Original Article

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Serum Aminotransferase Levels as a Marker in Assessment of Severity of Dengue Fever in Adult Patients- A Cross-sectional Study

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ABSTRACT

Introduction: Dengue Fever (DF) is the most common rapidly spreading mosquito-borne arboviral disease with a wide clinical spectrum that includes both severe and non severe clinical manifestations. Most patients with DF have liver involvement in the form of elevated serum Aminotransferase (AST). Severity of the disease is more in patients with elevated serum ASTs. Hence, this study was taken-up to assess the association of the elevated serum ASTs and severity of the disease.

Aim: To measure and associate the serum AST levels as a marker in assessing severity of DF in adults.

Materials and Methods: The cross-sectional study was conducted on 100 patients of DF admitted to General Medicine wards from May 2019 to April 2020 in Hassan Institute of Medical Sciences and hospital, Hassan, Karnataka, India. Serum AST levels were estimated at admission, on the day of discharge of the patient and related to prognosis of patient in DF. A predetermined proforma was used to record the details of history, physical examination and investigation and results were recorded in a Microsoft excel master chart. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software, version 20.0, Analysis of Variance (ANOVA) and student t-test were used for calculation of significance in all parameters within the groups while Fischer's-exact test was used to compare the significance between the groups. The p-value <0.05 was to be considered level of significance.

Results: In this study, 100 (mean age: 31.65±12.08 years) patients of dengue Non Structural protein 1/Immunoglobulin M (NS1/IgM) positive patients were studied. Out of this 60% NS1 Antigen (Ag), 22% NS1 Ag and IgM Antibody (Ab) and 18% were IgM Ab positive. 63 were males and 37 were females. The AST/SGOT values on day 1 was 203.76±360.87 U/L and on day of discharge was 55.96±31.76 U/L (p-value <0.001). And ALT/SGPT on day 1 was 109.63±146.01 U/L and on day of discharge was 41.05±20.78 U/L (p-value <0.001). The above results indicate that dengue patients had raised AST/SGOT values on day 1 as compared to day of discharge. There was a significant difference (p-value <0.001) found on day 1 and at the time of discharge.

Conclusion: Hepatic involvement, characterised by elevation of liver enzymes, is very common finding in Dengue Fever. Serum Aminotranferase (AST) levels are elevated more as compared to serum Alanine aminotransferase (ALT) . The present study found significant association of serum AST levels with the hospital stay and prognosis of the patient. Hence, serum AST levels are mandatory in DF to look for complications and it is of prognostic value.

Keywords: Arboviral disease, Liver dysfunction, Liver investigations, Poor prognosis, Severity marker

INTRODUCTION

The DF, also known as break-bone fever is the most common rapidly spreading mosquito-borne arboviral disease in the world. Dengue infection as a systemic disease, involves a wide variety of severe and non severe clinical manifestations and presentations in patients [1-3]. The severe form of disease presenting as plasma leakage with or without haemorrhage, follows a difficult clinical course for treatments, while most of the non severe patients are managed as normal criterias [2,4].

The causative agent, Dengue Virus (DENV) belongs to genus Flavivirus of the family Flaviviridae. It has a single-stranded ribonucleic acid genome surrounded by an icosahedra nucleo-capsid covered by lipid envelope. The DF and Dengue Haemorrhagic Fever (DHF) are caused by one among the four antigenically related dengue viruses DENV-1, DENV-2, DENV-3 and DENV-4. Infection with one serotype provides life-long immunity to that virus but not to the others [5].

The worldwide spread of dengue has increased dramatically nowadays, used to be endemic for various countries of South East Asia, Africa, South and North America and the Mediterranean regions. Inhabitants of tropical and sub-tropical regions also considered to be at high risk of DF infection. In the last 50 years, the incidence of dengue infection has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings with near about hundred million dengue infections occurring annually, and many billions people residing in dengue endemic countries [2].

