Research Article

ROLE OF C-REACTIVE PROTEIN LEVEL AS A BIOMARKER FOR SEVERITY ASSESSMENT IN DENGUE FEVER IN DISTRICT COMBINED HOSPITAL, NOIDA, INDIA

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ABSTRACT

Objective: Dengue fever is a mosquito-borne viral infection that affects millions of individuals worldwide. Timely identification of severe dengue cases is critical for appropriate management and reducing mortality. This prospective observational study aimed to assess the utility of C-reactive protein (CRP) as a biomarker for severity assessment in dengue fever. **Methods:** A prospective observational study was performed on dengue patients admitted to a district combined hospital, Noida, India. All patients of age above 18 years, diagnosed with dengue were included in the study. The detailed laboratory parameters pertaining to dengue were recorded. CRP levels were estimated and compared between groups *i.e.* severe and non-severe dengue. CRP cut-off value was detected using the receiver-operator curve. **Results:** Totally 100 patients with a mean age of 40 years were included. Among them, 12% of the patients suffered from severe dengue, 53% of the cases had non-severe dengue without warning signs, and 35% had non-severe dengue with warning signs. The median CRP was significantly higher in patients with severe dengue compared to patients with non-severe dengue (96.2 mg/dL *vs*. 5.3 mg/dL). Univariate logistic regression analysis showed that the odd's ratio *(OR)* of CRP was 1.053 (P≤0.001, 95% CI=1.029-1.078). CRP at a cutoff value of 21.6 mg/L (0.929 AUC) had excellent sensitivity (100%) and specificity (81.6%) in predicting severe dengue infection. **Conclusions:** CRP level could be used as a potential biomarker to predict severity of dengue in adults.

KEYWORD: Dengue Fever, CRP.

INTRODUCTION

Dengue is one of the most significant vector-borne viral diseases worldwide. Dengue fever is a viral infection transmitted by the Aedes mosquito. It is prevalent in tropical and subtropical regions, affecting millions of people each year. According to the World Health organization (WHO), dengue is a global public health challenge with higher prevalence in tropical and subtropical countries.[1] With symptoms ranging from mild flu-like illness to severe hemorrhagic fever, dengue can be life-threatening if not diagnosed and managed promptly. As the medical community continues to work towards improving diagnostic methods for dengue, the use of biomarkers has increasingly gained attention. One such biomarker that has shown promise is C-reactive protein (CRP). C-reactive protein (CRP) is an acute phase protein synthesized in all inflammatory conditions or in tissue injury. CRP is produced by the liver within six hours of onset of inflammation.[6] Nonetheless, the

level diminishes exponentially over 18-20 hours, close to the half-life of CRP, with the depletion of stimuli.[5] The increase in serum CRP level is as high as 1000 fold following infections of some specific bacteria.[5] CRP is a homo-pentameric protein, which dissociates irreversibly into five separate monomers at the sites of infection or inflammation. CRP, in presence of calcium binds with phosphocholine on microorganisms and stimulates the complement pathway of innate immunity resulting in inflammatory processes and host responses to infection.[5] Hence, CRP is considered as a biomarker for infection or inflammation.[11] CRP is clinically used to differentiate between viral and bacterial infection[6] and even to distinguish malaria from dengue fever in endemic areas.[12]

Several studies reported that CRP levels are altered in severe dengue. Additionally, significantly higher level of CRP was detected in early phase dengue patients with

plasma leakage compared to dengue patients without plasma leakage.[11] The objective of this study was to measure the CRP level in adult dengue patients in order to detect the importance of CRP in classifying dengue patients based on severity of illness, so as to consider CRP as a reliable, potential biomarker for predicting acute dengue at early phase.

MATERIALS AND METHODS STUDY DESIGN

A prospective observational study was conducted by clinical investigation in the Department of Internal Medicine at district combined hospital, Sec 39, in Noida, India for a 3 month period from Oct 2023 to Dec 2023.

Participants with selection criteria

Adult patients diagnosed with dengue, who were admitted in a District Combined Hospital, Sec 39, Noida, India were included in this prospective study. Inclusion criterion for the study was the patients more than 18 years of age and dengue-positive. Exclusion criteria for the study were: (1) patients below the age of 18 years, (2) patients discharged against medical advice (3) patients with secondary bacterial infections, and (4) patients with autoimmune disease.

Sample Size

The sample size of the participates was calculated using the formula, $n = Z_{\alpha/2} * p * (1-p)/MOE^2$, where $Z_{\alpha/2}$ is the

critical value of the normal distribution at a/2, MOE is the margin of error, p is the sample proportion, and n is the sample size. Considering the prevalence of dengue to be 8% with 6% margin of error and 95% confidence interval, the sample size was calculated. Total 100 patients were selected for this study. Demographic details such as age and sex, clinical characteristics such as symptoms, bleeding manifestations, comorbidities, general and systemic examination findings, and laboratory investigations including complete blood profile, renal function tests, liver function tests, CRP level, electrocardiogram, chest x-ray, ultrasound of abdomen, and coagulation parameters were recorded in a predesigned proforma.

