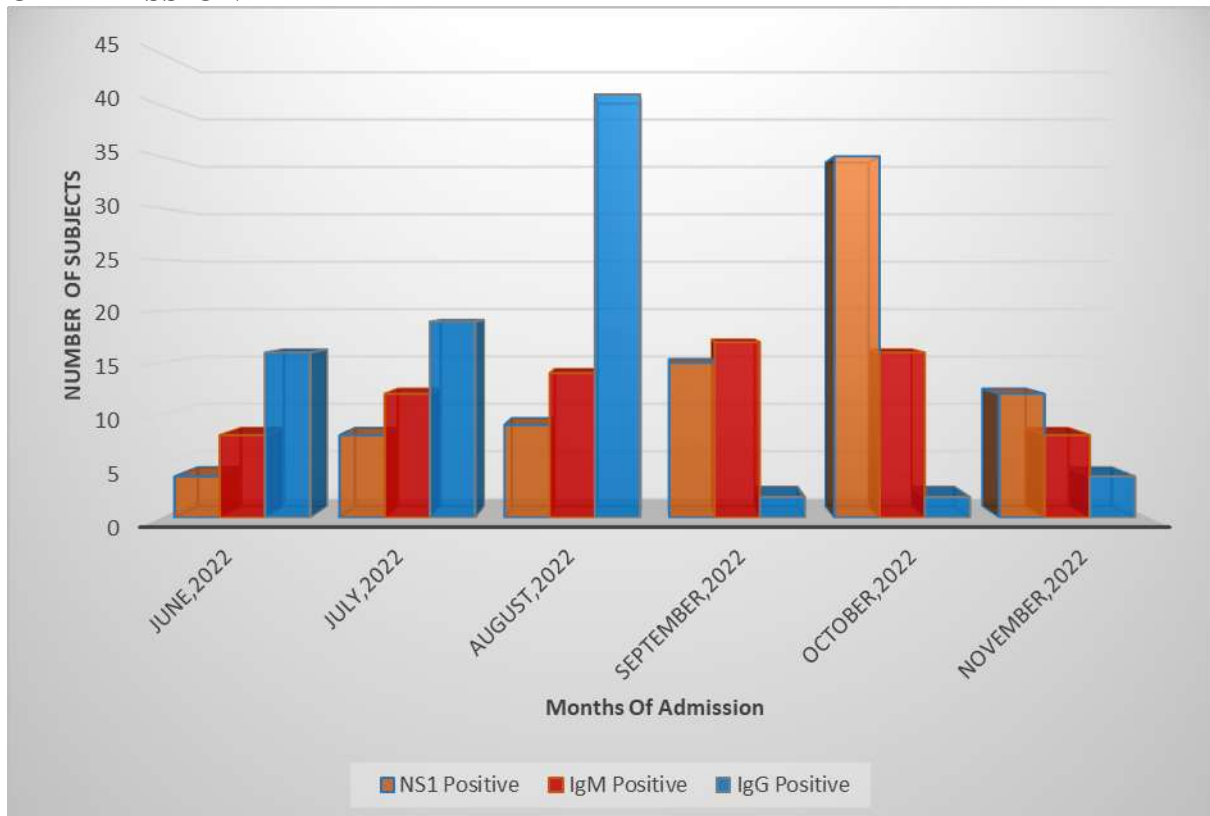
Table 1: CORRELATION BETWEEN NS1 ANTIGEN AND IgM SEROPOSITIVITY

	NS1 Antigen – IgM Seropositivity	Correlation	P-Value	Significance*
Positive |Positive	30			
Positive|Negative	53			
Negative |Positive	46			
Negative|Negative	369	0.2597079	0.000000004	Significant

*The Baseline P – Value is 0.05.

Table 2: CORRELATION BETWEEN NS1 ANTIGEN AND IgM SEROPOSITIVITY FOR EACH MONTH

Month	Correlation	P Value	Significance*
June,2022	0.278582	0.043391	Significant
July,2022	0.422029	0.000570	Significant
August,2022	0.004437	0.959731	Not Significant
September,2022	0.215166	0.038512	Significant
October,2022	0.184602	0.103385	Not Significant
November,2022	0.556109	0.000001	Significant
December,2022	-	-	-

*The Baseline P – Value is 0.05.

