

Role of C- reactive proteins and liver function tests in assessing the severity of dengue fever

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Abstract

Objective: To determine whether C-reactive protein and liver function tests can serve as severity markers for dengue fever.

Methods: The cross-sectional study was conducted in 2015-16 in Karachi and comprised patients with dengue fever visiting a tertiary care hospital. World Health Organisation classifications 1997 and 2009 were used to categorise patients according to clinical signs and symptoms. Receiver Operating Characteristics curve was used to determine discriminative ability and optimum cut-off value of biochemical markers. Comparisons were done through one-way analysis of variance using SPSS 17.

Results: Of the 218 patients, 133(61%) were males and 85(39%) were females. The overall mean age was 35.07 ±15.96 years. Levels of C-reactive protein and total bilirubin were significantly higher for dengue haemorrhagic fever compared to dengue fever; dengue shock syndrome compared to dengue fever; dengue shock syndrome compared to dengue haemorrhagic fever; and dengue shock syndrome compared to dengue fever / dengue haemorrhagic fever ($p<0.05$ each). Levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were significantly higher for dengue shock syndrome compared to dengue fever; dengue shock syndrome compared to dengue haemorrhagic fever; and dengue shock syndrome compared to dengue fever / dengue haemorrhagic fever ($p<0.05$ each). Levels of C-reactive protein, total bilirubin, alanine aminotransferase and alkaline phosphatase in patients with severe dengue were significantly higher compared to non-severe dengue.

Conclusion: C-reactive protein and liver function tests were found to be effective biochemical markers in assessing dengue fever severity.

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Introduction

Dengue fever (DF) is a mosquito-borne tropical disease caused in humans by the bite of an infected female aedes aegypti mosquito as well as aedes albopictus. The disease has been identified as one of the biggest emerging threats¹ and has an alarming annual incidence of 390 million cases² with people at risk of infection estimated at 3.9 billion spanning around 128 countries.³ In Pakistan, the disease has been reported in at least two confirmed outbreaks, the first of which was in 1994 while the second occurred in 2005. Since then, dengue epidemics occur annually in Karachi and various regions of Pakistan.^{4,5} In order to curb dengue-related mortality, early recognition is essential as it would pave the way for timely management. As the disease encompasses a broad clinical presentation, with a variety of warning signs, it is critical to evaluate biochemical markers that would be effective in diagnosing and classifying severity of the disease. The World Health

Organisation (WHO) has made various attempts for appropriate grading of DF.^{6,7} However, due to the unpredictable nature of the virus and its affinity for various organs, the disease calls for close monitoring to note its evolution. Various inhibitions were raised regarding the applicability and complexity of the 1997 criterion due to the variable outcome of the disease.^{8,9} Similarly, the 2009 criterion has received criticism about its limitations. It was noted that the sensitivity of the potential warning signs highlighted in the 2009 criterion was extremely low.¹⁰ For this reason, looking for alternative modalities to effectively grade the severity of the disease is crucial.

Dengue virus and its implications on the liver were reported as early as in 1968.¹¹ The most common liver abnormality is the derangement in transaminase levels which are enzymes involved in amino acid metabolism. This has been attributed to hepatic parenchymal lesions which result in elevated total bilirubin levels and release of liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase levels (ALP) in the bloodstream. Furthermore, inflammation of liver has been attributed to result in the increase of C-

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reactive protein (CRP). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) have also been noted to be deranged in case of dengue infection due to liver involvement and the inoculation of nonstructural protein 1 (NS-1).

The current study was planned to determine whether CRP and liver function tests (LFTs) can serve as severity markers for DF.

Patients and Methods

The cross-sectional study was conducted at the Department of Medicine, Civil Hospital, Karachi, in 2015-16. After approval from the ethics review board of the Dow University of Health Sciences (DUHS), Karachi, the sample size was calculated using the formula: $ss = \frac{Z^2 * p(1-p)}{e^2} / \{1 + \frac{Z^2 * p(1-p)}{e^2 N}\}$; with N = Karachi's population (greater than 1000000), e = margin of error 5%, and z = z -score 1.65 to represent the desired confidence level of 90%.¹² This confidence level was chosen to detect positive changes in a relatively smaller sample size.

