

Original Research Article

Biochemical markers as predictors of dengue severity

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ABSTRACT

Background: The objective of this study was to evaluate biochemical markers as predictors of dengue severity clinical outcome, bleeding severity, capillary leakage, supportive therapy requirement and duration of hospital stay.

Methods: In this observational study Patients from age more than 15 years with history of acute febrile illness Total 263 confirmed cases (based on the WHO criteria) of DF were included in this study, who have been admitted in our hospital. We measured levels of CK, LDH, AST and ALT with modified liquid-UV tests; semi-quantitative levels of CRP with a colorimetric rapid test; levels of albumin with colorimetric tests; and lipid profiles [cholesterol, triglycerides, Low-density lipoprotein (LDL) and High-density lipo-protein (HDL)] with a liquid-color test. Positive control human samples were included in all tests.

Results: We found that TG and LDL-C levels were significantly lower in dengue-positive patients compared to dengue-negative patients, and that LDL-C levels showed greater decreases and thus appeared to drive the reduction in total cholesterol. LDH, CPK, AST and ALT were significantly raised in DSS in compared to DF and other febrile illness. We found that lower total serum cholesterol and LDL-C levels at presentation were associated with subsequent development of DHF/DSS.

Conclusions: Assessment of lymphocyte, platelet counts, levels of LDL, TG, CPK, LDH, levels of AST and ALT are very significant and easily available and low-cost biochemical markers for prediction of dengue infection severity.

Keywords: Dengue, Biochemical marker, Predictors of dengue severity

INTRODUCTION

Dengue is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) also referred to as an arbovirus (arthropod-borneviruses) that belongs to the genus *Flavivirus* of the family *Flaviviridae*. It is a disease with a wide clinical spectrum and a wide variety of presentations, ranging from asymptomatic to an undifferentiated fever (viral syndrome) to the more severe forms such as Severe dengue (SD) or Dengue haemorrhagic fever (DHF).^{1,2} Lipoproteins may have the ability to modify inflammatory immune function and modify the host immune response during infections. Hypolipidaemia occurs in critically ill patients and is an independent predictor of clinical

outcome.³ In viral infections, like dengue, lipoproteins are postulated to bind to viruses, thus neutralizing their ill effects.⁴ Certain viruses use LDL receptors to enter the cell; thus, LDL may compete with viruses for these cellular receptors.

There is direct and indirect evidence of biochemical alterations related to severity of dengue. Studies have reported that patients with DHF have elevated serum levels of transaminases [Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), amylase, Lactate dehydrogenase (LDH), and creatine kinase (CK).⁵ Patients with DHF also have elevated levels of phospholipase A2, a protein whose concentration is correlated with that of C-Reactive protein (CRP). Cross-sectional studies have shown differences in serum levels of cholesterol and

triglycerides associated with severe forms of DHF studies. However, these potential biochemical markers have not been evaluated prospectively in early stages of dengue. Thus, the utility of bio-chemical alterations for timely identification of patients who will develop DHF is unknown. The objective of this study was to evaluate biochemical markers as predictors of dengue severity clinical outcome, bleeding severity, capillary leakage, supportive therapy requirement and duration of hospital stay.

METHODS

In this observational study patients from age more than 15 years with history of acute febrile illness and symptom and signs of dengue have been included. Total 263 confirmed cases (based on the WHO criteria) of DF were included in this study, who have been admitted in department of medicine G. S. V. M. Medical College Kanpur between July 2020 to November 2021. DF was diagnosed on the basis of the positive serum Immunoglobulin M (IgM) antibody and Non-structural protein 1 (NS1) antigen to DF. The serum IgM antibody was analyzed by the Enzyme-linked immunosorbent assay (ELISA) method using an IgM antibody capture Elisa (MAC-ELISA, National institute of virology, Pune, India).

It was a qualitative analysis and the titers were not measured. The baseline characteristics, including age, sex, occupation, and socioeconomic class, were noted. A detailed history, clinical evaluation was performed in all the patients. Systemic complications of DF including jaundice, lymphadenopathy, hepatosplenomegaly, cardiac failure, gastrointestinal, respiratory, and hematological manifestations were specifically examined. The routine laboratory investigations including hemoglobin level, blood count, platelet estimation and hematocrit were performed in all patients.

