

STUDY OF HEPATOREANL BIOMARKERS AND PLATELET COUNT IN PATIENTS WITH DENGUE INFECTIONS IN CHHATTISGARH POPULATION

Biochemistry

Dr. Shrikant Chandrakar

MBBS, MD (Biochemistry), FID, FCGP, Assistant Professor, BiochemistryCCMMC, Kachandur, Durg, Chhattisgarh

Dr. Sushma B J*

MBBS, MD (Biochemistry), FID, FCGP, Assistant Professor, BiochemistryCCMMC, Kachandur, Durg, Chhattisgarh *Corresponding Author

KEYWORDS

INTRODUCTION

Dengue is a mosquito borne viral infection found in tropical and sub-tropical regions of the world and is caused by one of the four serotypes of dengue virus (DENV1-DENV2) [1]. The WHO estimates 50 million dengue infections to be occurring annually and almost half the world's population lives in countries where dengue infection is endemic. Dengue is transmitted from human to human by the bite of female mosquito *Aedes aegypti*, *Aedes albopictus*, *Aedes polynesiensis* and the infection with one of these viruses characteristically results in fever, with headache and rash as a presenting complaints. Dengue affects human beings of all age groups worldwide. The dengue fever is widely prevalent in India and all the four serotypes are found Sharma et al., (2010). *Aedes aegypti* is the most common vector of dengue in India, followed by *A. albopictus*. The diagnosis of dengue fever is carried out based on clinical, epidemiological and laboratory data. The laboratory tests include both non-specific (Blood counts, platelets count, Haematocrit, liver function tests and renal function tests) and specific tests (Viral isolation tests, nucleic acid tests and serology - ELISA for antibody detection) [2]. The consequences of DENV infection range from asymptomatic condition, dengue fever [DF], or severe forms, such as dengue hemorrhagic fever [DHF] and dengue shock syndrome [DSS] [3]. Although dengue virus is a non-hepatotropic virus, liver injury due to dengue infection is not uncommon and has been described since the 1960s [4]. The degree of liver dysfunction in dengue infection varies from mild injury with elevation of aminotransferases alone to severe injury with jaundice and even fulminant hepatic failure [5]. The liver dysfunction could be a direct viral effect or an adverse consequence of dysregulated host immune response against the virus [6]. Thrombocytopenia is the most common finding, and has been one of the criteria used by WHO guidelines as a potential indicator of clinical severity. The Platelet counts tend to fall over the illness course, reaching a nadir in 7 to 10 days followed by a rapid recovery response. Lower counts (less than $50 \times 10^9/L$) are seen more frequently in severe disease (Muhammad umerkhan et al., 2014), further reduction in platelet counts (less than 20,000) indicates the requirement of platelet transfusions in these high-risk cases, due to increased risk of bleeding. In Renal function tests, Proteinuria has been detected in as high as 74% of patients with DHF (Emerson Q. Lima et al., 2007). Raise in blood urea and serum creatinine was also noted in first 7 days of illness. Several outbreaks of dengue infection have been reported from India [7-10]. However, large clinical studies documenting hepatic involvement in dengue infection, especially in adults, are scarce.

AIM OF THE STUDY:

To evaluate hepatorenal biomarkers, electrolytes and platelet counts in subjects with Dengue infections.

OBJECTIVES:

1. To evaluate hepatic function biomarkers like total bilirubin, albumin, ALT, AST, ALP and renal function biomarkers like urea, creatinine, electrolytes (sodium and potassium) in subjects with dengue infections.
2. To evaluate and compare platelet counts in different categories of Dengue Subjects.
3. To correlate liver function tests with severity of dengue infection.

MATERIALS AND METHODS:

The study included total of 81 clinically diagnosed and serologically

confirmed dengue cases admitted to our hospital during the period of 6 months from June 2018 to November 2018. Case definition: Patients will be included in the study: 1) If they had clinical symptoms such as fever, headache, arthralgia, myalgia, retro-orbital pain, nausea, vomiting & prostration. 2) Laboratory tests showing leukopenia, blood hemoconcentration, low platelet count & serologically positive for NS1 antigen or anti-dengue IgM antibodies or anti-dengue IgG antibodies. Patients with dengue infections were categorized into 3 as per WHO criteria. 1) Classical Dengue Fever if they had above mentioned clinical symptoms and laboratory evidence. 2) Dengue hemorrhagic fever [DHF]: If they had Thrombocytopenia $<100,000/mm^3$. Presence of spontaneous petechiae or positive tourniquet test. Hemoconcentration $>45\%$ in men and $>40\%$ in women. 3) Dengue Shock Syndrome: If they had rapid and weak pulse, narrow pulse pressure, age-specific hypotension, cold-clammy skin and restlessness.

Exclusion criteria:

Patients with liver disorders, Malaria and Typhoid fever were excluded from the study.