The sample was raised using convenience sampling. Those included were confirmed DF cases regardless of age and gender. Those excluded were patients with active inflammatory disease, chronic liver disease, hepatitis, cirrhosis, those receiving anticoagulation therapy and hepatotoxic drugs, and chronic alcoholics.

Informed written consent was taken before enrolment, and those who did not volunteer to participate were also excluded. Each patient was assigned an identification number to ensure anonymity.

The WHO classification of 1997 and 2009^{6,7,13} was used to categorise the patients. According to the 1997 classification, patients were divided into three categories: DF with presence of fever with two or more specific signs/symptoms; dengue haemorrhagic fever (DHF) with presence of fever for 2-7 days, thrombocytopenia, haemorrhagic symptoms, and indication of plasma leakage characterised by haemo-concentration or hypo-albuminaemia or ascites or pleural effusion; and dengue shock syndrome (DSS) with presence of DHF criteria plus rapid and weak pulse and hypotension with cold extremities. According to the 2009 classification, patients were divided into two categories: non-severe dengue (NSD) with or without warning signs i.e. mucosal bleeding, abdominal pain, hepatomegaly >2cm and ascites/pleural effusion; and severe dengue (SD) with shock and fluid accumulation with respiratory distress or severe bleeding or severe organ involvement.¹⁴

Data was collected using a questionnaire that had two sections: demographics and co-morbidities, and patients'

clinical features and laboratory values. All patients were subjected to a full physical examination and a detailed medical history was taken. Venous blood samples were obtained at the time of presentation or during hospitalisation to measure haematological indices, LFTs and CRP.

One step dengue immunoglobulin G and M (IgG/IgM) antibody whole blood / serum / plasma test cassette (Guangzhou Wondfo Biotech Co. Ltd), which is a rapid test for visual detection of dengue IgG/IgM antibody in whole blood / serum / plasma, was used for the diagnosis of dengue infection. Automated haematology system Sysmex XE-5000 (Sysmex America, Inc. One Nelson C. White Pkwy) was used to measure haematological indices. LFTs and CRP levels were measured using Roche Cobas C501 chemistry analyser (Roche Diagnostics).

Data was analysed using SPSS 17. Continuous variables were presented as mean + standard deviation and median + interquartile range (IQR). Comparison of the groups was done using one-way analysis of variance (ANOVA). All categorical variables were presented as frequencies and percentages. Data normality was assessed using Shapiro-Wilk test. Receiver Operating Characteristics (ROC) curve was used to determine the discriminative ability and optimum cut-off value of LFTs and CRP levels. The following guide was used for classifying the diagnostic test of area under curve (AUC) of ROC curve: 0.90-1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair, 0.60-0.70 = poor, and 0.50-0.60 = fail.¹⁵ A two-tailed p -value <0.05 was considered statistically significant.

Results

Of the 280 patients evaluated, 242(86.43%) met the inclusion criteria. Of them, 24(9.91%) patients refused to volunteer, and, therefore, the final sample stood at 218(90%); 133(61%) males and 85(39%) females. The overall mean age was 35.07 ± 15.96 years. Mean duration of hospitalisation was 5.82 ± 3.57 days. Of the total, 27(12.4%) patients had diabetes mellitus (DM), 40(18.3%) had hypertension (HTN) and 9(4.1%) had chronic kidney disease (CKD). The main presenting symptoms were fever 218(100%), lethargy 212(97.3%), myalgia 207(95%), chills 192(88.1%), abdominal pain 164(75.2%) and nausea/vomiting 159(72.9%). Clinical examination revealed jaundice in 102(55%) patients, hepatomegaly 81(37.2%), splenomegaly 69(31.7%) and ascites 29(13.3%). The gum was the most common site of haemorrhage 69(50.4%), followed by skin (rashes/petechiae) 32(23.4%), nose 18(13.1%) and gastrointestinal tract (GIT) 18(13.1%). According to 1997 WHO classification, 117(53.7%) had DF, 74(33.9%) had DHF and 27 (12.4%) had DSS, while

Table-1: Demographics and Severity of disease in patients (n=218).