Biomarkers

Biochemical tests were performed using acute-phase sera (obtained 48-96 hours after onset of symptoms) from all patients with a dengue infection (including those who would develop DHF) and from a random sample of 48 patients with other febrile (non-dengue) illnesses. We measured levels of CK, LDH, AST and ALT with modified liquid-UV tests; semi-quantitative levels of CRP with a colorimetric rapid test; levels of albumin with colorimetric tests; and lipid profiles (cholesterol, triglycerides, LDL and HDL) with a liquid-color test. Positive control human samples were included in all tests.

Data analysis

Biochemical test results (except for those for semi-quantitative CRP) were compared using Student's t-test. We determined the frequency of biochemical alterations using accepted normal upper limits for CK (70 U/l in women and 90 U/l in men), lipids (HDL 35 mg/dl, LDL 80

mg/dl, triglycerides 160 mg/dl), and LDH (570 U/l); and a three-fold normal upper limit for transaminases (105 U/l) and albumin (4 g/dl) as cut-off values. For CRP, values greater than 6 mg/l were considered increased.

Statistical analysis

Data was compiled using Microsoft excel and analyzed using SPSS 22.0. Quantitative data was analyzed using mean and standard deviation. Comparison of mean values was done using One Way ANOVA and Tukey post hoc test. P value less than 0.05 was considered significant.

RESULTS

We have included 263 patients in this study. Out of 263 patients 134 (50.85%) patients were diagnosed to have DF, 121 (46%) patients were diagnosed as DHF and 8 (3.16%) patients were diagnosed as DSS based on WHO criteria. Out of 263 patients 153 patients were male and 110 were female. Among the 263 patients, the patients in the age group 21-30 years were the most commonly affected (34.2%). The ages ranged from 13-70 years with a mean of 41.5 years. The incidence were less (3%) in the age group more than 60 years (Figure 1).

Among 121 DHF patients 41 patients were DHF-I (15.6%) and 80 patients had DHF-II (30.40%). Hematological value ranged from 11-54% with mean 37%. It was increased in 193 (73%) patients. It was increased in 102 (39%), 86 (33%), 5 (1%) patients with DF, DHF and DSS respectively. The TLC ranges from 1400-53000 cells/cumm with a mean of 5980 cells/cumm. 136 (51.7%) patients had leucopenia (<4000 cells/cumm) which ranged from 1400-4000 cells/cumm. 63 (24%) of patients with DF had leucopenia, 69 (26%) of DHF patients had leucopenia and 4 (2%) of DSS patients had leucopenia. In this study 43% patients had relative lymphocytosis (>45%). 60 (23%) patients with DF had lymphocytosis while 64 (24%) of patients with DHF and 3 (1%) of patients with DSS had relative lymphocytosis. ESR was raised in 83% of patients. ESR ranged from 12-46 mm/hr with a mean of 19 mm/hr. 110 (42%), 104 (40%) and 7 (1%) of patients with DF, DHF, and DSS had raised ESR respectively.

Platelet count range was from 8000-333500 with a mean platelet count of 59482 cells/cumm. Thrombocytopenia (<100,000) was seen in 85% of cases. 27 (10%) patients had mild thrombocytopenia (60,000-100,000 cells/cumm), 141 (53%) patients had moderate thrombocytopenia (20,000-60,000 cells/cumm), 56 (21%) patients had severe thrombocytopenia (<20,000 cells/cumm). Thrombocytopenia was seen in 99 (37%), 118 (45%) and 7 (3%) patients with DF, DHF and DSS respectively.

Analysis of serological diagnostic investigation in dengue infection

IgM antibody was positive in 86.89% of patients. NS1Ag was positive in 40.30% of patients. The variability in the

incidence of dengue with IgM and NS1Ag was due to presentation of patients at different times. Thus, patients

who presented between 2-7 days were NS1Ag positive and those presenting after 7 days were IgM antibody positive.

Table 1: Serological investigation.

