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Original Research

Serological markers of dengue infection & platelets

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ABSTRACT:
Background: The dengue virus, a member of the genus Flavivirus of the family Flaviviridae, is an arthropode-borne virus. The present study was conducted to assess correlation between serological markers of dengue and platelet counts. **Materials & Methods:** 110 patients suspected of dengue fever was enrolled. Serum samples were collected from the suspected dengue fever patients. The samples were tested for NS1 antigen, IgM, and IgG antibodies using the ICT test kit. **Results:** NS1 only was detected in 58, IgM only in 22, IgG only in 11, NS1 and IgM only in 8, NS1 and IgG only in 5 and IgM and IgG only in 6 cases. The difference was significant (P< 0.05). 58 patients that were positive for NS 1, thrombocytopenia was seen in 50, 14 out of 22 IgM positive patients, 9 out of 11 IgG positive patients, 6 out of 8 NS1 and IgM only positive patients, 2 out of 5 NS1 and IgG only positive patients and 4 out of 6 IgM and IgG only positive patients were detected. The difference was significant (P< 0.05). **Conclusion:** The use of NS1 is useful indicator of dengue virus infection. Hence, the diagnosis should include detection of NS1.
Key words: Dengue, Flavivirus, IgG

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INTRODUCTION

The dengue virus, a member of the genus Flavivirus of the family Flaviviridae, is an arthropode-borne virus that includes four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[1] The World Health Organization (WHO) consider dengue as a major global public health challenge in the tropic and subtropic nations. Dengue has seen a 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities.[2]

The resource poor health care system has to depend upon simple to perform and easy to interpret laboratory tests for diagnosis. It is known that early and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality.[3] Dengue virus gains entry into the host organism through the skin following an infected mosquito bite. Humoral, cellular, and innate host immune responses are implicated in the progression of the illness and the more severe clinical signs occur following the rapid clearance of the virus from the host organism. Hence, the most severe clinical presentation during the infection course does not correlate with a high viral load. Alterations in endothelial microvascular permeability and thrombo-regulatory mechanisms lead to an increased loss of protein and plasma.[4]

The three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation, detection of the viral genomic sequence by a nucleic acid amplification technology assay (RT-PCR), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test (ICT). Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage due to its long half-life in blood.[5] The present study was conducted to assess correlation between serological markers of dengue and platelet counts.

MATERIALS & METHOD

The present study was conducted in the department of Microbiology among 110 patients suspected of dengue fever of both genders. All patients were informed regarding the study and written consent was obtained.

Data such as name, age, gender etc. was recorded. Serum samples were collected and tested for NS1 antigen, IgM, and IgG antibodies using the ICT test kit. The platelet count was recorded in dengue parameter-positive and -negative cases. Results were tabulated and subjected to statistical analysis. P value less than 0.05 was considered significant.

RESULTS

Table I Distribution of patients

Total- 110		
Gender	**Male**	**Female**
Number	50	60

Table I shows that out of 110 patients, males were 50 and females were 60.

Table II Assessment of dengue parameters

Parameters	Number	P value
NS1 only	58	0.01
IgM only	22	
IgG only	11	
NS1 and IgM only	8	
NS1 and IgG only	5	
IgM and IgG only	6	

Table II, graph I shows that NS1 only was detected in 58, IgM only in 22, IgG only in 11, NS1 and IgM only in 8, NS1 and IgG only in 5 and IgM and IgG only in 6 cases. The difference was significant (P< 0.05).

