

# Evaluation of Different Laboratory Test Methods Helpful in Diagnosis of Dengue Fever Admitted in Tertiary Care Hospital

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## Abstract

Dengue is the most common arthropod borne endemo-epidemic arboviral infection in many tropical and sub-tropical regions of the world. An estimated 50-100 million dengue infections occur annually. Case fatality rate vary from 1 to 5% but can be less than 1% with appropriate diagnosis and treatment (WHO dengue) 1. As the laboratory diagnosis is confusing, the study was conducted to evaluate the different laboratory test done in microbiology, biochemistry and pathology. This study was conducted at Princess Esra Hospital, Deccan College of Medical Sciences. Total 1266 cases were studied in the age group of 5 yrs to 80 yrs (both children and adult) from July 2018 to December 2018. During this 6 month of study the total numbers of positive cases were 385 and negative cases were 881. The laboratory tests which were performed were NS1 Antigen, Dengue IgM, Dengue IgG, CBP and Platelet count, Prothrombin Time, INR, Activated partial Thromboplastin Time, Alanin Transaminase, Aspartate Transminase.

## Keywords

CBP (Complete Blood Picture); INR(International Normalized Ratio); Prothrombin Time

## Introduction

Dengue is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, Den-3, Den-4) also referred to as an arbovirus (arthropod-borne viruses) that belongs to the genus Flavivirus of the family Flaviviridae (Anderson CR) (Chambers) [1]. Patients were classified into classic dengue fever (DC) and Dengue hemorrhagic fever (DH) according to (WHO Criteria 2009) [2]. Transmissions to humans occur by the bite of the female Aedes aegypti mosquito infected by one of the four serotypes of the virus. The period of transmission from humans to mosquitoes begins one day before the start of fever up to the sixth day of illness corresponding to the Viremia phase. After a

female bites an individual in the Viremia phase, viral replication (extrinsic incubation) begins in the vector in from eight to twelve days. In humans, the incubation period ranges from 3 to 15 days (intrinsic incubation) with an average of 5 days (Oishi) (LinCF) [3].

The objective of this study was to correlate laboratory tests during the evolution of dengue fever Haemorrhagic manifestations in dengue infection seen clinically are petechiae, ecchymosis, purpurae, hematuria, hemoptysis, subconjunctival haemorrhages, epistaxis, hematemesis, malena, gum bleeds, minorrhagia, positive tourniquet test (Anuradha S)7 (Kabra SK)8. Bleeding in dengue fever is multifactorial in etiology. Several factors like thrombocytopenia, prolonged shock with capillary leak, derangement of Coagulation profile are known causes of bleeding.

## Method

This study was done in Princess Esra Hospital with included all the laboratories microbiology, pathology and biochemistry. A total of 1266 patients of suspected dengue presented during the period from July 2018 to December 2018, which included both children and adults in the age group of 5 yrs to 80 yrs. The laboratory tests which were performed were NS1 Antigen, Dengue IgM, Dengue IgG, CBP and Platelet count, Prothrombin Time, INR, Activated partial Thromboplastin Time, Alanin Transaminase, Aspartate Transminase. In the microbiology lab the total samples 1266 were processed using Igm and IgG captive ELISA and NSI ELISA according to manufacturer's instructions. (J.Mitra and Co.Pvt. Ltd) Intensity of colour/optical density was monitored at 450nm. For quality control each kit had a positive and a negative control. Calculations and interpretations were done as per kit literature.

A total of 385 cases were found to be positive. These 385 were further classified into classic Dengue Fever which was 227 cases and Dengue Haemorrhagic Fever which was 158 cases based on the clinical manifestation.

In the biochemistry lab the patients of Dengue Haemorrhagic Fever were tested for Prothrombin Time, INR, Activated Partial

thromboplastin Time, Alanine Transaminase, Aspartate Transaminase.

Prothrombin Time (PT) was done in Thrombostat using reagent Liquiplastin (clacified thromboplastin). Samples were collected in light blue cap vacutainer with buff sodium citrate. It was immediately centrifuged at 1500g for 15 min and plasma was tested.