Each year near about 100 million cases reported of dengue, 5 lakhs cases reported of DHF and more than 10,000 deaths because of dengue occurs throughout the world. Mortality in dengue cases had been much more commonly reported in children. Bangladesh, Myanmar, Sri Lanka, India, Thailand and other South East Asian countries are currently considered as parent areas to DF and DHF [4]. India, Indonesia and Myanmar have reported, focal outbreaks, away from the urban areas with unexpectedly high case-fatality rates of 3-5%, as against expected case fatality rate of 1% in these regions [4].

Characteristic features are fever and minimal constitutional symptoms to shock and bleeding tendencies or DHF/Dengue Shock Syndrome (DSS) [5,6].

Close monitoring of vital parameters, platelet count and haematocrit is primarily required in management of Dengue fever. It has been shown that most patients with DF have liver involvement in the form of elevated serum ASTs. Reactive hepatitis as well as direct injury to hepatocytes by the virus itself, both are the mechanisms considered in elevation of liver enzymes [7]. Patients with hepatitis are more likely to have increased risk of bleeding tendencies in the patients along with other abnormalities like, renal failure, encephalopathy or acalculous cholecystitis. In addition to thrombocytopenia, deranged

liver function plays a significant role in bleeding. Therefore, evaluation of liver function, particularly the ASTs, needs to be checked routinely during management of patients with dengue. Biochemical alterations in body have been related to the severity of DF [7]. Atypical forms of dengue infection are numerous. Notable one is liver dysfunction. Liver injury is due to either direct injury to hepatocytes by virus itself or indirectly due to an immune mediated damage to hepatocytes (consequence of deregulated host immune response) [8].

Although liver is not the major target organ, still many related changes like centrilobular necrosis, steatosis, kupffer cells hyperplasia, acidophilic bodies, and monocytic infiltration of portal tracts have been reported many times [9]. Hence, the present study was conducted with the objective to measure and to associate the serum AST levels as a marker in assessing severity of DF in adults.

MATERIALS AND METHODS

The cross-sectional study was conducted with patients of DF admitted under General Medicine wards of Hassan Institute of Medical Science, Hassan, Karnataka, India. The total duration of the study was one year, from May 2019 to April 2020, after obtaining approval from Ethical Committee of Hassan institute of medical science, Hassan (Ref:IEC/HIMS/RR67/21-05-2019).

Inclusion criteria: Both male and female patients of all age groups ranging from 18 to 80 years, Dengue NS-1 Antigen or IgM Ab positive cases and who gave written informed consent were included in the study.

Exclusion criteria: Patients of age <18 years, or with chronic liver disease due to any cause and those with positive Hepatitis-B surface antigen (HBsAg), Immunoglobulin M (IgM) to Hepatitis A Virus (HAV) or Hepatitis C Virus (HCV), who had taken any alternative medication or were on any other known hepatotoxic drug for the last six months and patients with Malaria and Typhoid fever, with history of alcohol abuse and those not willing to participate in the study were excluded.

A total of 100 patients of DF were included in the study. After admission of cases based on dengue serology (NS1 antigen IgM, Ab), detailed history and clinical examination were done along with platelet count. Routine haematological investigations along with liver function tests and platelet count on day one and on day of discharge were carried out. Serum AST levels were estimated at admission, on the day of discharge of the patient and its association to prognosis of patient in DF. The lab values of serum AST levels has been analysed with the clinical profile and outcome in these study groups.

STATISTICAL ANALYSIS

Descriptive and inferential statistical analysis has been carried out in the present study. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more group of patients, student t-test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. Results were taken in a Microsoft excel master chart and statistical analysis was done using SPSS, version 20.0.

RESULTS

Out of 100 patients studied, most of them were in the age group of 21-30 years (n=57) and 31-40 years (n=20). The mean age was 31.65±12.08 [Table/Fig-1].