Sample Collection and Analysis:

After taking informed consent under aseptic precautions about 5ml of blood samples will be drawn into plain vacutainers from the antecubital veins of dengue patients. The blood was centrifuged at 2000 rpm/15min for clear separation of serum. All the assays were performed on the same day.

Biochemical Analysis:

Liver function tests [Total bilirubin, Total protein, Albumin, Alanine amino transaminase, Aspartate amino transferase], Renal function tests like urea and creatinine were measured by using fully automated chemistry analyzer. and serum electrolytes [sodium, potassium, chloride] were measured by ion selective electrode method. After clinical evaluation, the patients with diagnosis of dengue were admitted in the wards depending upon the severity of the disease and given guidance for outpatient follow-up. The admitted patients were mainly treated symptomatically with antipyretics, analgesics, maintained of proper hydration & antiemetics. Platelet Count was measured using 5-part automated blood cell counter (Beckman Coulter) and was counterchecked on peripheral blood smear.

RESULTS:

A total of 81 subjects with dengue infections were enrolled in the study during the period of 6 months from June 2018 to November 2018. Out 81 subjects, 58 were males 71.6% and 23 were females representing 28.4% and 28.3% respectively.

Table 1: Shows the Agewise and Genderwise Distribution of Subjects with Dengue Infections

	Males	Females
Number of Subjects with Dengue Infections	58 (71.6%)	23 (28.3%)
Age in years (Mean SD)	28.811.65	28.511.48

From the table 1, it is evident that the, mean age in years in males is 28.811.65 compared to females 28.511.48.

Table 2: Categorization of Dengue Subjects Based on Serology Test

Total number of Dengue Subjects	81
NS1 antigen positive	81
IgG positive	4
IgM positive	4
IgG and IgM positive	Nil

From the table 2, it is evident that out of 82 dengue subjects, all of them were positive for NS1 antigen test, 4 were positive for IgM antibodies and 4 were positive for IgG antibodies.

Table 3: Shows division of Dengue subjects into 3 categories according to WHO criteria

Total number of Dengue Subjects	81
Classical Dengue Fever (CDF)	46 (56.7%)
Dengue Hemorrhagic Fever (DHF)	31 (38.2%)
Dengue Shock Syndrome (DSS)	4 (4.9%)

From the table 3, it is evident that out of total 81 subjects with dengue infections 46 were suffering from CDF, 31 were DHF and 4 were DSS representing 56.7%, 38.2% and 4.9% respectively.

Table 4: Comparison of liver function tests, renal function tests, serum electrolytes and platelet counts among the 3 categories of subjects with dengue infections

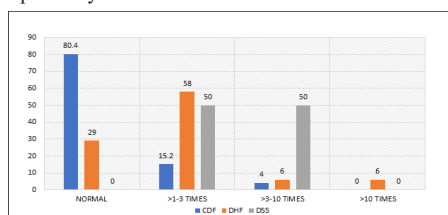
	CDF (n = 46)	DHF (n = 31)	DSS (n = 4)
Age in years (Mean SD)	28.311.3	28.1612.15	28.911.1
Total Bilirubin (mg/dL)	0.700.30	0.740.38	0.900.33
Alanine Transaminase in U/L (ALT)	39.828.9	69.442.9	74.540.3
Aspartate Transaminase in U/L (AST)	55.436.6	149.2161.4	269.528.7
Alkaline Phosphatase in U/L (ALP)	188.748.7	198.273.57	201.4133.2
Albumin in gm/dL	3.550.3	3.110.2	2.890.3
Urea in mg/dL	25.59.11	25.311	25.38.5
Creatinine in mg/dL	0.990.23	1.00.27	1.20.27
Sodium in mEq/L	139.12.88	130.23.2	126.52.43
Potassium in mEq/L	3.860.32	3.80.2	3.80.14
Platelet Counts lakh/mm ³	1.280.58	0.790.12	0.3520.07

From the table 4, it is evident that Total Bilirubin, ALT, AST, ALP, Creatinine levels are increased maximum in DSS, then in DHF compared to CDF. Serum Albumin, Sodium and Platelet Counts are decreased maximum in DSS, then in DHF compared to CDF. There was no change in Serum Urea and Potassium levels in all the three categories.

Table 5: Evaluation of ALT and AST in subjects with Dengue Infections (n=81)

	ALT & AST	ALT	AST
Grade A	Normal	47 (58%)	33 (40.7%)
Grade B	>1-3 times	28 (34.5%)	35 (43.2%)
Grade C	>3-10 times	5 (7.4%)	10 (12.3%)
Grade D	>10 times	Nil	3 (3.7%)

From the table 5, it is evident that the ALT were normal in 47 (58%) dengue subjects, where as it was increased to >1-3 times and >3-10 times in 28 (34.5%) and 5 (7.4%) subjects respectively. AST levels were normal in 33 dengue subjects, whereas it was increased to <1-3 times, >3-10 times and >10 times in 35 (43.2%), 10 (12.3%) and 3 (3.7%) respectively.