International Normalized Ratio (INR) was calculated. The Activated Partial Thromboplastin Time (APTT) was done in Thrombostat using reagent Liquicelin E (Activated Cephaloplastin). The activator used was Ellagic acid to test the liver function Alanine Transaminase (ALT) or Glutamate Pyruvate Transaminase (GPT) and Aspartate Transaminase (AST) or Glutamate Oxaloacetate Transminase (GOT) was performed. These samples were collected in a yellow cap vacutainer with clot activator and gel for serum separation. It was done in auto analyzer cobas C311 by UV Kinetic method.

In the pathology lab, slides were prepared and complete blood picture and platlet count reports were clinically correlated with reports from microbiology and biochemistry lab.

Month	Positive	Negative
July (34)	14	20
August (97)	34	63
September (263)	61	202
October (310)	94	216
November (421)	134	287
December (141)	48	93

**Table:1** Total number of Positive and Negative cases

## Results and Discussion

Blood samples from 1266 patients during the period from July 2018 to December 2018 with clinical features suggestive of Dengue fever were processed using IgM and IgG capture ELISA and NSI ELISA. Out of these 1266 samples, 385 were serologically positive by at least one of the above test. These 385 cases were further classified into classic Dengue fever cases 227 and Dengue Haemorrhagic fever cases 158 based on the bleeding manifestations.

Dengue is most frequent arboviral infection with more than 100 million infections through the world annually. Fever, vomiting, abdominal pain, periorbital pain, headache were the most common symptoms. Bleeding manifestations were seen in 41% of cases, more common in children with Dengue Haemorrhagic fever compared to Dengue fever. The virus can infect vascular endothelium and reticuloendothelial cells which can cause diverse clinical picture and bleeding manifestation.

Leucopenia is the most prominent haematological change. In the present study the Mean Total Leucocyte count (Cells/cumm) was found to be 6,234. However there are reports of mild leucocytosis at the onset of the disease with neutrophilia. Lymphocytosis is a common finding with the presence of

atypical lymphocytes during the course of the disease. Leucocytosis was observed in patients with the classic Dengue fever in the first few days of the illness, followed by leucopenia. In Dengue Haemorrhagic fever, thrombocytopenia was observed from the beginning. The mean platelet count in the present study was 84,096/cumm. This suggest that other factors like platelet dysfunction or disseminated intravascular coagulation may have a role in bleeding in dengue fever cases. However studies which include only Dengue Haemorrhagic Fever cases show correlation between low platelet count and bleeding manifestation (Aggarwal A) [4]. The studies by (Gomber et al) 10 and (Narayan et al) 11 documented the same. The mechanisms underlying the bleeding in DHF are multiple including vasculopathy, thrombopathies and Disseminated Intravascular coagulation. Thrombopathy consists of thrombocytopenia and platelet dysfunction (Srichaikul T) [5]. AST levels increased at the onset of symptoms in all clinical forms and remained at varying but high levels during disease evolution. ALT started with above normal values in the severe form and remained steady throughout the course of the disease. In both the classic Haemorrhagic form the increase occurred progressively. Similar results were obtained by chen et al. who showed that both AST and ALT exhibited higher than average values in children chen et al. found a significant increase in transaminases, especially AST in children with dengue when compared to the control group with other febrile illness (Other than dengue fever). For an increase in ALT > 40 IU in children with dengue fever can be considered as a predictive marker for shock syndrome. The liver is one of the target organs for dengue and clinical manifestations of hepatic dysfunction can occur during the course of this disease [6]. The liver is deprived of oxygen leading to lesions of the parenchyma, in which the injured hepatocytes release transaminases that is detectable in the peripheral blood [7]. In the present study the mean ALT was 144 U/L and AST was 1220/L. In all the patients with dengue fever PT, INR, APTT was done. PT is a sensitive indicator of synthetic function. The prolonged APTT in the acute phase may be due to hepatic injury and a low grade DIC. In the present study the values for PT, INR, APTT was found to be increased. The mean PT was 103.7 sec, INR was 7.3 and APTT was 12 [8].

## Conclusion

Thus majority of om cases (61%) were detected exclusively by the presence of viral NSI antigen compared to IgM (7%) antibodies in patients sera. It is known that early detection of Dengue fever cases by NSI essay helps in diagnostic detection and confirmation of cases. It is a known fact that during a primary infection, individuals develop IgM after 5 -6 days and IgG antibodies after 7 -10 days. Thus it can be stated that supporting clinical symptoms along with early detection of viral NSI antigen can help speed up diagnosis of Dengue fever during the first 5 days of fever and fever beyond that can be diagnosed by IgM Elisa alone.

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