BIOCHEMICAL EVALUATION OF LIVER FUNCTIONS IN DENGUE PATIENTS

Indira Bhaskar Biswas¹, Sandip Ghosh², Nibedita Basu³, Chittaranjan Maity⁴

¹Professor, Department of Biochemistry, KPC Medical College & Hospital, Kolkata.

²Assistant Professor, Department of Biochemistry, KPC Medical College & Hospital, Kolkata.

³Professor, Department of Biochemistry, KPC Medical College & Hospital, Kolkata.

⁴Professor, Department of Biochemistry, KPC Medical College & Hospital, Kolkata.

ABSTRACT

BACKGROUND

Dengue fever is a self-limited, systemic viral infection transmitted between humans by mosquito. It is a global health problem.

MATERIAL AND METHODS

In our study, liver functions were assayed in dengue patients admitted in KPC Medical College and Hospital. All the patients were selected randomly irrespective of age and sex. The diagnosis was established by clinical signs and symptoms, blood platelet count and serological tests such as NS1 antigen (ELISA), and IgM level (MAC-ELISA).

RESULT

Out of 98 patients, 6 had dengue haemorrhagic fever. 82 patients had enlarged liver of about <2 cm. In all cases, platelet count was low, NS1 was reactive and IGM was high. Liver enzymes such as Alanine transferase (ALT), Aspartate transferase (AST), Alkaline Phosphatase (ALP) were increased. Serum Protein levels (Total Protein), Albumin, globulin were less than normal level and A:G was altered, whereas serum bilirubin level there was no significant change. After seven days when there was complete recovery and the patient was waiting to be discharged, the parameters were repeated and it was found that the values were 2 to 3 times more than the normal.

CONCLUSION

It was concluded that in dengue patients, liver was affected. Besides that in few patients particularly in dengue haemorrhagic fever, Prothrombin time and Prothrombin level and platelet counts were also correlated.

KEYWORDS

Dengue, Hepatic Functions, Liver Enzymes.

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INTRODUCTION

Dengue fever also known as break bone fever is an infectious tropical disease caused by dengue virus. Dengue is now a global threat and is endemic or epidemic in almost every country located in tropical and subtropical areas. Incidence of dengue has grown dramatically around the world in recent decades. A recent estimate indicates 390 million dengue infection per year (95% credible interval 284-528 million) of which 96 million (95% credible interval 67-136 million) manifest clinically.^[1] Existence of dengue like disease was reported in 1779 when an epidemic swept Asia, Africa and North America.^[2] In India, DENV (dengue virus) was isolated in 1946 and many outbreaks were reported.^[3] Dengue haemorrhagic fever was first reported in Kolkata, West Bengal in 1963.^[4] The rapidly expanding global foot print of dengue is a public health challenge with an economic burden that is currently unmet by licensed vaccines, specific therapeutic agents or efficient vector control.

Incidence of dengue has increased due to urbanisation, population growth, increased international travel, global warming.

Dengue fever is caused by RNA flavivirus transmitted to people through the bite of a female *Aedes aegypti* mosquito. Infection with dengue virus can be present in mild form as classic dengue fever or more severe form of disease: the dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue virus an arbovirus, circulates as four distinct serological types DENV-1,2,3,4. Contracting one form of dengue fever provides lifelong immunity from that serotype but not from other serotypes. If a person is infected a second time by a different strain, the antibodies from the first attack can only partially bind to the virus and are unable to prevent infection. The antibodies with the virus loosely attached then shuttles into an immune cell would normally kill the virus, but because the virus is not properly attached it breaks free once it gains entry to the human immune cell where it replicates into more viral particles and thereby enhancing the infection.^[5] Some evidence has suggested that there is greater involvement of liver infection with DENV-2 compared to other 3 serotypes. Monotypic infection with DENV-1 was 9.5%; with DENV-2 60.8% and with DENV-3 was 29.7%.^[6] A study carried out in Brazil showed that DENV-3 was isolated in more number of cases than DENV-2 in the classic form of the disease.^[7]

Diagnosis of dengue is based on the findings of fever plus two of the following symptoms: rash, generalised pain, nausea, vomiting, low WBC count, positive tourniquet test or any warning signs like abdominal pain, mucosal bleeding,

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Corresponding Author:

Dr. Indira Bhaskar Biswas,

Professor,

Department of Biochemistry,

KPC Medical College & Hospital, Kolkata.

