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Original Article

Pathology Section

Immunological Diagnosis of Dengue Fever with Antigen and Antibody Detection Methods

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ABSTRACT

Introduction: Dengue fever or infection is a potentially fatal infection that can occur in people of all age groups. Dengue virus is distributed worldwide and represents a serious public health problem; it affects over 100 million people worldwide, annually. Detection of Non-structural Protein 1 (NS1) antigen helps in diagnosing dengue infection in the early stages and helps in prevention of Dengue Shock Syndrome (DSS) and Dengue Haemorrhagic Fever (DHF).

Aim: To identify the dengue specific antigen (NS1) and antibody (Immunoglobulin M (IgM) and Immunoglobulin G (IgG)) and correlate them with the platelet and White Blood Cell or Total Leukocyte Count (WBC or TLC) and also to assess the effectiveness of platelet transfusion in these patients.

Materials and Methods: This was a prospective study done for two consecutive years involving only second half of the year 2018 and 2019 i.e., June-December 2018 and June-December 2019. Dengue infection or fever incidence is more during second half of the year during monsoon and winter seasons. A total of 587 samples were included in this study (positives) out of 4477 samples received for dengue testing. Statistical analysis was done with the help of SPSS version 14.0 software. A p-value <0.05 was taken as significant for interpretation.

Results: A total of 587 cases were included in this study, males were 349 (59.45%) and females 238 (40.55%). Out of the 587 positive tests, NS1 antigen was positive in 473 cases, IgM was positive in 188 cases and IgG was positive in all active and recovered cases. Out of the 587 cases, only 423 cases (72%) showed thrombocytopenia during the first three days of the clinical symptoms or NS1 positivity, total 561 (95.6%) cases showed thrombocytopenia during the first five days of positivity or symptoms.

Conclusion: Association of thrombocytopenia in dengue positive cases was highly significant. NS1 antigen detection is the only means of diagnosis of dengue infection in the first few days of fever, which helps in minimising the complications and early management of complications.

Keywords: Dengue haemorrhagic fever, Dengue shock syndrome, Thrombocytopenia

INTRODUCTION

The World Health Organisation (WHO) classifies dengue as an important public health disease. The epidemiology and ecology of dengue infection is strongly related with human habits and activities [1]. Before the year 2000, severe forms of dengue virus infection, such as DHF and DSS, were seen and were restricted to only few countries [2]. Dengue fever is now endemic in more than 100 countries such as various parts of Africa, the United States of America, and the Mediterranean region of the Middle East, Southeast Asia, and the Western Pacific [2,3]. According to WHO recent statistics and estimation, over 390 million people are infected with dengue fever per year globally [4], resulting in over half a million haemorrhagic dengue fevers that leads to 25,000 deaths annually around the world [5]. Clinical features of dengue infection include sudden onset of moderate to high grade fever, hot flushes, moderate to severe headache, back pain, myalgia and arthralgia involving various small and big joints, retro-orbital pain, nausea and vomiting and rash ranging from petechial haemorrhages to generalised erythematous rash. Rash typically begins between fourth to seventh day in most of the cases after the mosquito bite and typically lasts for 3-10 days. Thrombocytopenia sets in during febrile phase and the platelet count is progressively reduced. As per WHO guidelines, thrombocytopenia is used as one of the diagnostic criteria for DHF [6]. The early diagnosis of dengue infection is most crucial for timely clinical intervention, aetiological investigations, and also control of the disease and complications [6]. There are varieties of methods for dengue diagnosis, including virus isolation and identification, nucleic acid detection, detection of antigens, serological tests, haematological tests, nucleotide detection, and serological tests for NS1, IgM or IgG seroconversion. Few diagnostic methods listed above have limitations in terms of their ability in giving immediate results, some of them require expertise, sophisticated facilities and expensive laboratory equipments, viral isolation and Ribonucleic Acid (RNA) purification are time-taking procedures. Presently immunological methods such as antigen (NS1) and antibodies detection (IgM and IgG) are most commonly used globally because of fast results with good sensitivity and specificity. The aim of this study is to identify the dengue specific antigen (NS1) and antibody (IgM and IgG) and correlate them with the platelet and total WBC or TLC and also to assess the effectiveness of platelets transfusion in these patients.

MATERIALS AND METHODS

This was a prospective study done for two consecutive years from January 2018 to December 2019, more number of cases were reported in the second half of the year 2018 and 2019 i.e., June-December 2018 and June-December 2019. Total number of cases reported during these two years were 587. In the first year of the study, 247 cases and 340 cases in the second year. This study was done at Aakash Pathlab, Aakash Healthcare Superspeciality Hospital, Dwarka, New Delhi. Ethical clearance and informed consent of patients for the sample testing and study was obtained by the hospital (Number: IEC/AHCSSH/21).

