A clinical study of serum alanine and aspartate aminotransferase levels in dengue haemorrhagic fever

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Abstract:

Background: Early recognition and prompt initiation of treatment are vital if dengue related morbidity and mortality are to be limited. Dengue when left untreated, the mortality rate is as high as 20%. The case fatality rate in patients with Dengue hemorrhagic fever and Dengue shock syndrome can be as high as 40%. The exact clinical and laboratory profile is crucial for diagnosis as well as successful management of the patients.

Objective: To evaluate frequency ofliver involvement and aminotransferase levelchange in dengue fever and to determine relationof transaminase level change with diseaseseverity

Methods:A singe centered cross-sectional study was conducted among 60 known cases of dengue. Serum aminotransferase levels were analyzed by Modified IFCC Method (International Federation of clinical chemistry).

Results: Majority belonged to 21-30 years (46.7%). Males constituted 68.3%. Most common clinical manifestation was fever (88.3%). Alainine aminotransferase, aspartate aminotransferase were significantly and negatively correlated with platelet count. They were significantly and positively correlated with hematocrit values but platelet count was negatively correlated with the hematocrit which was significant.

Conclusion: Hepatic dysfunction is very common in all forms of dengue infection, with AST rising significantly more than ALT. When considered as prognostic marker, there is a direct correlation. The higher the derangement in serum transaminase level, more is the chance of the disease being severe. And also, the higher the derangement in the level of transaminases, more was the number of days of hospitalization.Therefore, use of liver tests to evaluate degree of liver damage is of great importance, and markers such as AST and ALT may be used as parameters to evaluate severity of disease, and also a cost effective way for a better therapeutic approach.

Key words: alanine, aspartate, dengue, fever

INTRODUCTION

Dengue is a mosquito borne acute viral illness caused by infection withone of the 4 serotypes of Dengue virus (DENV), an arthropod borne singlestranded RNA virus of genus Flavivirus.Dengue is transmitted by mosquitoes of the genus Aedes, mainly Aedesaegypti with an incubation period of 4-7 days which may range from 3-14days.The seasonal transmission of dengue is more in monsoon and post monsoon. [1]

Dengue is primarily an urban disease. The incidence of dengue indeveloping nations, like India, has increased due to deficient watermanagement, unplanned urbanization, and migration of

population from rural tourban areas with complete lack of proper sanitation facilities. DF and DHF arealso now spreading to rural areas worldwide. [2]

Today, dengue ranks as the most important mosquito borne viral diseasein the world. Currently, at least, 112 countries are endemic for dengue andabout 40% of the world population (2.5-3 billion people) are at risk in tropicaland sub-tropical countries. Annually, 100 million cases of DF and half amillion cases of DHF occur worldwide. In the past 50 years, incidence ofDengue has increased 30-fold with significant outbreaks occurring in five of the sixWorld Health Organization (WHO) regions. South East Asia is one of the regionswith highest risk of DF/DHF accounting for 52% of the global risk.[3]

The first virologically proven epidemic of DF in India occurred in Calcutta andEastern Coast of India in 1963-1964. Outbreaks are now reported quitefrequently from different parts of our country. Incidence of dengue is rising inIndia from 6.34 per million population between 1998 and 2009 to 34.81 permillion population between 2010 and 2014.The incidence of DF hasincreased many folds in the last four decades.Initially, Dengue infection may be asymptomatic (50-90%),may resultin a non-specific febrile illness or may produce symptom complex of classicDengue fever (DF) to Dengue Shock syndrome (DSS) and multi organdysfunction as a part of severe illness. A small percentage of people who have previously been infected by one dengue serotype develop bleeding and endothelial leak upon infection with another dengue serotype. This syndrome is termed as Dengue Hemorrhagic fever (DHF). Characteristic laboratory findings of Dengue fever include thrombocytopenia, leucopenia, and elevated liver enzyme.[4]

Atypical manifestations of Dengue fever are hepatitis, acute kidney injury, ARDS, pancreatitis, diarrhea, acalculous cholecystitis, myositis, myocarditis, conduction abnormalities, disseminated intravascular coagulation, and atrial fibrillation. Atypical manifestations also include neurological manifestations such as seizures, encephalitis, meningitis, transverse myelitis, and Guillain–Barré syndrome.[5]