Serological investigation	Total (N=263)		DF (N=134)		DHF-I (N=41)		DHF-II (N=80)		DSS (N=08)	
	N	%	N	%	N	%	N	%	N	%
NS1Ag	106	40.30	57	42.53	14	34.15	32	40	3	37.5
IgM antibody	228	86.89	113	84.33	35	85.37	73	91.25	7	87.5
Both NS1Ag/IgM	71	27.0	36	6.87	8	19.51	25	31.25	2	25.0

Table 2: Value of biochemical markers.

Biochemical markers	Other febrile illness (N=48)	DF (N=134)	DHF (N=121)	DSS (N=8)
AST	58.6	1249 (46-2452)	1965 (58-3872)	2207 (178-4236)
ALT	52.8	495.5 (36-955)	946 (52-1840)	1257 (276-2238)
LDH	382.6	568.8(324-814)	674 (456-892)	782 (456-1099)
CPK	187 (140-234)	216 (168-264)	197 (156-238)	284 (226-342)
LDL	84.2	82 (66-98)	49 (42-56)	44(36-52)
HDL	43.6	47 (38-46)	40 (38-46)	39 (32-46)
TG	171 (126-216)	153 (124-182)	116 (84-148)	84 (72-96)
BUN	48 (12-84)	35 (14-56)	(57.38-76)	68 (42-94)
S. creatinine	1.32 (1.20-1.44)	1.15 (0.9-1.40)	1.53 (1.26-1.80)	1.84 (1.22-2.46)
S. albumin	4.2 (3.8-4.6)	3.5 (3.2-3.8)	3.2 (2.8-3.6)	2.5 (1.8-3.2)

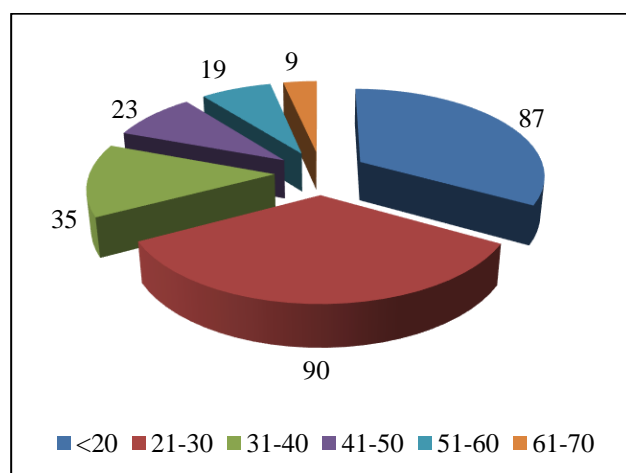


Figure 1: Age distribution.

Biochemical parameters

Serum creatinine was raised in 22 patients (8%). S. creatinine was raised in 7 (3%) patients with DF while it was raised in 13 (4%) and 2 (1%) of the patients with DHF and DSS respectively. Serum urea in this study was raised in 55 (21%) patients ranging from 12-284 mg/dl with a mean of 38 mg/dl. It was raised in 20 (8%), 32 (12%) and 3 (1%) in patients with DF, DHF and DSS respectively. SGPT was raised in 199 (76%) of patients with a range from 36-2955 U/l with a mean of 1495 U/l. SGPT was raised in 99 (38%), 93 (35%) and 7 (3%) patients with DF, DHF and DSS respectively. SGOT was increased in 233 (89%) of patients with a range from 36-7270 U/l with a mean of 3653 U/l. SGOT was increased in 118 (45%), 109 (41%) and 6 (3%) patients with DF, DHF and DSS