Graph I Assessment of dengue parameters

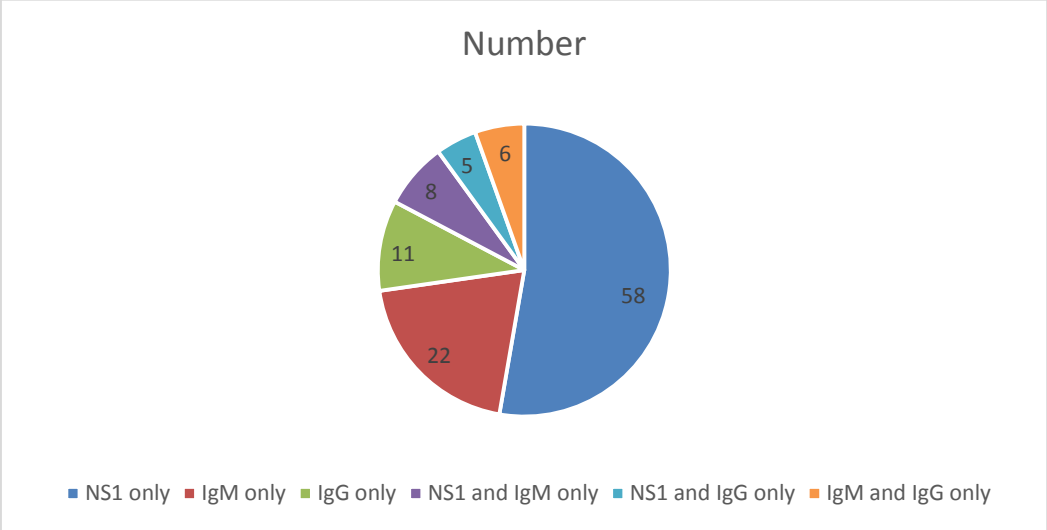


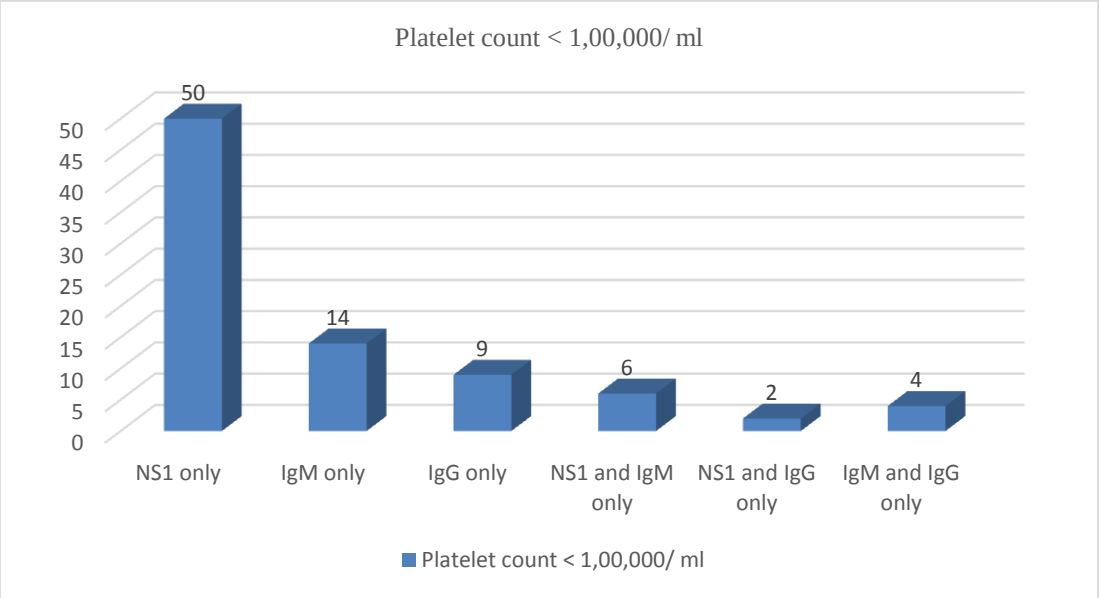
Table III Comparison of dengue parameter and platelet count

Parameters	Platelet count < 1,00,000/ ml	P value
NS1 only	50	0.01
IgM only	14	
IgG only	9	
NS1 and IgM only	6	
NS1 and IgG only	2	
IgM and IgG only	4	

Table III, graph II shows that 58 patients that were positive for NS 1, thrombocytopenia was seen in 50, 14 out of 22 IgM positive patients, 9 out of 11 IgG positive patients, 6 out of 8 NS1 and IgM only positive patients, 2

out of 5 NS1 and IgG only positive patients and 4 out of 6 IgM and IgG only positive patients were detected. The difference was significant (P< 0.05).