Dengue virus is a non-hepatotropic virus but liver injury due to dengue infection is not uncommon and has been described since the 1960s.Hepatic involvement can lead to acute hepatitis and characterized by pain in the right hypochondrium, hepatomegaly, jaundice, and raised aminotransferase levels. In hepatitis, the levels of these enzymes reach a maximum on the ninth day after the onset of symptoms, and they gradually return to normal levels within three weeks. Histopathological findings of liver involvement include centrilobular necrosis, fatty alteration, hyperplasia of Kupffer cells, acidophil bodies, and monocyte infiltration of portal tract.[6]

Early recognition and prompt initiation of treatment are vital if disease related morbidity and mortality are to be limited. Dengue fever is typically a self-limited disease with a mortality rate of less than 1% when detected early. When treated, severe Dengue has a mortality rate of 2%-5%, but, when left untreated, the mortality rate is as high as 20%. The case fatality rate in patients with DHF and DSS can be as high as 40%. [7]

The exact clinical and laboratory profile is crucial for diagnosis as well as successful management of the patients. This study was done to evaluate the frequency ofliver involvement and aminotransferase levelchange in dengue fever and to determine relationof transaminase level change with the diseaseseverity.

METHODOLOGY:

A singe centered prospective cross-sectional study was conducted at Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad to study the relationship between liver transaminases i.e., serum alanine and aspartate transaminases and dengue hemorrhagic fever,for which, a total

number of 60 patients were included in the study. The study was done over a period of 24 months from September 2019 - August 2021. In the present study of 60 patients of age 18-75 years were included.

Institution Ethics Committee permission was obtained. Written informed consent was taken from all the study participants.

Patients attending Malla Reddy Hospital with complaints of fever, who fulfil the diagnostic criteria of WHO case definition for dengue hemorrhagic fever were included in the study. Those not willing to be a part of the present study, cases with classical dengue fever and cases with dengue shock syndrome were excluded.

Once the diagnosis was clinically made suspecting dengue hemorrhagic fever, it was confirmed by dengue serology test for Ns1 antigen, IgM and IgG antibodies against the antigen using ELISA method.

In diagnosed patients, venous blood (2ml) was collected into plain tube(red) using standard aseptic precautions.Then the sample was allowed to clot, retracted, and centrifuged at a rate of 3000 revolutions per minute for 5min and serum aminotransferase levels were analyzed by Modified IFCC Method (International Federation of clinical chemistry).

DENGUE HAEMORRHAGIC FEVER: DIAGNOSTIC CRITERIA[8]

1) Fever or history of acute fever lasting 2 to 7 days.

2) Thrombocytopenia (1 lakh/cu mm or less)

3) Haemorrhagic tendencies evidenced by at least one of the following:

      a) A positive tourniquet tests

      b) Petechiae, ecchymoses, Purpura.

      c) Bleeding from mucosal, GIT, Injection sites

      d) Hematemesis / Malena.

4) Evidence of plasma leakage: at least one of the following:

      a. Rise in HCT $\geq 20\%$

      b. Drop in HCT $\geq 20\%$ of baseline

      c. Pleural effusion/Ascites/Hyponatremia.

TOURNIQUET TEST:[9]

• It was performed by inflating a blood pressure cuff to a point midway between systolic and diastolic pressures for 5 minutes.

• This test was considered positive when 10 or more petechiae per square inch were observed.

• In DHF, the test usually gives a definitive positive result with 20 or more petechiae.

Statistical Analysis:

Data entry was done using M.S. Excel and statistically analyzed using Statistical package for social sciences (SPSS Version 16) for M.S Windows. Descriptive statistical analysis was carried out to explore the distribution of several categorical and quantitative variables. Categorical variables were summarized with n (%), while quantitative variables were summarized by mean ± S.D. All results were presented in tabular form and are also shown

graphically using bar diagram or pie diagram as appropriate. Pearson correlation test was used to find the correlation between the parameters. P-value less than 0.05 considered to be statistically significant.

RESULTS:

Table 1: Distribution of patients based on the baseline characteristics

Variables		Frequency	Percent
Age Group	<20 years	8	13.3%
	21-30 years	28	46.7%
	31-40 years	13	21.7%
	41-50 years	2	3.3%
	51-60 years	5	8.3%
	>61 years	4	6.7%
Gender	Male	41	68.3%
	Female	19	31.7%
Clinicalmanifestations	Fever	53	88.3%
	Retro-orbital pain	17	28.3%
	Headache	37	61.7%
	Myalgias	36	60.0%
	Arthralgia	16	26.7%
	Vomiting	22	36.7%
	Jaundice	4	6.7%
	Altered Sensorium	0	0.0%
	Pain Abdomen	9	15.0%

