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Study of association of thrombocytopenia and serological parameters in dengue fever with special reference to NS1 antigen in hilly Region of Northern India

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ABSTRACT

Background: Dengue fever is an arthropod-borne disease transmitted to humans by *Aedes aegypti* mosquitoes and one of the leading causes of arthropod-borne viral disease in the world. **Aims and Objectives:** The present study aims to analyze any association between platelet count and immunochromatography -based dengue serology tests. **Materials and Methods:** This was a cross-sectional study conducted in Government Doon Medical College and Hospital. A total of 19,208 clinically suspected cases of dengue who reported in various outpatient departments, emergency services, and inpatient departments of our hospital between June 2023 and November 2023 were included in this study. Samples were tested by rapid immunochromatographic test for dengue non-structural protein 1 (NS1) antigen, immunoglobulin M (IgM), IgG, and platelet count was also obtained and a comparison was made. **Results:** Out of total 19,208 samples tested, 4876 (25.38%) samples were positive for NS1, 589 (3.06%) samples were positive for IgM. Majority of the patients were in the age group of 21–40 years 9274 (48.28%). 11,753 (61.19%) were males and 7455 (38.80%) were females. A strong correlation was seen between NS1 and thrombocytopenia where a total of 2737/4876 (n = 56.13%) patients showed positivity in NS1 with low platelet counts. **Conclusion:** Detection of NS1 antigen helps in early diagnosis of dengue to avoid complications significantly. In confirmed dengue cases with fever, thrombocytopenia is more consistently found and can be used as a predictor to reduce the morbidity and mortality of dengue disease.

Key words: Immunochromatographic test; Dengue; Non-structural protein 1

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INTRODUCTION

Dengue fever (DF) is an acute febrile arboviral disease that affects tropical and subtropical regions of the world including India. The incidence of the disease has increased over the past 50 years, and 2.5 billion people live in areas where dengue is endemic.[1]

Dengue virus (DENV) belongs to the Flaviviridae family, which includes more than 70 major human disease-causing pathogens affecting mostly inter-tropical regions, where 3.9 billion people live.[2]

The disease has been described since 1779–1780; however, there are evidence that a similar disease occurred earlier on several continents.[3]

It is an arboviral disease that is mostly transmitted to humans by the bite of mosquitoes, especially those of the Aedes genus, primarily by Aedes (Stegomyia) aegypti (Linnaeus, 1762) and in some rare cases by Aedes (Stegomyia) albopictus (Skuse).[4]

Most DF diseases are self-limited with low mortality (<1%) when detected early and provided with proper medical care.

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Infection with one serotype confers lifelong immunity to that serotype only. Nonneutralizing antibodies when binding to new DENV serotype facilitates its entry into susceptible host cells and lead to antibody-dependent enhancement. These patients might develop severe diseases (including dengue hemorrhagic fever/decision support system) with a mortality rate of around 2–5% after receiving treatment; when left untreated, the mortality rate is as high as 20%.[5,6]

Rapid and sensitive laboratory methods are required for early detection of the disease to reduce its morbidity and mortality.[7] More specific methods such as virus isolation, genomic RNA detection by polymerase chain reaction, antigen, and antibody detection by enzyme-linked immunosorbent assay (ELISA) are available, but it needs a well-trained staff and an expensive setup which is not feasible in peripheral hospital settings.[8]

Aims and objectives

This study was planned to evaluate the role of immunochromatography (ICT) based tests in dengue management with the following objectives.

Objectives

1. To study the correlation between decreasing platelet counts and serological tests for non-structural protein 1 (NS1) antigen and antibody detection for early diagnosis of DF.
2. To assess the role of decreasing platelet count keeping in view of centers where only ICT-based tests are available for diagnosis of DF.

MATERIALS AND METHODS

This cross-sectional study was conducted in a tertiary care hospital for 6 months from June 2023 to November 2023. The study group included a total of 19,208 samples which were received in the Microbiology division of the central laboratory of Government Doon Medical College and hospital, Dehradun. Ethical Approval of the study was obtained from Ethical Committee of Government Doon Medical College and Hospital.