Variable	
Demographics and co-morbidities, n (%):	
Mean age (years)	35.07±15.96
Male	133 (61%)
Married	132 (60.6%)
Diabetes	27 (12.4%)
HTN	40 (18.3%)
Chronic Kidney Disease	9 (4.1%)
Dengue Severity, n (%):	
WHO 1997 criteria	
DF	117 (53.7%)
DHF	74 (33.9%)
DSS	27 (12.4%)
WHO 2009 criteria	
Non-severe dengue	151 (69.3%)
Severe dengue	67 (30.7%)

SD: Standard deviation; HTN: Hypertension; DF: Dengue fever; DHF: Dengue haemorrhagic fever; DSS: Dengue shock syndrome; WHO: World Health Organization

Table-3: Clinical features of patients (n=218).

Clinical features, n (%):	
Mean fever in Fahrenheit	101.44±1.24
Mean duration of fever in days	5.71±3.85
Fever	218 (100%)
Presence of chills with fever	192 (88.1%)
Abdominal pain	164 (75.2%)
Orbital pain	33 (15.1%)
Nausea/Vomiting	159 (72.9%)
Lethargy	212 (97.3)
Myalgia	207 (95.0%)
Rashes/ Petechiae	32 (14.7%)
Ascites	29 (13.3%)
Jaundice	120 (55.0%)
Hepatomegaly	81 (37.2%)
Splenomegaly	69 (31.7%)
Epistaxis	18 (8.3%)
Gum bleeding	69 (31.7%)
GI Bleeding	18 (8.3%)
Mean duration of hospital stays (±SD), days	5.82±3.57
Fatality/Mortality	22 (10.1%)

GI: Gastrointestinal.

Table-5: Liver function tests (LFTs) and C-reactive protein (CRP) level distribution according to the 2009 World Health Organisation (WHO) dengue classification for non-severe dengue.

Variables	AUC	SE	CI	p-value	Cut-off
Total BR [mg/dl]	0.394	0.047	0.303-0.486	0.020	64.5
ALP [U/L]	0.296	0.037	0.225-0.368	0.001	114.5
ALT [U/L]	0.416	0.042	0.333-0.498	0.063	36.5
AST [U/L]	0.323	0.039	0.247-0.398	0.001	34.6
PT [sec]	0.462	0.052	0.360-0.565	0.409	11.25
aPTT [sec]	0.366	0.046	0.276-0.457	0.003	28.8
CRP [mg/L]	0.268	0.040	0.190-0.347	0.001	4.75

Liver function tests (LFTs) and C-reactive protein (CRP) level distribution according to the 2009 World Health Organisation (WHO) dengue classification for non-severe dengue.

Table-2: Laboratory features of patients.

Laboratory values:	
Leucopenia [WBC<4000 cells/uL], n (%)	68 (31.2%)
Hb [gm/dl]	12.22±3.18
MCV [fL]	82.50±7.64
Haematocrit [%]	42.90±8.15
RBC count [x106/uL]	5.17±1.94
Total leukocytes count [x109 /L]	5.1 (4.0)
Platelet count [x109 /L]	43.5 (60.5)
LFTS:	
Total Bilirubin [mg/dl]	0.95(1.93)
ALP [U/L]	113.0 (61.0)
ALT[U/L]	54.0 (52.7)
AST [U/L]	28.50 (36.0)
PT [sec]	13.71±3.91
aPTT [sec]	34.20±10.86
CRP [mg/L]	15.0 (10.0)

Hb: Hemoglobin; MCV: Mean corpuscular volume; RBC: Red blood cell; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransaminase; PT: Prothrombin time; aPTT: Activated partial thromboplastin time; CRP: C-reactive protein.