Out of 100 patients studied, 63 patients were males and 37 patients were females. Eighteen patients were found to have bleeding signs, 09 patients were with hepatosplenomegaly 60 patients, were detected with NS1 Antigen positive, 22 patients were detected with both NS1 Antigen and IgM Ab positive and 18 patients were detected with IgM Ab positive.

Out of 100 patients studied, 35 patients were detected with free fluid in the abdomen, four patients were detected with splenomegaly, three patients were detected with hepatosplenomegaly and three patients were detected with gall bladder pathology in the form of thickening of wall and acalculous cholecystitis [Table/Fig-2].

Age (years)	Frequency (n)	Percentage (%)
18-20	4	4.0
21-30	57	57.0
31-40	20	20.0
41-50	10	10.0
51-60	6	6.0
61-70	2	2.0
>70	1	1.0
Total	100	100.0

[Table/Fig-1]: Age distribution. Mean age 31.65±12.08 years, N=100.

Ultrasound abdomen	No. of patients	Percentage (%)
Free fluid	35	35.0
Gall bladder pathology	3	3.0
Hepatosplenomegaly	3	3.0
Normal	54	54.0
Polycystic ovarian disease	1	1.0
Splenomegaly	4	4.0
Total	100	100.0

[Table/Fig-2]: Ultrasound abdomen findings of patients studied.

Out of 100 patients studied, 85 patients were found to have thrombocytepenia (<100,000 Lac/cumm) and 13 patients had (<150,000 Lac/cumm) on day one of admission and there was a significant increase in platelet count on day of discharge with a change of 84.0% as depicted in [Table/Fig-3].

Platelet count (1.5-4.5 Lac/cumm)	Day 1	Day 5/Discharge	Percentage (%) change
<1.0	85 (85%)	1 (1%)	84.0%
1-1.5	13 (13%)	11 (11%)	2.0%
1.5-2	2 (2%)	26 (26%)	24.0%
>2.0	0 (0%)	62 (62%)	62.0%
Total	100 (100%)	100 (100%)	-

[Table/Fig-3]: Platelet count distribution of patients studied.

Ninety-four of the total patients stayed about 4-7 days in the hospital. Among them 61 patients were males and 33 patients were females [Table/Fig-4].

Duration of stay in hospital (in days)	Gender		Total
	Female	Male	
1-3	2 (5.4%)	0 (0%)	2 (2%)
4-7	33 (89.2%)	61 (96.8%)	94 (94%)
8-15	2 (5.4%)	2 (3.2%)	4 (4%)
Total	37 (100%)	63 (100%)	100 (100%)

[Table/Fig-4]: Duration of stay in hospital (in days) distribution of the patients studied.

Eighty two patients were diagnosed with classical DF, 16 patients were diagnosed with DHF and 02 patients were diagnosed with DSS [Table/Fig-5].

Diagnosis	No. of patients	Percentage (%)
DF	82	82.0
DHF	16	16.0
DSS	2	2.0
Total	100	100.0

[Table/Fig-5]: Diagnosis based on severity of the disease.
DF: Dengue fever; DHF: Dengue haemorrhagic fever; DSS: Dengue shock syndrome

Student t-test (paired) was applied to test the significance among the parameters of complete blood count. There was a significant difference (p<0.001) found among the parameters on day 1 and at the time of discharge of the patients, like Haemoglobin 14.14±2.09 mg/dL and 13.63±1.63 mg/dL, Packed Cell Volume (PCV) 42.29±6.01% and 38.85±4.78%, Total Leucocyte Count (TLC) 5033.30±3414.86 cells/cumm and 6344.50±1974.80 cells/cumm, and platelet count 0.50±0.38 Lac/cumm and 2.40±0.79 Lac/cumm, respectively indicating dengue patients had raised haematocrit and decreased TLC and platelet count on day 1 [Table/Fig-6]. There was a significant difference (p-value<0.001) found among the liver parameters on day 1 and at the time of discharge of the patients, like total bilirubin 0.74±0.70 mg/dL and 0.63±0.50 mg/dL, AST/SGOT 203.76±360.87 U/L and 55.96±31.76 U/L and ALT/SGPT 109.63±146.01 U/L and 41.05±20.78 U/L, respectively indicating dengue patients had raised AST/SGOT on day 1. The AST levels were found to be higher than ALT levels [Table/Fig-6].