**Figure 1: Shows Comparisons of pattern of rise in ALT in patients with CDF, DHF and DSS expressed in percentage (%)****Figure 2: Shows Comparisons of pattern of rise in AST in patients with CDF, DHF and DSS expressed in percentage (%)**

DISCUSSION

In this study, we evaluated various biomarkers pertaining to liver function, renal function and platelet counts in clinically diagnosed and serologically confirmed cases dengue infection during an outbreak in central India from June 2018 to November 2018. In this study, CDF, DHF and DSS were present in 46 (56.7%), 31 (38.2%) and 4 (4.9%) respectively. This finding was similar to the studies¹⁰

found derangement in liver function biomarkers, there was elevation in liver enzymes like ALT, AST and there was decrease in the levels of albumin. There was no significant derangement in total bilirubin levels and ALP levels. Hepatic dysfunction is common in patients with dengue infections since DENV primarily targets Hepatocytes and Kupffer cells. DENV directly attacks the receptors present on the surface of host cells and viral attachment on the receptors acts as a limiting step for infecting the cells [11-14]. The other important mechanism of infecting the target cells may be due to unregulated host immune response against the virus. The commonest abnormality found in dengue infections is raised transaminase levels (ALT/AST). In majority of the cases AST elevation is more common than ALT during the first week of infection, with a tendency to gradually decrease within 3 weeks [15].

In our study, also we found 59.2% of the dengue subjects showing elevated AST levels compared to ALT which was about 41.9%. The etiology of elevated aminotransferase levels during acute illness is unclear since AST is expressed in heart, skeletal muscle, red blood cells, kidneys, brain and liver while ALT is secreted primarily by liver. Because dengue infection can cause acute damage to these non-hepatic tissue types that express AST raised AST levels may not be solely due to liver involvement. Therefore, it is possible that the patients with high AST are more likely to be classified as severe dengue under the 2009 criteria due to the common pathways to non-hepatic tissue damage, even though there is no association with poorer outcome [16]. Hence measurement and serial monitoring of AST & ALT is mandatory to look for liver involvement thus to prevent early development of hepatic failure. We found hypoalbuminemia in 34.5% of the dengue subjects. Other studies have reported hypoalbuminemia ranging from 12.9% - 76%. The wide range of variation may be due to the heterogeneity of the population studied and the severity of the disease [17-20].

Several forms of renal involvement have been identified in patients with dengue, including elevation of the serum creatinine level, AKI, acute tubular necrosis, hemolytic uremic syndrome, proteinuria, glomerulopathy and nephrotic syndrome [20, 21]. AKI is a significant, albeit poorly studied, complication of dengue. The data available are heterogeneous and mostly originate from retrospective case series and case reports. The reported frequency of this association exhibits wide variation in accordance to the specific population being assessed, severity of dengue, criteria used for the diagnosis of AKI and time of evaluation. Lao-prasopwattana et al. [22] reported an incidence of 0.9% among children in Thailand. In our study, we found only 3.7% DSS subjects showing elevated urea and creatinine levels, this finding was similar to the study conducted by Lee et al reported an incidence of 3.3% among adults in Taiwan [23].

In this study, we found decreased platelet count in 80% of the dengue subjects which include 37%, 38.2% and 4.9% respectively in CDF, DHF and DSS. The reasons for low platelet count in dengue fever are as follows. Dengue virus induces bone marrow suppression. Since bone marrow is the manufacturing centre of blood cells its suppression causes deficiency of blood cells leading to low platelet count. Anaemia

and spontaneous severe bleeding are the other resultant factors of bone marrow suppression. Studies suggest that dengue virus can even bind to platelets of human blood in the presence of virus-specific antibody. When vascular endothelial cell that are infected with dengue virus gets combined with platelets they tend to destroy platelets. This is one of the major causes of low platelet count in dengue fever. Even the antibodies that are produced after infection of dengue virus can contribute in destruction of platelets, thus lowering the platelet count. Low platelet count in dengue fever may lead to life-threatening condition known as hemorrhagic dengue fever that is categorized by spontaneous bleeding tendency and shock.

CONCLUSION:

Dengue fever is usually associated with mild to moderate elevations aminotransferases levels and in most cases with acute hepatitis. Liver damage is most frequent among women with sequential infection. The disease course is self-limiting but still some patients may be prone for hepatic failure hence serial monitoring and a timely measurement of aminotransferases is needed along with platelet counts, renal biomarkers and serum electrolytes. Care must be taken not to treat patients with hepatotoxic drugs

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