Table-4: Liver function tests (LFTs) and C-reactive protein (CRP) level distribution according to the 1997 World Health Organisation (WHO) dengue classification.

Variables	DF (n=117)	DHF (n=74)	DSS (n=27)	p-value
Total bilirubin [mg/dl]	0.72 (2.00)	0.95 (1.59)	2.40 (4.78)	-
ALP [U/L]	110 (62.00)	112.00 (72.00)	120.00 (359)	0.738
ALT[U/L]	54.0 (52.7)	57.00 (44.30)	66.00 (282.0)	0.108
AST [U/L]	26.0 (27.0)	28.0 (21)	84.0 (65)	0.706
PT [sec]	13.11±2.86	14.02±4.37	15.47±5.67	0.093
aPTT [sec]	33.97±9.68	34.84±12.62	33.47±10.81	0.78190
CRP [mg/L]	10.0 (5.0)	17.0 (4.63)	29.5 (7.0)	<0.001

DF: Dengue fever; DHF: Dengue haemorrhagic fever; DSS: Dengue shock syndrome;

ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransaminase;

PT: Prothrombin time; aPTT: Activated partial thromboplastin time;

Table-6: Liver function tests (LFTs) and C-reactive protein (CRP) level distribution according to the 2009 World Health Organisation (WHO) classification for severe dengue.

Variables	AUC	SE	CI	p-value	Cut-off
Total BR [mg/dl]	0.606	0.047	0.514-0.697	0.020	1.22
ALP [U/L]	0.704	0.037	0.632-0.775	0.001	121.5
ALT [U/L]	0.584	0.042	0.502-0.667	0.063	64.5
AST [U/L]	0.677	0.039	0.602-0.753	0.001	41.1
PT [sec]	0.538	0.052	0.435-0.640	0.640	11.25
aPTT [sec]	0.634	0.046	0.543-0.724	0.003	30
CRP [mg/L]	0.322	0.041	0.242-0.402	<0.001	15.75

ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransaminase;

PT: Prothrombin time; aPTT: Activated partial thromboplastin time; AUC: Area under curve;

SE: Standard error; CI: Confidence interval.

according to 2009 classification, 151(69.3%) had NSD and 67(30.7%) had SD (Tables 1-3). Overall mortality in those with DSS and SD was 59.2% (n=16) and 29.9% (n=20) respectively.

CRP and total bilirubin differed amongst the three groups, and ALT, AST and ALP levels did not differ in line with the severity of dengue (Table 4).

There were significantly higher levels of CRP ($p<0.001$), total bilirubin ($p=0.004$), ALT ($p<0.001$) and ALP ($p<0.001$) in SD patients compared to NSD, while PT and aPTT levels were similar in the two groups (Table 5).

ROC curve indicated that CRP, ALT and ALP were fair discriminants between NSD and SD (Table 6).

Discussion

The study noted a sharp rise in CRP with increasing disease severity according to both 1997 and 2009 WHO classifications. The rise in CRP levels and its association with dengue severity has been documented in various studies.¹⁶⁻¹⁸ In a study, the role of CRP in discerning between mild and severe dengue was evaluated and a positive association was established¹⁹ which is similar to the results of the current study. Similarly, in another study it was noted that CRP levels present in patients suffering from DHF were greater than those with DF.²⁰

The present study concluded that a CRP cut-off value of 19.60 mg/L was an excellent discriminant in predicting DSS. This value is in stark contrast with another study¹⁹ which showed a CRP cut-off value of 30.1 mg/L. This difference could be due to inclusion of only 6 patients of DSS in the total sample of the other study.¹⁹ Moreover, 4 out of 6 patients had extremely high CRP values ($>100\text{mg/L}$).