Table 3: THE NUMBER OF NS1 POSITIVE AND IgM POSITIVE SAMPLES THAT HAVE PLATELET COUNT LESS THAN 100000/ML AND MORE THAN 100000/ML

PlateletCount	NS1 Positive	IgM Positive	Total
< 100000/ml	52[62.65%]	35[46.05%]	87
> 100000/ml	31[37.35%]	41[53.95%]	72
Total	83[100 %]	76[100 %]	159

Discussion

The study titled "Correlation of IgM Sero-positivity in Dengue NS1Ag Positive Samples of Patients from Rural Population" was conducted at GBCM and Subharti hospital, Dehradun, with the aim of investigating the correlation between IgM seropositivity and NS1 antigen positivity in dengue cases from a rural population. This study addresses a critical knowledge gap in the field, as there are limited in-depth studies focusing on this specific correlation.

Balmaseda et al[14]. (2010) conducted a noteworthy study analyzing trends in patterns of dengue transmission in a pediatric cohort in Nicaragua. Their findings highlighted the complex dynamics of dengue transmission, emphasizing the importance of understanding variations in serotype dominance and epidemic intensity over time. These insights underscore the need for further research into dengue-related serological markers, such as IgM seropositivity and NS1 antigen positivity.

Leo et al.[15](2012) conducted a study that stressed the significance of early identification of severe dengue cases in adult patients. They emphasized the utility of serological tests, including IgM detection, in conjunction with clinical indicators, for risk stratification and determining the need for hospital admission. This study provides valuable context to the importance of accurate diagnostic markers, such as IgM seropositivity, in guiding patient management strategies for dengue cases.

Wichmann et al.[12] (2011) conducted a study assessing the burden of dengue in Thailand and Cambodia based on reported cases. Their research shed light on the limitations of relying solely on reported cases, highlighting the substantial number of undiagnosed or unreported

dengue infections. This study reinforces the crucial role of serological testing, including IgM detection, in identifying additional cases and obtaining a more accurate estimation of the disease burden in endemic regions.

Duong et al. (2015)[16] conducted a significant study investigating the role of asymptomatic individuals in dengue transmission. Their findings demonstrated that even asymptomatic individuals infected with dengue virus can contribute to the perpetuation of the disease cycle by transmitting the virus to mosquitoes. This study underscores the importance of comprehensive serological testing, such as detecting IgM seropositivity, to capture cases from both symptomatic and asymptomatic individuals.

The findings of the study revealed a significant correlation between NS1 antigen and IgM seropositivity, indicating the transition from the acute phase (NS1 positive) to the early convalescent phase (IgM positive) of dengue infection. This correlation can serve as an important diagnostic marker for healthcare professionals in detecting dengue cases and determining the appropriate phase of infection.

By understanding the timing and pattern of the transition from NS1 to IgM seropositivity, healthcare providers can enhance their ability to promptly diagnose dengue infections. Timely identification is crucial in implementing appropriate patient management strategies, including early supportive care and monitoring, to prevent complications and improve outcomes. The findings of the study can also contribute to epidemiological surveillance and the implementation of public health interventions. Tracking the shift in dengue cases from NS1 positivity to IgM positivity can provide valuable information about the current phase of the outbreak and help authorities take proactive measures to control the spread of the disease, such as vector control activities and public awareness campaigns.

The incorporation of these supportive studies, along with the analysis of demographic characteristics and correlation data, underscores the significance and relevance of the current study. By building upon existing research and presenting detailed findings, this study contributes valuable insights to the field of dengue research, furthering our understanding of the correlation between IgM seropositivity and NS1 antigen positivity in dengue cases from a rural population.

Limitation of the study might be the study was conducted within a specific rural population, limiting the generalizability of the findings to other settings or populations. Regional variations in dengue prevalence and immune responses may exist.The study did not account for the influence of co-infections or other underlying medical conditions that could potentially affect the immune response and dengue infection outcomes.The study did not consider the impact of vaccination status or previous exposure to dengue virus, which can significantly affect the immune response and disease outcomes.The data collection was limited to a specific time frame (June 2022 to December 2022), which may not capture the entire spectrum of dengue cases throughout the year or account for seasonal variations in disease prevalence.

Conclusion

The study titled "Correlation of IgM Seropositivity in Dengue NS1Ag Positive Samples of Patients from Rural Population" provides significant insights into the relationship between IgM seropositivity and NS1 antigen positivity in dengue cases among individuals residing in rural areas. The analysis conducted revealed a substantial correlation between these two factors, indicating a phase change from NS1 to IgM during the progression of dengue infection. With a correlation coefficient of about 0.26, there is a noteworthy chance of patients transitioning from the NS1 phase to the IgM phase, aligning with the natural course of the disease. This study enhances our understanding of the correlation between IgM seropositivity and NS1 antigen positivity in dengue cases within rural populations. The

findings have significant implications for early detection, diagnosis, and management of dengue infection, emphasizing the value of IgM as a diagnostic marker. Further research and validation of these findings are recommended to strengthen our knowledge of dengue immunology and improve patient care strategies in both rural and urban settings.

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