Majority of the patients belonged to the age group of 21-30 years (46.7%) followed by 31-40 years (21.7%), <20 years (13.3%), 51-60 years (8.3%), >61 years (6.7%) and 41-50 years (3.3%). Mean age was 32.60 ±13.69 years, ranging from 18 to 75 years. Males constituted(n=41) 68.3% of the study population while females constituted(n=19) 31.7% of the study population.Most common clinical manifestation was fever (n=53) (88.3%) followed by headache(n=37) (61.7%), myalgias (n=36) (60%), retro orbital pain (n=17) (28.3%), arthralgia (n=16) (26.7%), pain abdomen (n=41) (15%) and jaundice (n=4) (6.7%). (Table 1)

Table 2: Laboratory parameters

Laboratory parameters	Mean	Std. Deviation
HB (g/dl)	12.85	2.86
Platelet count (/mcL)	40,200.00	24,568
Hematocrit (%)	45.20	6.61
Alanine aminotransferase (U/L)	176.83	90.38
Aspartate aminotransferase (U/L)	190.80	122.90

Mean hemoglobin was 12.85 ±2.86 g/dl., Mean Platelet count was 40,200 ± 24,568/ mcL., Mean Hematocrit was 45.20 ± 6.61%., Mean Alanine aminotransferase was 176.83 ± 90.38 U/L., Mean Aspartate aminotransferase was 190.80 ± 122.90 U/L. (Table 2)

Table 3: Correlation of alainine aminotransferase, aspartate aminotransferase with platelet count

	Correlation	Sig.

Alainine aminotransferase & Platelet count	-0.495	0.001
Aspartate aminotransferase & Platelet count	-0.463	0.001

Alainine aminotransferase, aspartate aminotransferase were significantly and negatively correlated with platelet count. As the platelet count decreased, the alainine aminotransferase, aspartate aminotransferase levels increased. (Table 3; figure 1&2)

Figure 1: Correlation of alainine aminotransferase with platelet count:
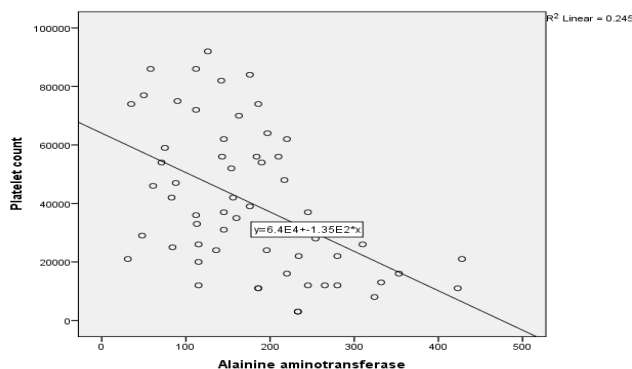

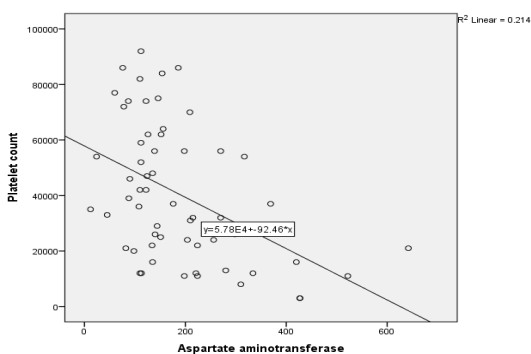Figure 2: correlation of aspartate aminotransferase with platelet count:

Table 4: Correlation of alainine aminotransferase, aspartate aminotransferase with hematocrit

	Correlation	Sig.
Aspartate aminotransferase & haematocrit	0.28	0.02
Aspartate aminotransferase &haematocrit	0.234	0.02
Platelet count& Hematocrit	-0.463	0.001

Alainine aminotransferase, aspartate aminotransferase were significantly and positively correlated with the hematocrit values but platelet count was negatively correlated with the hematocrit which was statistically significant. (Table 4; figure 3&4)

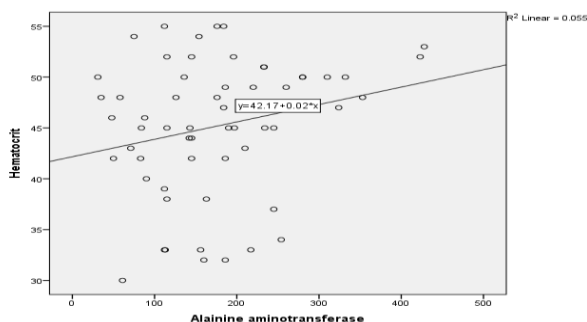Figure 3: correlation of alainine aminotransferase with hematocrit:

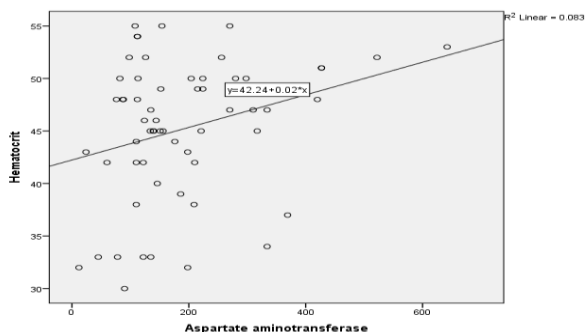Figure 4: correlation of aspartate aminotransferase with hematocrit:

Table 5: Distribution of patients based on the tourniquet Test

		Frequency	Percent
Tourniquet Test	Positive	30	50.0%
	Negative	30	50.0%
	Total	30	100.0%

Tourniquet test was positive in 50% patients.(Table 5)

Table 6: Distribution of patients based on the serology findings

serology findings	Frequency	Percent
Positive NS1	47	78.3%
Positive IgM	28	46.7%
Positive IgG	11	18.3%

Dengue NS1 was positive in 78.3% patients, Dengue IgM was positive in 46.7% patients, Dengue IgG was positive in 18.3% patients (Table 6)

DISCUSSION

Majority of the patients in the present study belonged to 21-30 years (46.7%). The mean age was 32.60±3.69 years ranging from 18-75 years.When compared to a study conducted by Balakumar J et al[10]most of the patients were 15-25 years (34%) and the mean age was 35.94±5, which is closely similar to the present study. These results can also the patients was 36.86±13.9 years.

In the present study, males were 41 (68.3%) and females were 19 (31.7%). This is comparable to the study by Shams N et al[12]in which majority were males (77.3%). And also in the study done by Balakumar J et al[10]there were 54% males.

In the present study, the most common clinical manifestation was fever (88.3%) followed by headache (61.7%), myalgias (60%), retro orbital pain (28.3%), arthralgia (26.7%), pain abdomen (15%) and jaundice (6.7%) which is similar and comparable to previous studies. Narayanan M et al[13] who observed that fever and vomiting were the most frequent symptoms.

In the present study, the most common bleeding manifestation observed was melena (43.3%) followed by Petechiae (38.3%), epistaxis (15%), hematuria (15%), bleeding gums (13.3%), hematuria + melena (5%), bleeding gums + melena (3.3%), hematoma at injection site (1.7%), hematuria + bleeding gums (1.7%) and menorrhagia (1.7%).This is in contrast to studies done by Narayanan M et al[13]andAggarwal A et al[14]whereinhematemesis was the most common bleeding manifestation observed.

In the present study, the platelet count between 50,000 – 1,00,000 was observed in 35% (n=21) of patients, while platelet count between 20,000 – 50,000 was observed in 46.3% (n=26) of patients, while platelet count of < 20,000was observed in 21.7% (n=13) of patients. Similarfindings were observed in astudy by Malavige GN et al[15], wherein the platelet count between 50,000- 1,00,000 was 24.2%, 20,000-50,000 was 46% and < 20,000 in 30%.

In the present study, mean Hematocrit was 45.20 ± 6.61%.In comparison, the mean hematocrit was 35.5% in a study by Gurudeep DS et al. [16]

Discussing about the hepatic dysfunction, the extent of ALT and AST elevation revealed good correlation with each other. Levels of ALT and AST increased significantly with increasing severity of DF especially in patients with dengue hemorrhagic fever. Where aspartate aminotransferase (AST) levels are usually higher than alanine aminotransferase (ALT) levels. A similar finding was also observed in the present study. The similar pattern is observed not only in dengue fever but also comparable to alcoholic hepatitis but different from other viral hepatitis. The specific underlying cause is unknown however it has been postulated that excessive AST release from injured myocytes during dengue infection might be to blame. [16]

Study done by Souza et al[17] have proposed similar data, by comparing two different groups, however, there was no significant difference in the degree of liver enzyme rise between the DF and DHF groups [18, 19] while in our study we included only DHF patients and found significant correlation of aminotransferases with the severity of disease.