All specimens from clinically suspected patients attending various outpatient and inpatient departments with complaints of fever, headache, nausea, muscle pain, retro-orbital pain, hemorrhagic condition, and rashes after proper prescription from clinicians were included in the study.

Under all aseptic precautions, the venous blood sample was collected individually into the plain vial from clinically suspected patients attending various outpatient and inpatient departments with complaints of fever, headache, nausea, muscle pain, retro-orbital pain, hemorrhagic condition, rashes after proper prescription from clinicians. Samples were tested by rapid immunochromatic test (Q-line rapid dengue combo) at the central laboratory for dengue NS1 antigen, immunoglobulin M (IgM), and IgG as per manufacturer's guidelines. The platelet count of all the serologically positive samples was obtained and a comparison was made. Samples that got hemolyzed, insufficient in quantity were excluded from the study. The relevant clinical and laboratory data were collected from the hospital Laboratory Information system and our laboratory records for all the patients in a standardized format. Statistical analysis was done by 2×2 contingency table analysis.

RESULTS

Out of the total 19,208 suspected serum samples, majority (4876/19,208, 25.38%) were positive for NS1 antigen, 589 (3.06%) samples were positive for IgM by rapid ICT-based test. A total of 472 (n=2.4%) showed positivity for both IgM and NS1, while a total of 229 (n=1.19%) were positive for both IgM and IgG parameters (Table 1). Also amongst these out of total 19,208 samples tested 11,753 (61.19%) were males and 7,455 (38.80%) were females (Figure 1).

A strong positive correlation was seen between NS1 and thrombocytopenia (platelet count ≤100,000/μL)[7] where

Table 1: Efficacy and percentage positivity of various dengue serological parameters (NS1, IgM, IgG)

Dengue parameter	Total positive
NS1	4876 (25.38%)
IgM	589 (3.06%)
IgG	376 (1.95%)
NS1+IgM	472 (2.4%)
IgM+IgG	229 (1.19%)

IgM: Immunoglobulin M, NS1: Non-structural protein 1, IgG: Immunoglobulin G

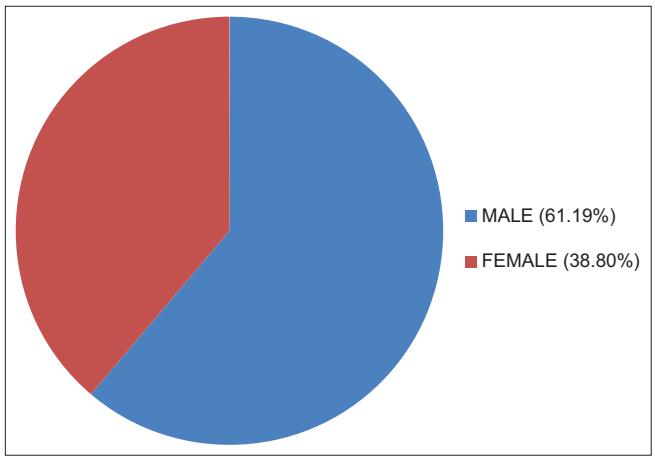

Figure 1: Gender wise distribution of total suspected Patients(N=19208)

a total of 2737/4876 (n=56.13%) NS1 positive cases had thrombocytopenia. The association of thrombocytopenia with NS1 positive cases was found to be significant (P<0.00001) (Tables 2 and 3). The predominant age group in this study was found to be between 21 and 40 years i.e. 9274 (48.28%) (Table 4). In case of IgM positive cases thrombocytopenia was seen in 203/589 (n=34.46%) cases, while in other cases with common parameters the counts were 142/472 (n=30.08%) for IgM and NS1 positive cases, 76/229 (n=33.18%) for IgM and IgG-positive cases (Table 4).

The highest cases of thrombocytopenia were seen in the age group of 40–60 (1072/2931; 36.57%), followed by the age group of 20–40 (937/2931; 31.96%). The age group of 60–80 comprised 631 cases (21.52%), age group of 0–20 comprised 102 cases (3.48%) (Table 5).

