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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, Alanine Transaminase, Aspartate Transminase.

Keywords: CBP (Complete Blood Picture); INR (International Normalized Ratio); Prothrombin Time; Activated partial thromboplastin 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. Patients were classified into classic dengue fever (DC) and Dengue hemorrhagic fever (DH) according to. Transmissions to humans occur by the bite of the female Aedes algypti mosquito infected by one of the four serotypes of the virus [1]. 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 [2].

Methods

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, Alanine Transaminase, and Aspartate Transminase [3-7]. In the microbiology lab the total samples 1266 were processed using Igm and IgG captive ELISA and NSI ELISA according to manufacturer's instructions. Intensity of colour/optical density was monitored at 450 nm. 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 Transminase, 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 1500 g for 15 min and plasma was tested [8-11].

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 [5]. It was done in auto analyzer cobas C311 by UV Kinetic method. In the pathology lab, slides were prepared and complete blood picture and platelet count reports were clinically correlated with reports from microbiology and biochemistry lab [12].

Month	NSI	NSI+IgM	NSI+IgG	IgM	IgG	IgM+IgG	NSI+IgG +IgM
July	5	1	-	-	4	4	-
August	13	3	3	1	2	9	4
Septe mber	36	2	-	3	7	8	5
Octob er	65	2	-	6	3	18	-
Nove mber	80	1	-	10	8	30	5
Dece mber	15	1	1	2	2	12	1

Table 1: Shows the serological distribution of these 385 positive cases.

Result 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 classific 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. Leucopoenia 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, thrombocytoperia 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.

Conclusion

Thus majority of cases (61%) were detected exclusively by the presence of viral NSl antigen compated to IgM (7%) antibodies in patient's sera. It is known that early detection of Dengue fever cases by NSl 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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