Transaminase levels rise in approximately all dengue patients, according to Rajni R et al[20]. Because AST and ALT levels are negatively correlated with the platelet count, they rise with increasing dengue severity, which is indicated by a decrease in platelet count.In our study, ALT <40 IU/L was observed in 2 patients (3.3%) with mean platelet count being47,500/mcl in this cohort.Similarly,ALT 41-120IU/L was seen in 17 patients (28.3%) with mean platelet count of 48,529/mcl, ALT 121-400 IU/L was seen in 39patients (65%) with mean platelet count of 37,435/mclandALT >400 IU/L was seen in 2 patients (3.3%) with mean platelet count of 16,000/mcl. It was observed that as the alanine aminotransferase levels are increasing there is a significant decrease in platelet count which is statistically significant.

Kittitrakul et al[21] and Srivastava et al [22]observed mean ALT levels of 152 U/L and 121 U/L, respectively, in their studies, while in our study the observed mean Alanine aminotransferase (ALT/ SGPT) was 176.83 ± 90.38 U/L seen in 60 DHF patients.

Transaminase levels have increased in substantially all dengue patients, according to the studies. Because AST and ALT levels are negatively correlated, the rise with increasing dengue severity, which is indicated by a reduction in platelet count.This negative correlation, however, cannot be used to differentiate dengue patients with platelet counts of less than 1,00,000/cmm from those with counts of more than 1,00,000/cmm.

When compared to other studies,in the present study, all the patients 100% (n=60) had platelet count<1,00,000/cmm, and all the patients had significant raise in the AST and ALT levels. The mean platelet count was 40,200/cmm.

The AST and ALT values obtained were categorized into four subgroups, <1 ULN (<40 IU/L), 1-3 ULN (40 - 120 IU/L),3-10 ULN (121 – 40 IU/L) and >10 ULN (>400 IU/L). In the present study none of the patients had AST and ALT values > 1000 IU/L, with the highest recording being, AST: 642 IU/L and ALT: 428 IU/L which depicts the elevation of the aminotransferases above 400 IU/L but <1000 IU/L.

In patients with < 1 ULN of ALT i.e.< 40 IU/L, the average platelet count was 47,500/mcl, in 1-3 ULNi.e. 40 – 120 IU/L was 48,529/mcl, 3-10 ULN i.e., 121-400 IU/L was 37,435/mcl, > 10 ULN i.e. >400 IU/L was 16,000/mcl.

Whereas< 1 ULN of AST i.e., < 40 IU/L, the average platelet count was 44,500/mcl, in 1-3 ULN i.e., 40 – 120 IU/L was 50,294/mcl, 3-10 ULN i.e., 121-400 IU/L was 39,277/mcl, > 10 ULN i.e.,>400 IU/L was 10,800/mcl which depicts that with the decrease platelet count, there is in significant increase in AST and ALT values.Therefore, liver damage is a common dengue complication, and AST and ALT levels should be used as biochemical markers to detect and monitor hepatic dysfunction in those patients.

According to a study done by Fernando S et al[15]the levels of aspartate transaminase (AST), alanine transaminase (ALT), and gamma glutamyl transferase (GGT) were raised throughout the illness in individuals with dengue infection. On days 5 and 6, the maximum AST levels were seen, and both AST and GGT levels were substantially greater in patients with severe dengue (SD) compared to those with non-severe dengue (NSD).

Hepatic dysfunction is very common in all forms of dengue infection, with AST rising significantly more than ALT. Serum aminotransferase levels appear to have a directly proportional correlation with grading of dengue infection (p<0.001).

In Balakumar J et al study, [10]out of 50 patients 32 (64%) had reduced platelet count (less than one lakh) and all the 32 patients had elevated serum aminotransferase level. There was significant negative correlation between serum aminotransferase level and platelet count (P value < 0.001), in our study 60 DHF pts are included, all of them had thrombocytopenia less than 1,00,000/mcl and significant negative correlation between serum aminotransferase level with platelet count and significant positive correlation between serum aminotransferase level with hematocrit is observed.

LIMITATIONS

Present study had small sample size. We did not check for dengue virus sub types and antibody titres were not quantitated.

CONCLUSION

Hepatic dysfunction is very common in all forms of dengue infection, with AST rising significantly more than ALT. Rise in serum transaminase levelalso has a correlation with the decrease in the platelet count in patients with DHF. Rise in the levels of liver enzymes (AST and ALT) along with the increase in severity of the disease also correlates with the raise in

hematocrit.When considered as prognostic marker, there is a direct correlation. The higher the derangement in serum transaminase level, more is the chance of the disease being severe. And also, the higher the derangement in the level of transaminases, more was the number of days of hospitalization.Therefore, the use of liver tests to evaluate the degree of liver damage is of great importance, and markers such as AST and ALT may be used as parameters to evaluate the severity of the disease, and also a cost effective way for a better therapeutic approach.

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