Table 2: Correlation between dengue serological parameters and thrombocytopenia (Platelet count<1 lakh/μL)

Dengue parameter	Thrombocytopenia
NS1	2737 (56.13%)
IgM	203 (34.46%)
IgG	12 (3.1%)
NS1+IgM	142 (33.18%)
IgM+IgG	229 (30.08%)

IgM: Immunoglobulin M, NS1: Non-structural protein 1, IgG: Immunoglobulin G

Table 3: Comparison of platelet counts and its correlation with dengue NS1 serological parameter

NS1 status	Platelet count<1 lakh/μL	Platelet count>1 lakh/μL	Total count
NS1 positive	2737	2139	4876
NS1 negative	444	750	1194

P<0.00001. NS1: Non-structural protein 1

Table 4: Age wise distribution verses low platelet count

Age wise distribution	Thrombocytopenia cases (2931)
0–20	102 (3.48%)
21–40	937 (31.96%)
41–60	1072 (36.57%)
61–80	631 (21.52%)
>80	189 (6.44%)

Table 5: Age wise distribution of study population

Age wise distribution	Number of patients (19,208)
0–20	4710 (29.52%)
21–40	9274 (48.28%)
41–60	3337 (17.37%)
61–80	1404 (7.3%)
>80	483 (2.5%)

DISCUSSION

It is important to establish the diagnosis of acute DENV infection during the initial few days of manifestation of the clinical symptoms to provide timely guidance for the management of patients and to control early outbreaks.[9]

Virus isolation and characterization are considered the gold standard for laboratory diagnosis of acute DENV infection. However, it is expensive and takes at least 6–10 days for the virus to replicate in a cell culture or laboratory mosquitoes. Detection of the viral genomic sequence by reverse transcription-polymerase chain reaction is also an expensive method and is not available in most hospital diagnostic laboratories.[10]

NS1 is a highly conserved glycoprotein that is essential for the viability of DENV and is produced both in membrane-associated and secretory forms by the virus. The NS1 antigen is a highly specific marker of dengue infection. It is considered to be a very important diagnostic test in the early febrile stage of illness as this antigen appears early in blood. In comparison to the NS1 antigen, the dengue IgM appears late i.e. on 5th day of infection. The dengue IgM as well as IgG antibodies show some cross-reactivity with other members of the flaviviridae family, and are thus not considered as reliable.[7,11]

In our study out of a total 19,208, 4876 (25.38%) samples were positive for NS1, and a strong correlation was seen between NS1 and thrombocytopenia where a total of 2737/4876 (n=56.13%) NS1 positive cases had thrombocytopenia. We also compared platelet counts with dengue IgM and found that 589 (3.06%) samples were positive for thrombocytopenia. Similarly, with IgG 376 (1.95%) samples were positive for thrombocytopenia. This is in concordance with a study conducted by Kulkarni et al., where NS1 alone and with IgM correlated well with thrombocytopenia.[12]

Similar findings were seen in another study conducted by Badave et al., 2015 where a significant correlation was noted between NS1 antigen and thrombocytopenia.[13] Another study conducted by Mehta et al., (2016), showed a strong correlation of 74% between IgM and thrombocytopenia.[14] This was in contrast to our study and the reason for this may be that patients in our hospital presented in early stages of illness. Similar studies conducted by Pachori et al. and Shankarappa found that thrombocytopenia was strongly associated with serologically positive dengue patients.[15,16]

Limitations of the study

This study was done for a short duration of 6 months and can be planned for long durations so that results can be compared on a large number of samples.

CONCLUSION

There is a significant increase in the global burden of Dengue cases, which calls for improved diagnostic methods for early detection to avoid complications in patients of acute febrile illness like dengue infection. The ELISA test has a greater sensitivity as well as specificity in the detection of dengue-specific antigen and antibodies, but it is time-consuming and requires specialized training. On the other hand, ICT-based tests are quick, convenient, cost-effective, and easy to interpret. To conclude, the detection of NS1 in association with thrombocytopenia provides an early insight into predicting a patient's outcome.

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NN- Defining the objective and Literature search, Preparation of first draft of manuscript, implementation of study protocol, manuscript preparation and submission of the article; **AN**- Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **YR**- Design of study, statistical Analysis and Interpretation; manuscript revision; **SP**- Review Manuscript and overall supervision; **RH**- Literature survey and preparation of Figure and table.

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