E-mail: indu170@rediffmail.com

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increased haematocrit with low platelets, lethargy and liver enlargement in someone who lives in endemic area.^[8] Decreased level of consciousness occurs in 0.5-6% of severe cases which is attributed either to infection of the brain by the virus or indirectly as a result of impairment of vital organs, for example the liver.^[9]

Liver involvement is universally present in children and in adult patients with dengue infection (DI).^[10] Hepatocytes are the site of dengue virus replication and hence the involvement of liver in dengue disease.^[11] Liver damage is a common complication of dengue infection and aminotransferase levels are a valuable marker for monitoring dengue cases.^[7]

The present study is aimed to evaluate the hepatic functions in DENV patients who were seen in Kali Pradip Chaudhuri Medical College Hospital, Kolkata.

MATERIALS AND METHODS

A prospective study of 98 patients diagnosed and treated at our institute during an outbreak of dengue infection in Kolkata. Patients were selected randomly irrespective of all age group and both sex. All clinically suspected DVI patients as per WHO guidelines criteria were screened and the probable diagnosis was based on 2 or more of the symptoms with high fever, a detailed clinical examination, serological and haematological tests were conducted to confirm the diagnosis of DVI. And in DHF patients who presented with high fever of sudden onset, retro-orbital pain, arthralgia, generalised malaise, bleeding particularly in skin (Petechiae), occasionally in gums and nose, melena and low blood pressure were included. Patients diagnosed with malaria, enteric fever, hepatitis by relevant investigations were excluded.

SEROLOGY

Test for the detection of anti-dengue Abs were carried out in serum samples collected after 5th day –10th day following the onset of symptoms. Viraemia in dengue lasts for less than 5 days and that IgM antibody response takes 5-10 days to develop in cases of primary dengue viral infection and 4-5 days in case of secondary dengue virus infection.^[8] MAC-ELISA & NS1 Antigen tests were conducted in accordance with the manufacturer's instructions. When results were positive, patients were considered to be currently infected with Dengue virus. Platelet count and haematocrit also supported the diagnosis of Dengue virus. Liver enzymes such as ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline Phosphatase), Serum protein levels–Total proteins, Albumin, Globulin, A: G, Serum Bilirubin levels were measured on the day of admission (on the 5th day after the onset of symptoms) and also the tests were repeated before the patient was fit to be discharged from hospital.

Analysis of Liver Function Tests

AST, ALT, Total Protein, ALP was analysed by Autoanalyser Mindray BS-390. Kit used was Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China.

Method for AST

Aspartate aminotransferase kit {International Federation of Clinical Chemistry (IFCC)} without pyridoxal phosphate activation.

Method for ALT

Alanine aminotransferase kit {(International Federation of Clinical Chemistry (IFCC)} without pyridoxal phosphate activation.

Method for Alkaline Phosphatase

IFCC and Laboratory medicine modified method. Dengue fever may be diagnosed by Virus isolation in cell culture, nucleic acid detection by PCR, viral antigen detection or specific antibodies (serology).^[12] PCR and Viral antigens detection are more accurate in the first 7 days, not widely available due to their greater cost.^[13] Tests for dengue-virus specific Abs, types IgM & IgG can be useful in confirming a diagnosis in later stages of infection. In a person with symptoms, the detection of IgM is considered diagnostic.^[14]

RESULTS

The prospective study is an attempt to elucidate the clinical profile and laboratory findings of dengue infected patients seen in our hospital. 92 patients (94%) presenting with classic dengue features and 6 (6%) were DHF; 3 of these patients went into DSS. Out of 98 patients, 41 were males and 57 females. Aged between 09 to 65 years with majority of cases ranging between 10-19 yrs. Adults were 83 and Children 15. The common clinical symptoms by the dengue patients were fever, headache, GI symptoms is shown in fig 1. The routine lab investigations done in patients with DVI are shown in the Table 1. Laboratory parameters in patients with dengue viral infection is shown in Table 2. Serological tests showed 81% NS1 reactive and 100% IgM reactive and 7% showed IgM & IgG positive. Liver function tests showed increased levels of aminotransferases in all our 98 cases (Table -3). Total protein was <6 g% in (74%) and Albumin <3.5 and A:G ratio was <1. But the Serum bilirubin level was within normal limits.