respectively. Serum bilirubin was raised in 7% of the patients with a range of 1.2-18.6 mg/dl and a mean of 9.9 mg/dl. Serum bilirubin was raised in 8 (3%), 8 (3%) and 1 (1%) patients with DF, DHF and DSS respectively. Serum cholesterol level was decreased in 108 (41%) of the patients ranging from 55-64 mg/dl with an average mean of 59.5 mg/dl. It was decreased in 2 (1%), 98 (37%) and 8 (3%) patients with DF, DHF and DSS respectively. Serum triglyceride level was decreased in 204 (77%) patients with a range of 84-182 mg/dl with a mean of 117 mg/dl. It was decreased in 103 (40%), 93 (35%) and 8 (2%) patients with DF, DHF and DSS respectively (Table 2). Serum HDL was decreased in 104 (39%) of patients with a range from 3-24 mg/dl with a mean of 13.5 mg/dl. It was decreased in 60 (23%), 6 (2%) and 38 (15%) in DHF, DSS and DF respectively. Serum LDL was decreased in 112 (42%) patients with a range from 25-32 mg/dl with a mean of 28 mg/dl. It was decreased in 2 (1%), 102 (38%) and 8 (3%) patients with DF, DHF and DSS respectively. Serum LDH was raised in 132 (50%) of patients with a range of 280-2420 U/l with a mean of 1350 U/l. It was increased in 68 (26%), 58 (23%) and 6 (1%) of the patients with DF, DHF and DSS respectively (Table 2).

DISCUSSION

Several biochemical compounds are shown to be either elevated or decreased in serum/plasma of patients with severe dengue and quantifying them might serve as biomarkers of severe dengue disease. Study conducted by Van et al showed levels of total plasma cholesterol, HDL and LDL were significantly decreased in children with the severest disease compared with patients with mild DHF.⁶ We found that TG and LDL-C levels were significantly

lower in dengue-positive patients compared to dengue-negative patients, and that LDL-C levels showed greater decreases and thus appeared to drive the reduction in total cholesterol (Table 2).

Total cholesterol, LDL-C, and TG levels were significantly lower in severe (DHF and DSS) compared to mild dengue during the course of illness regardless of severity classification scheme (Table 3). Finally, we found that lower total serum cholesterol and LDL-C levels at presentation were associated with subsequent development of DHF/DSS. Microbial translocation occurs during severe DENV infection and Lipopolysaccharide (LPS) levels are significantly increased in dengue patients, which are indicated by elevated levels of LPS binding protein (LBP) and soluble CD14 (sCD14). Elevated LPS levels in dengue patients were found to correlate with clinical disease severity.⁷ Liver injury is associated with severe dengue disease with the increase in serum AST, ALT, gamma-glutamyl trans-peptidase, alkaline phosphatase, and serum albumin concentrations. In our study AST and ALT level were significantly raised in compare to other febrile illness (p value<0.001) (Table 3). Reports showed that the AST and ALT levels were high in severe dengue, like DHF and DSS and may serve as predictors of severe disease.⁸⁻¹⁰

Liver damage caused by DENV infection could be contributing to the lower cholesterol levels we observed in dengue patients. The liver is a major site of cholesterol synthesis in humans, and the rate of cholesterol production depends on the cellular level of cholesterol, for which LDL and HDL, among other lipoproteins, are responsible through their roles in cholesterol transport.¹¹ Children with DSS and liver injury have lower zinc levels and the low levels were probably caused by loss from diarrhoea and from zinc trans locating to liver cells.¹²

The Inter- α inhibitor proteins (IaIp) belong to a family of serine protease inhibitors and its concentrations in pediatric patients suffering from severe DENV infection were significantly lower than in patients with mild DF and healthy controls.¹³ NO is known to have a strong immunoregulatory role and in adjusting the diameter of blood vessels, remodeling blood vessels, inhibiting leukocyte adhesion, platelet aggregation, and contractile

cell proliferation.¹⁴ Serum NO levels in DHF patients were shown to be significantly lower than those of the DF patients.¹⁵ Thus, increased levels of LPS, AST, ALT and decreased levels of lipids, IaIp and NO might serve as markers of severe dengue disease. LDH is raised in DSS/DHF in compared to Ofi and it is statistically significant in our study (Table 3).