Graph II Comparison of dengue parameter and platelet count



DISCUSSION

Dengue is an acute, potentially fatal viral infection that can culminate into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It is spread through the bite of infected Aedes aegypti mosquito. Most primary infections are uneventful. Infection with one serotype confers an individual life-long immunity to that serotype and cross-reactivity to the other serotypes.[6] The complications like DHF and DSS are usually attributed to this cross-reactivity. Dengue is almost endemic throughout India. Proposed theories suggest that endothelial cell activation caused by monocytes, T-cells, the complement system, and various inflammatory molecules mediate plasma leakage.[7] Thrombocytopenia may be related to alterations in megakaryocytopoiesis, manifested by infection of human hematopoietic cells and compromised progenitor cell growth. This may cause platelet dysfunction, damage, or depletion, leading to significant hemorrhages. The NS1 protein was found to be highly conserved in all dengue serotypes, circulating in high levels during the first few days of illness. It correlates with the development of DHF. There is no cross-reaction of the dengue NS1 protein with those of other related flaviviruses.[8]

The WHO classifies DF into two groups: Uncomplicated and severe. Severe cases are linked to excessive hemorrhage, organ impairement, or severe plasma escape, and the remaining cases are considered uncomplicated. According to the 1997 classification, dengue can be divided into undifferentiated fever, DF, and DHF. DHF was further subdivided into grades I–IV. Grade I: Only mild bruising or a positive tourniquet test. Grade II: Spontaneous bleeding into the skin and elsewhere. Grade III: Clinical sign of shock and Grade IV: Severe shock - feeble pulse, and blood pressure cannot be recorded. Grades III and IV comprise DSS.[9] The present study was conducted to assess correlation between serological markers of dengue and platelet counts.

In present study, out of 110 patients, males were 50 and females were 60. Jyothy et al[10] in their study a total of 520 serum samples were collected from the suspected dengue fever patients. Sixty-two samples tested positive for one or more dengue-specific parameters. Out of the 62 samples, 39 (62.9%) were positive for the NS1 antigen, only seven (11.3%) were positive for IgM, and only three (4.9%) were positive for IgG. A platelet count < 1,00,000/ml was observed in 32 cases (51.6%). When the platelet count was done in 100 dengue parameter-negative fever patients (controls), thrombocytopenia was observed in 30% of the cases.

We found that NS1 only was detected in 58, IgM only in 22, IgG only in 11, NS1 and IgM only in 8, NS1 and IgG only in 5 and IgM and IgG only in 6 cases. Paranavitane et al[11] 186 adult patients with confirmed dengue were enrolled during day 3–8 of illness. Clinical and laboratory parameters were recorded during the course of the illness and NS1 antigen levels were determined using both the Panbio dengue early ELISA and a NS1 rapid antigen detection kit. 59.1% of patients presented to hospital on day 5–6 of illness when NS1 antigen positivity was significantly associated with severe dengue and the NS1 antigen levels were significantly higher in those who went on

to develop shock. Serum NS1 antigen levels significantly and inversely correlated with the total white cell counts and lymphocyte counts. The bedside NS1 test showed comparable sensitivity (97.4%) and specificity (93.7%) to the laboratory NS1 test.

It was seen that 58 patients that were positive for NS 1, thrombocytopenia was seen in 50, 14 out of 22 IgM positive patients, 9 out of 11 IgG positive patients, 6 out of 8 NS1 and IgM only positive patients, 2 out of 5 NS1 and IgG only positive patients and 4 out of 6 IgM and IgG only positive patients were detected. Cautious attention should be directed at DF if a patient suffers from high fever within 2 weeks of being in the tropics or subtropics. A decreased number of white blood cells (leukopenia), accompanied by a decreased number of platelet count (thrombocytopenia) and metabolic acidosis are the initial changes on laboratory examinations. Microbiological laboratory testing confirms the diagnosis of DF. Virus segregation in cell cultures, nucleic acid demonstration by polymerase chain reaction (PCR), and serological detection of viral antigens (such as NS1) or particular antibodies are the preferred microbiological assays. Viral segregation and nucleic acid demonstration provide precise diagnosis, although the high cost limits the availability of these tests.[12]

CONCLUSION

Authors found that use of NS1 is useful indicator of dengue virus infection. Hence, the diagnosis should include detection of NS1.

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