When the presenting symptoms had subsided, the tests were repeated and we found aminotransferase levels did not come to normal levels.

STATISTICS

Median values for all groups and both Median and Mean values for DHF groups. 95% Confidence Interval (CI) of median for the selected parameters of Dengue cases have been determined along with 95% CI of mean values of the parameter in DHF to understand the range of 95% CI. For DF, Median has been used as a representative average along with its 95% CI. Median was used because fluctuation is less, more appropriate for large sample >30 regarded. For DHF n=6. Thus, for small number of values of the parameter mean being better representative average has been used along with 95% CI.

Fig. 1: Percentage of Dengue patients with Clinical Symptoms

Parameter	Values
Haemoglobin	<10.5 g/dL (37%)
Platelet count	< 100,000/cu. mm (87%) <50,000/cu. mm (13%)
White Blood Cells	< 3000/cu. mm (24%)
Prothrombin time	>17 seconds (27%)
Table 1: Laboratory Parameter in Patients with Dengue Viral Infection (n=98)	

Liver Enzymes	DF (n=92)				DHF (n=6)		
	Median	95% CI of Median <200	Median	95% CI of Median <400	Median	Mean	95% CI of Mean >400
AST	121	n=38 115-172	309	n= 54 252 -331	578	619	510 -728
ALT	135	n= 30 102 -161	322	n=62 284 -355	612	653	547 -759
ALP	152	n= 42 130 -188	297	n= 50 220 -346	1017	1033.5	988 - 1078
Table 2: Comparison of Liver Enzymes between classic Dengue (DF) and DHF							

AST-aspartate transaminase, ALT-alanine transaminase, ALP-alkaline phosphatase.

DISCUSSION

The results of the present study showed that liver injury was present in almost all cases with dengue infection as indicated by the abnormal liver function tests and clinical manifestations of liver disease namely hepatomegaly, pain in the right hypochondrium, ascites and pedal oedema.

In our study, 85% patients had hepatomegaly which includes 15 children and 67 adults, 17% had pedal oedema, 15% had ascites on clinical examination. Wahid SF et al has reported that in children, liver involvement is more profound in severe forms of DI such as DHF, DSS.^[15]

Kuo et al reported that approximately 90% had abnormal AST, ALT, ALP, bilirubin and GGT.^[16] Liver involvement occurred through an inflammatory process in parenchyma, provoked directly or indirectly by the virus, reducing the diameter of the lumen, biliary canaliculi causing obstruction. In our study, AST, ALT values were more than 5 times in classic dengue infection and ALP levels were also increased. And more than 10 times in DHF patients, bilirubin levels were within normal limits. The tests were repeated before discharge and it showed the enzyme values did not reach the normal limits.

In children, the aminotransferase levels were more than 10 times indicating children are at higher risk of hepatic involvement and possibility of developing hepatic encephalopathy.^[17]

Hypoalbuminaemia in 74% and reduction of serum globulin may be an important factor of fluid loss into third space which is an indicative of severity of DVI.

None of our patients had jaundice, serum bilirubin levels were within normal limits, but 23% had itching.

Mechanism of liver injury in dengue may be due to direct effects of the virus or host immune response on liver cells, circulatory compromise, metabolic acidosis and hypoxia caused by hypotension or localised vascular leakage inside the liver.^[10,18]

In our study, all our patients had two and more symptoms of DI. Liver profile showed increased values to support that there was liver involvement in DF and severe in DHF or DSS, but there were no deaths.

CONCLUSION

Hepatic dysfunction was observed in both classic dengue and severe forms DHF and DSS. Liver enzyme showed significant rise in children and also in adult. The abnormalities of liver function and degree of hepatic enlargement did not correlate. Even after 2 weeks, aminotransferase levels were much above the reference value. In geographical areas where dengue is endemic, patient presenting with high fever, tender hepatomegaly and increased liver enzyme levels, should be strongly considered as DI. To define the mechanism of liver injury in DI, further studies are required. Limitation of our study was ultrasound, and biopsy of liver was not done to confirm the diagnosis. Dengue haemorrhagic fever patients number are too small, it is hard to make conclusion difference of these two groups with these results.

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