Severe dengue haemorrhagic fever (DHF) patients also develop shock and experience a certain degree of hepatic injury, implicating that serum lactate and LDH may be elevated in Dengue shock syndrome (DSS).¹⁷ Serum lactate and LDH was found to be elevated in DHF and/or DSS patients. Lactate may be used as a predictor of DSS if the level is >2 U/l on day 0. LDH can be used to differentiate patients with or without dengue in the early febrile phase, if the level is >500 IU. If the level of LDH is increased to approximately 1,000 IU on day 0, it may be a predictor of severe dengue infection or DHF and DSS with plasma leakage.¹⁷

Dengue infection has been associated with a variety of renal disorders. Acute renal failure complicates severe dengue infection in 2-5% of the cases and carries a high mortality rate.¹⁸ Acute renal failure is typically associated with hypotension, rhabdomyolysis, or haemolysis. CPK and CPK-MB can be raised in DHF and DSS without apparent cardiac involvement. Some studies showed increased level of CPK even without cardiac involvement.^{19,20}

We found that CPK level was significantly raised in our DSS patients in compared to DHF, DF and OFI patients (Table 3). This could be due subclinical cardiac involvement and involvement of striated muscle and of the cardiac muscle. For this reason, the monitoring of these enzymes should be considered as part of the monitoring of patients with dengue. CPK may be a strong predictor of severity of dengue infection because it significantly raised in DSS.

The limitation of our study was the small sample size, hence further studies with large sample size is needed to understand the mechanism of lower lipid level in severe dengue patients. Pediatric population has not been included in this study, which is also a limitation of this study.

Table 3: Comparison of levels of biochemical markers.

Bio-chemical markers	Other febrile illness (N=48)		DF (N=134)		DHF (N=121)		DSS (N=8)		P value			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	OFI vs. DF	DF vs. DHF	DF vs. DSS	DHF vs. DSS
AST	58.6	26.4	1249	32.6	1965	32.8	2207	29.1	<0.001*	<0.001*	<0.001*	<0.001*
ALT	52.8	15.8	495.5	18.6	946	26.8	1257	28.5	<0.001*	<0.001*	<0.001*	<0.001*
LDH	382.6	56.4	568.8	68.1	674	82.2	782	84.1	<0.001*	<0.001*	<0.001*	<0.001*
CPK	187	21.1	216	28.2	197	14.5	284	21.3	>0.05	>0.05	>0.05	<0.05*
LDL	84.2	35.6	82	38.5	49.2	20.2	44.4	19.5	>0.05	<0.05*	<0.05*	>0.05
HDL	43.6	11.5	47	12.4	40	15.3	39	14.9	>0.05	<0.05*	>0.05	>0.05

Continued.

Bio-chemical markers	Other febrile illness (N=48)		DF (N=134)		DHF (N=121)		DSS (N=8)		P value			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	OFI vs. DF	DF vs. DHF	DF vs. DSS	DHF vs. DSS
TG	171	48.5	153	49.1	116	46.2	84	39.8	>0.05	<0.01*	<0.01*	>0.05
BUN	48	9.5	35	5.8	57	9.2	68	5.8	<0.001*	<0.001*	<0.001*	<0.001*
S. creatinine	1.32	0.08	1.15	0.05	1.53	0.06	1.8	0.06	<0.001*	<0.001*	<0.001*	<0.001*
S. albumin	4.2	1.1	3.5	0.5	3.2	0.8	2.5	0.4	>0.05	>0.05	>0.05	>0.05

Note: OFI- Other febrile illness; DF- Dengue fever; DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome; AST- Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; CPK: Creatine phosphokinase; LDL: Low density lipoprotein; HDL: High density lipoprotein; TG: Triglyceride; BUN: Blood urea nitrogen, *-p value<0.05 is significant.

CONCLUSION

The usage of the four classes of biomarkers has advantages and limitations. Molecular markers can be accurate. However, it involves high cost for the sequencer and reagents. Immunological markers which are also seen in other inflammatory diseases require flow cytometry analysis. Endothelial activation and biochemical markers are easy to estimate and the cost is low. However, the levels are modified in other disease conditions as well. Therefore, determination of combination of biomarkers will be beneficial in predicting severe dengue disease. Our study clearly demonstrates that assessment of lymphocyte, platelet counts, levels of LDL, TG, CPK, LDH, levels of AST and ALT are very significant and easily available and low-cost biochemical markers for prediction of dengue infection severity.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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