Inclusion Criteria

A total of 587 samples were included in this study (NS1 and IgM positives) out of 4477 samples received for dengue testing. All the patients irrespective of age with clinical features suggestive of dengue fever which were confirmed by serological test were included in the study.

Exclusion Criteria

Patients who presented with fever due to other conditions such as upper or lower respiratory tract infections, abscess etc., were excluded from this study.

Sample Collection Method

Two blood samples per patient were received in the lab with proper details of the patient such as name with surname, Inpatient (IP) or Outpatient (OP) number, Unique Health Identity Number (UHID) etc. A proper requisition was obtained with clinical history along with the sample from the treating clinician for each and every case. Minimum 2 mL of venous blood sample in each tube i.e., one Ethylene Diamine Tetracetic Acid (EDTA) tube (Violet/Purple Top) for complete blood count/picture and for making peripheral smear for platelet count and other Serum Seperatoe (SSE) tube (Yellow Top) for NS1 antigen and IgM (Immunoglobulin M) and IgG (Immunoglobulin G) testing by Enzyme Linked Immunosorbent Assay (ELISA) method using serum obtained by centrifugation of the SSE sample tubes, procedure of the testing done according to the manufacturer's instructions (J-Mitra ELISA kits for dengue serology). All the required investigations were done at the time of admission and at the time of discharge, complete blood counts or platelet counts were done on daily basis by haematology analyser (cell counter)-5 part SYSMEX XN 550 XN-L series and by peripheral smear method wherever required, usually when the count was below 1,50,000 cu.mm. Daily transfusion activity such as Platelet Concentrate (PC), Random Donor Platelets (RDP) or Single Donor Platelets (SDP) was noted and correlated with the patient previous platelet count.

RESULTS

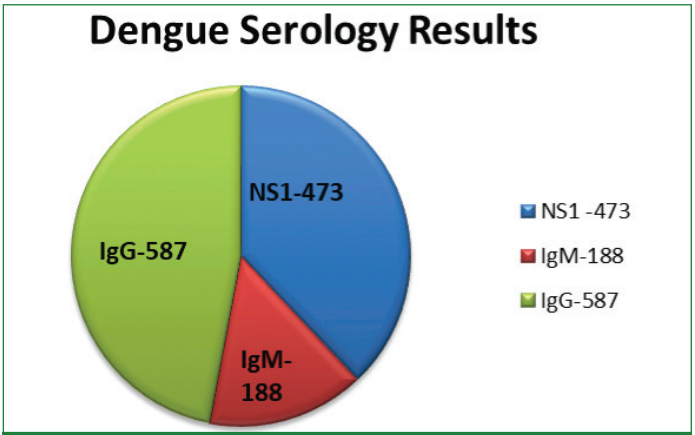
A total of 587 cases were included in this study, males were 349 (59.45%) and females 238 (40.55%). Youngest patient in this study was 3-month-old and the oldest 72 years, mean age was 34.6 years. Age-wise distribution has been tabulated in the [Table/Fig-1]. Most of the cases were in the age group of 30 to 39 years (135 cases), followed by 20-29 years age group (114 cases) and least dengue cases were noted in the age group of of 70 plus years (19 cases).

Age	Number of cases	Percentage
Upto 9 years	74	12.60
10-19 years	89	15.16
20-29 years	114	19.43
30-39 years	135	22.99
40-49 years	76	12.94
50-59 years	45	07.67
60-69 years	35	05.97
Above 70 years	19	03.24
Total cases	587	100%

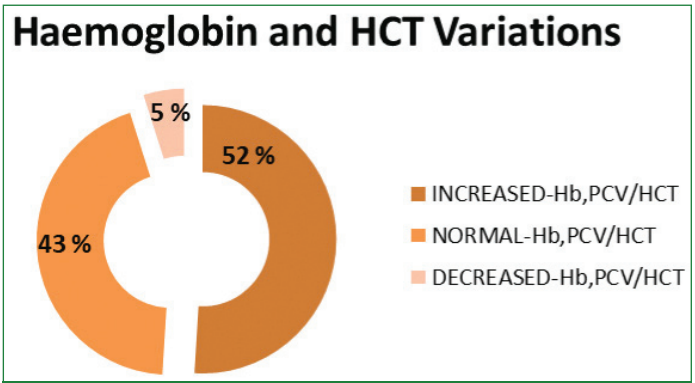
[Table/Fig-1]: Showing age-wise distribution of the cases.