CBC	Normal ranges	Day 1	Day of discharge	Difference	t-value	p-value
Haemoglobin (mg/dL)	14-18	14.14±2.09	13.63±1.63	0.512	4.619	<0.001
PCV (%)	40-54	42.29±6.01	38.85±4.78	3.439	7.727	<0.001
Total leucocyte count (cells/cumm)	4000-11000	5033.30±3414.86	6344.50±1974.80	-1311.200	-4.182	<0.001
Platelet count (Lac/cumm)	1.5-4.5	0.50±0.38	2.40±0.79	-1.897	-25.633	<0.001
LFT	**Normal ranges**	**Day 1**	**Day of discharge**	**Difference**	**t-value**	**p-value**
Total bilirubin (mg/dL)	0.1-0.8	0.74±0.70	0.63±0.50	0.113	3.410	0.001
AST/SGOT (U/L)	0-38	203.76±360.87	55.96±31.76	147.800	4.409	<0.001
ALT/SGPT (U/L)	0-41	109.63±146.01	41.05±20.78	68.584	5.150	<0.001

[Table/Fig-6]: Assessment of Complete Blood Count (CBC) and Liver Function Test (LFT) on admission, and Day of Discharge for the subjects. Student's t-test used for analysing the level of significance. p-value <0.05 to be considered significant. PCV: Packed cell volume.
AST/SGOT: Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase: ALT/SGPT: Alanine aminotransferase/Serum glutamic pyruvic transaminase

Fisher-Exact test was applied to test the significance between platelet counts in relation to AST levels in dengue patients. There was a statistically significant association between AST levels and platelet count (p-value 0.009). This implies that increase in AST levels is associated with significant decrease in platelets in dengue patients [Table/Fig-7].

Platelet count (1.5-4.5 Lac/cumm)	AST values (units/L)				Total
	0-38	39-100	101-200	>200	
<1.0	0 (0%)	20 (76.9%)	36 (83.7%)	29 (96.7%)	85 (85%)
1-1.5	0 (0%)	6 (23.1%)	6 (14%)	1 (3.3%)	13 (13%)
1.5-2	1 (100%)	0 (0%)	1 (2.3%)	0 (0%)	2 (2%)
>2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	1 (100%)	26 (100%)	43 (100%)	30 (100%)	100 (100%)

[Table/Fig-7]: Platelet count in all subjects in relation to AST values: Fischer-exact test used to find significance.

The ANOVA test was applied to test the significance among the clinical variables according to AST levels. There was a significant difference (p-value <0.001) found among the platelet count with average of 1.88±0.00 Lac/cumm when AST were normal and with average of 0.41±0.24 Lac/cumm when AST were >200. There was a significant association between levels of AST/SGOT and platelet count [Table/Fig-8]. The AST/SGOT were found to be 203.76±360.87 U/L and 55.96±31.76 U/L (p-value 0.001) and ALT/SGPT 109.63±146.01 U/L and 41.05±20.78 U/L (p-value <0.001), respectively indicating dengue patients had raised AST/SGOT than ALT levels [Table/Fig-6].

DISCUSSION

Currently, dengue is causing major public health concern throughout the World particularly in South East Asian countries. Dengue outbreaks, caused significant morbidity and mortality in certain parts of India. Hepatic involvement in DF, measured in the form of elevated serum AST and is very common in the patients. The most common mechanisms considered for the same are both direct injury by virus to the hepatocytes as well as reactive hepatitis. The involvement of liver and its severity had been indicated by elevated serum AST, hence can be considered a good marker [10,11]. Those patients with elevated liver enzymes, mainly AST are more likely to have associated with disease severity in the form of increased risk of bleeding tendencies, shock, and acalculous cholectystitis. In addition to decreased platelet count, hepatic dysfunction plays a significant role in bleeding [12]. Hence, it is mandatory to evaluate serum ASTs in all patients with DF and it serves as an early indicator of dengue infection. It is found that, out of 100 patients, 91 patients had elevated liver enzymes and these patients had more complications like hepatitis, shock and in some cases bleeding. Among the liver enzymes, AST levels are higher compared to the ALT levels [13].