Possible elevation of CRP could be explained by the fact that CRP has been identified as a biomarker for inflammation, especially in cases of bacterial infections.²¹⁻²³ However, its role in viral infections has also been recognised and it is assumed that CRP levels within the range of 10-40mg/L hint towards viral inflammation; titers $>40\text{mg/L}$ are generally seen in bacterial infections of an acute nature.²¹⁻²⁴ Even though dengue virus is not hepatotropic in nature, the virus has been documented to cause liver damage that varies from mild lesions to fulminant hepatitis.²⁵⁻³⁰ It has been observed that liver involvement occurs through an inflammatory process in the liver parenchyma which can explain the rise in CRP levels.

The current study also pointed towards a progressive rise in total bilirubin levels with increasing disease severity. This is in line with various studies.³¹⁻³³ In contrast to a study³⁴ where total bilirubin values were not different amongst the three groups, the current study indicated a rise in total bilirubin which is specific for each group. Another research documented the trend of rising total bilirubin levels as the severity increased.³⁵

The inflammation occurring in liver parenchyma has been observed to shorten the diameter of the biliary canaliculus which results in obstruction. The concomitant rise in total

serum bilirubin levels with progression in severity can be attributed to this obstruction, which is a manifestation of the dengue virus pathogen.^{11,18}

The current study noted that ALT, AST and ALP levels did not increase as the severity of dengue increased. The markers have been used to adequately distinguish DSS from DHF and DSS from DF. However, the present study did not support these findings. On the other hand, increase in these liver enzymes has been noted previously.³⁶ The current study observed a trend of higher hepatic injury in patients of the DHF group and the DSS group which was established by observing the LFTs. This is in accordance with previous literature.^{26,27,37-39} With patients suffering from DHF, there was a notable abnormality in ALT enzymes whereas in patients suffering from DSS, all biochemical tests pertinent to the liver were deviated. This is in line with observations made earlier.^{28,29} However, some studies^{34,40,41} observed that there was no significant difference in LFTs of patients having DHF and DSS. Another study²⁸ noted there was a difference in the AST value amongst females and males, but the current study reported no such observation. A study⁴² reported that AST served as a good, early indicator of the prognosis of DF. Another research²⁶ noted that dengue patients had higher AST Levels compared to ALT levels, an observation which was similar to that of the current study. AST, ALT and ALP enzymes are concentrated in the liver. The inoculation of dengue virus in the system leads to the development of parenchymatous lesions in the liver. The shortening of the biliary canaliculus yields biliary obstruction which is manifested by releasing these markers into the bloodstream, thereby resulting in an increase in serum levels. It should also be noted that the AST, ALT and ALP enzymes are found in varying concentrations in other tissues of the body. The concentration of AST is high in the heart and skeletal muscle whereas both ALT and AST are found in low concentration in the kidneys. The occurrence of ALP is noted in virtually all tissues of the body with high concentration being noted in the liver, bile duct and kidneys. A study noted that in a few cases dengue virus had shown tropism for the heart and kidney, causing myocarditis and acute tubular injury [43]. Therefore, damage to these organs can also explain the rise in AST, ALT and ALP levels.

The current study concluded that PT and aPTT did not hold any significance when discriminating among DF, DHF and DSS, but it was observed that both PT and aPTT were tediously protracted in the study groups, which is a finding in line with other studies.^{32,44-46}

PT and aPTT values were prolonged in both NSD and SD patients, but, as the values were similar across the two

groups, they could not be used as discriminators.

The prolongation in PT and aPTT is credited to a host of factors that occur during the course of dengue infection, most notable of which is the binding of NS-1 to both prothrombin and thrombin which are responsible for the maintenance of the coagulation cascade pathway. It was noted that binding of NS-1 with prothrombin results in inhibited activation which results in a prolongation of PT and aPTT.⁴⁶ Furthermore, liver damage is also postulated as a contributing factor as it results in a decline in the production of coagulation factors.⁴⁷

The current study has limitation in terms of being a single-centre study with a small sample size. CRP and LFT values were not measured at difference phases of the illness, like febrile phase, critical phase and convalescent phase. The lack of serotyping of the dengue virus was another limiting factor.

Conclusion

CRP and LFTs were found to be effective biochemical markers in assessing DF severity.

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