Out of the 587 positive tests, NS1 Antigen was positive in 473 cases, negative in 73 cases and in 41 cases NS1 antigen report was not available or not done (Due to the lack of knowledge about the dengue fever, not reported to the hospital during the first 3-5 days of fever, NS1 done but report lost). The IgM was positive in 188 cases, negative in 77 cases, and in 322 cases IgM was not done and treated only on NS1 positivity report or status and based on other haematological parameters mainly thrombocytopenia. The IgG was positive in all active and recovered cases [Table/Fig-2].

In the present study, raised haemoglobin, Packed Cell Volume (PCV) or Haematocrit (HCT) was observed in 305 cases (52%), Normal haemoglobin, PCV/HCT was seen in 252 cases (43%) and decreased haemoglobin, PCV/HCT was also seen in 30 cases (05%) [Table/Fig-3]. Haemoconcentration was more common in the patients with more than five days of continuous fever.



[Table/Fig-2]: Dengue serology test results-Positives.



[Table/Fig-3]: Haemoglobin and Haematocrit (HCT) variations in dengue cases.

In this study, mild to severe leucopenia was noted in 511 (87%) cases with 59 cases (11.55%) showing mild leucopenia (white cell count or WBC count between 3100-4000/cu.mm), 402 cases (78.67%) showing moderate leucopenia with WBC count ranging from 1100-3000/cu.mm and remaining 50 cases (9.78%) showed severe leucopenia with less than 1000 cells/cu.mm. Out of the 587 cases, only 423 cases (72%) showed thrombocytopenia during the first three days of the clinical symptoms or NS1 positivity, total 561 (95.6%) cases showed thrombocytopenia during the first five days of positivity or symptoms, remaining 26 (4.42%) cases did not show thrombocytopenia except mild to moderate symptoms and NS1 or NS1 and IgM positivity. Out of these cases, 511 cases showed both leucopenia and thrombocytopenia of various grades depending upon the serological positivity [Table/Fig-4].

	Number of cases/Total	Percentage	Serology positive
WBC count			
<1,000/cu.mm	50/511	09.78 %	NS1+IgM
1,100-3,000/cu.mm	402/511	78.67 %	NS1+IgM
3,100-4,000/cu.mm	59 / 511	11.55 %	NS1+IgM
>4,000/cu.mm	76 / 587	12.94 %	IgM Only
Platelet count			
<50,000/cu.mm	394/561	70.23 %	NS1+IgM
51,000-1,00,000/cu.mm	124 /561	22.10 %	NS1+IgM+IgG
>1,01,000-1,50,000/cu.mm	43/561	07.66 %	NS1+IgM+IgG
>1,50,000/cu.mm	26 /587	04.42 %	IgM+IgG

[Table/Fig-4]: Leucopenia and thrombocytopenia, serology of dengue cases.
*Severe Leukopenia- WBC less than 1,000/cu.mm, Moderate Leukopenia- WBC count between 1,100 and 3,000/cu.mm, Mild Leukopenia- WBC count between 3,100 and 4,000/ cu.mm;
*Severe thrombocytopenia- Platelet count less than 50,000/cu.mm, Moderate thrombocytopenia- Platelet Count between 51,000 and 1, 00,000/cu.mm, Mild thrombocytopenia- Platelet count between 1, 01,000 and 1, 50,000/ cu.mm

In this study, patients with platelet count below 20,000/cu.mm or active bleeding were transfused with 1-6 units of PC or RDP or Platelet Rich Plasma (PRP) depending on the availability of

the product (316 cases-53.83%). In majority of the cases, where the donor was available, SDP was extracted and transfused (108 cases-34.20%). Most of the patients recovered from thrombocytopenia after transfusion of one of the above product within 25-48 hours. Faster recovery of the thrombocytopenia and leucopenia was observed in the patients who received SDP, counts almost doubled within 24-48 hours of transfusion. In this study, it was observed that lower the pre transfusion platelet count the better was the post transfusion platelet count recovery after SDP transfusion. Out of 587 cases, only two deaths have occurred due to late presentation to the hospital, severity, marked thrombocytopenia and various complications such as DSS, pneumonia and hepatic injury.

DISCUSSION

In the past few decades, dengue infection and dengue fever incidence has increased by many folds in various countries and universally. According to WHO, one modeling estimate indicates 390 million dengue virus infections per annum (95% credible interval 284-528 million), of which 96 million (67-136 million) manifest clinically (with any severity of disease) [4]. Another study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection with dengue viruses. Despite a risk of infection existing in 129 countries, 70% of the actual burden is in Asia [7]. The number of dengue cases reported to WHO increased over eight fold over the last two decades, from 5,05,430 cases in year 2000, to over 2.4 million in 2010, and 4.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4,032 [4,7].