Chen HC et al., observed about three tenth of dengue patients had hepatic involvement [14]. Liver involvement is significantly higher in Asian populations and the rate of hepatic dysfunction in shock patients was somewhat higher than that of non shock patients. Pancharoen C et al., also observed that mean values of AST and ALT were significantly increased in patients with DHF [15]. Kuo CH et al., reported increased levels of AST and ALT to be found associated with many complications like severe bleeding manifestations in dengue patients [16].

Nguyen TL et al., found that damaged liver function plays a significant role in bleeding in addition to thrombocytopenia [17].

In the present study, out of 100 patients, 98 patients had thrombocytopenia. Out of 98 patients with thrombocytopenia, 22 patients had bleeding tendencies whereas out of 91 patients with elevated liver enzymes, 18 patients had bleeding tendencies. Out of

Variables	AST (units/L)				Total	p-value
	0-38	39-100	101-200	>200		
Haemoglobin (mg/dL)	15.00±0.00	13.90±2.03	14.14±2.28	14.32±1.94	14.14±2.09	0.870
PCV (%)	43.00±0.00	41.72±5.97	42.59±6.38	42.33±5.77	42.29±6.01	0.951
Total leucocyte count (cells/cumm)	8000.00±0.00	5042.31±3043.70	4994.19±2919.81	4982.67±4378.07	5033.30±3414.86	0.861
Platelet count (Lac/cumm)	1.88±0.00	0.63±0.37	0.46±0.40	0.41±0.24	0.50±0.38	<0.001
Total bilirubin	0.30±0.00	0.86±1.17	0.67±0.39	0.75±0.50	0.74±0.70	0.669
AST/SGOT (U/L)	36.00±0.00	74.27±17.14	140.92±27.68	411.65±613.70	203.76±360.87	0.001
ALT/SGPT (U/L)	40.00±0.00	45.65±26.10	81.49±29.98	207.75±236.48	109.63±146.01	<0.001

[Table/Fig-8]: Different clinical variables compared with AST values in subjects. Values expressed as mean±SD. ANOVA test applied to calculate the significance between groups.
p-value <0.05 to be considered significant; PCV: Packed cell volume; AST/SGOT: Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase; ALT/SGPT: Alanine aminotransferase/Serum glutamic pyruvic transaminase

100 patients, two patients were presented in shock. In both patients liver enzymes were elevated with AST levels more than ALT levels.

Sedhain A et al., study reported significant increase of AST and ALT levels in DHF patients as compared to those in DF patients and also found that AST levels were greater than ALT levels in contrast to viral hepatitis. The same study reported the importance of Ultrasound (USG) findings like hepatomegaly, gall bladder thickening and third space loss, in detecting the extent of liver involvement. They were higher significantly in DHF as compared to DF patients [18].Thus USG findings correlated with serum elevated enzymes and clinical presentation of the patient suffice the complete prognostic analysis in the DF and DHF patients.

Limitation(s)

The sample considered for the study was less and also confined to a single centre, hence the results could not be generalised.

CONCLUSION(S)

Hepatic involvement in DF, characterised by elevated liver enzymes, AST more than ALT levels and the condition is associated with complications like bleeding, shock and organ impairment. In addition to thrombocytopenia, hepatic involvement plays a significant role in bleeding. Elevated liver enzymes have been observed to have prognostic value by authors in the present study. With the high prevalence of serum AST levels in DF, and risk for poor prognosis in such patients, frequent laboratory monitoring is recommended, especially in high-risk groups.

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