Early diagnosis of the dengue infection is most important; delays lead to severe thrombocytopenia and coagulopathies which ultimately leads to shock and death of the patient. NS1 antigen testing is most important and most favoured because of its ease and rapid results. NS1 can be detected in the early febrile period, even from the day one of infection; NS1+IgM can be detected during the mid-febrile period; IgM in mid-febrile period: IgM+IgG in the recovery phase [8].

The standard diagnostic tests for dengue detection in a symptomatic patient are evidently the dengue virus detection, virus isolation and virus identification by cell culture. However, this is gradually being replaced by real time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method for more rapid diagnosis and accuracy [9].

In this study, a total of 587 cases were included, males were 349 and females 238. Youngest patient in this study was 3-month-old and the oldest 72 years. This study is in tandem with the study conducted by Ingale SV et al., where only less than 15% cases were in the age below 15 years and remaining were above 15 years age group [10]. In another study by Cheah WK et al., where extremities of ages were done, the age of youngest patient was eight months and oldest was 89 years; in present study youngest was 3-month-old and the oldest was 72 years [11]. Males were 349 (59.45 %) and females 238 (40.55 %) in the present study which was in close relation with the studies conducted by authors Kulkarni SK, Mohan DK and Shiddappa DM [12,13].

Out of the 587 positive tests, NS1 antigen was positive in 473 cases (80.6%), negative in 73 cases (12.45 %) and in 41 cases NS1 antigen report was not available or not done or presented late to the hospital (after four days of fever). IgM was positive in 188 cases, negative in 77 cases, and in 322 cases IgM was not done and treated only on NS1 positivity report or status and on the basis of other on other haematological parameters mainly thrombocytopenia. IgG was positive in all active and recovered cases. According to the authors Kulkarni SK, Banerjee A et al., and Kanthikar SN et al., the positivity of NS1 was 68%,81% and 76%, respectively [12,14,15].

In the present study, raised haemoglobin, PCV/HCT was observed in 52% of the cases, normal haemoglobin, PCV/HCT was seen in 43% of the cases and decreased haemoglobin, PCV/HCT was also seen in 05% of the cases. In a study done by Kulkarni SK, out of 100 positive cases, raised HCT values were observed in 49 (49%) patients while 51 (51%) had HCT values within normal range [12]. Doddamane K and Jayalakshmi MK, reported that platelet count at the time of admission or during the first few days of fever was below 10,000 in 7.5% of the patients and above 1,00,000 in 22.5% of the patients which was in correlation with this study [16]. In this study, most of the patients recovered well with platelet transfusions and supportive therapy, only two deaths occurred [Table/Fig-5].

Author/Effect and positivity	Platelet count number (%)	Haematocrit number (%)	Total leukocyte count number (%)
Ingale SV et al., [10]			
Normal (IgM+IgG)	NA	NA	NA
Raised (IgG/IgM+IgG)	NA	49 (49%)	06 (06%)
Decreased (NS1/IgM+NS1)	100 (100%)	51 (51%)	94 (94%)
Present study			
Normal (IgM+IgG)	26 (4.42%)	305 (52%)	76 (12.94%)
Raised (IgG/IgM+IgG)	00 (0.00%)	253 (43%)	00 (00.00%)
Decreased (NS1/IgM+NS1)	561 (95.58%)	29 (29%)	511 (87.06%)

[Table/Fig-5]: Comparison of Haematological parameters of the present study with the other studies.

The association of thrombocytopenia in dengue positive cases was highly significant when compared to thrombocytopenia in dengue negative fever cases. Inclusion of NS1 for evaluation of all cases of fever, either in endemic or non-endemic zones is a major boon in detecting the cases as the antibodies take nearly one week to appear in the blood therefore, antigen detection by the Immuno-Chromatographic Test (ICT) is the only means of diagnosis of dengue infection in the first few days of fever, which helps in minimising the complications and early management of complications.

Limitation(s)

This study showed the importance of early diagnosis of the dengue infection with NS1 and IgM. IgG is not of much importance as it can be seen in reinfection cases and recovered cases. The mortality rate in this study was very low and the complications arising from the dengue infections could not be evaluated totally. Further studies are recommended with extended note on complications and management.

CONCLUSION(S)

Dengue fever is rapid spreading arthropod borne infection, general awareness among the public regarding the prevention measures and constant vigilance by the healthcare officials in eliminating breeding sites of mosquitoes could go a long way in combating dengue especially in the prevention of large outbreaks by monitoring dengue viral activity by serological and molecular tests.

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