Dengue illness severity

All the patients were classified according to the severity of dengue based on the 2009-revised WHO criteria[10] as non-severe and severe dengue. The non-severe dengue group was further categorized as dengue with warning signs and dengue without warning signs. The number of cases in severe dengue group, dengue with warning signs and dengue without warning signs were 12, 35 and 53 respectively. CRP level of all the recruited patients were estimated and werecompared among each group based on dengue severity.
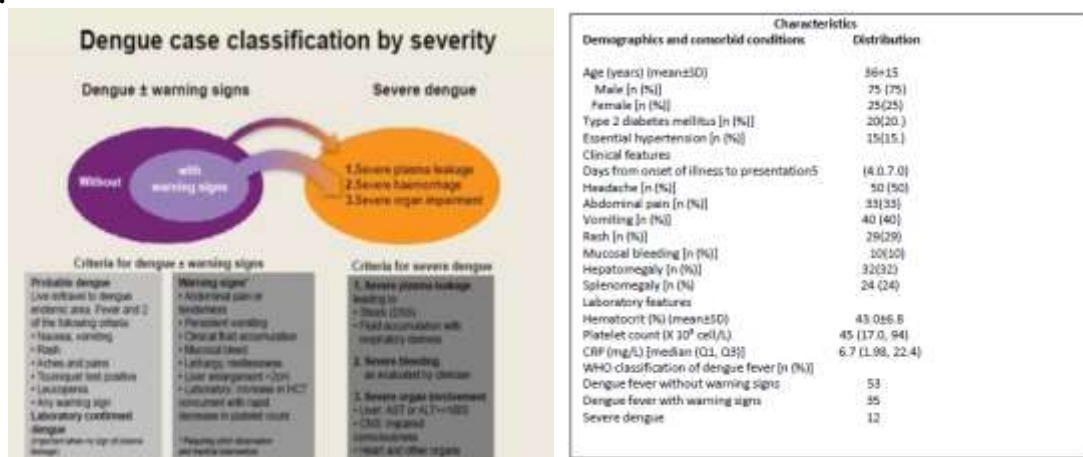
Table 1.

Table 0
WHO revised criteria for classification of severity of dengue.

Laboratory methods

Serum samples from all the study participants were tested for DENV infection. Confirmation of dengue positivity was done by assessing dengue-specific immunoglobulin M or IgM antibody using a kit from National Institute of Virology, Pune.

Statistical analysis

All categorical variables were analyzed and reported in the form of frequency and percentages and continuous

variables were presented as mean±standard deviation and median, where required. Receiver operating characteristic curve was used to derive the cutoff value of CRP for severe and non-severe dengue group and also to know the specificity and sensitivity of the test. The area under the curve (AUC) between 0.50-0.60, 0.60-0.70, 0.70-0.80, 0.80-0.90 and 0.90-1.00 was defined as failing, poor, fair, good and excellent, respectively. The P-value <0.05 was considered to be statistically significant. Data was analyzed using SPSS 23.0 software.

RESULTS

Patient demographic and baseline characteristics

As shown in (Table 0), the mean age of patients who

were diagnosed with dengue fever was (36±15) years with male preponderance; 75 out of 100 (75%) were males and 25 (25%) were females. The most common symptoms were headache (50.0%, 50/100), abdominal pain (33%, 33/100) and vomiting (40%, 40/100). Mucocutaneous rash was seen in approximately 29% of the cases, with mucosal bleeding in around 10% of the cases. Hepatomegaly (32%) and splenomegaly (24.%) were common findings on systemic examination. Fifty-four patients (54%) had dengue fever without warning signs and 35 (35%) had dengue fever with warning signs. Severe dengue accounted for only 12% (12/100) of the cases.

Predictors of severity of dengue
Univariate logistic regression analysis was done for all categorical and continuous variables. Only those significant have been mentioned. Total four parameters were included *i.e.* ALT, serum albumin, total leucocyte count and CRP with OR=1.005 (*P*=0.001, 95% *CI*= 1.002-1.009), OR=0.114 (P≤0.001, 95% CI=0.034-0.381), OR=1.000 (*P*=0.001, 95% CI=1.0-1.0) and OR=1.053 (P≤0.001, 95% *CI*= 1.029-1.078) respectively. Multivariate logistic regression analysis showed that CRP (OR=1.089, 95% CI=1.018-1.166, *P*=0.013) and ALT (OR=1.010, 95% CI=1.001-1.018, *P*=0.034) were statistically significant independent

predictors of dengue severity. Serum albumin and total leucocyte count were not included in the multivariate regression analysis model due to correlation with CRP levels.

CRP levels
The mean CRP level was 22.2 mg/L. The median CRP level of 96.2 mg/L (38.9,124.0) in the severe dengue group was significantly higher compared to CRP level of 5.3 mg/L (1.6,8.3) in the non-severe dengue group with P<0.001(Mann- Whitney *U* test). The CRP cutoff value of 5 mg/L (0.748 AUC) for dengue without warning signs (*n*=53/100) versus dengue with warning signs (*n*=35/100) (Figure2) had a sensitivity of 76.5% and a specificity of 64.2% demonstrating that CRP level could be used to differentiate these two groups. The CRP cutoff value of 28 mg/L (0.877 AUC) for dengue with warning signs (n=35/100) versus severe dengue (n=12/100) had a sensitivity of 90.9% and a specificity of 70.6% (Figure 2). The CRP cutoff value of 21.6 mg/L (0.929 AUC) for non-severe dengue(n=88/100) versus severe dengue (*n*=12/100) as in (Figure 2) had an excellent sensitivity of 100% and a specificity of 81.6% highlighting the usefulness of CRP level in differentiating severe form of dengue from a milder form.
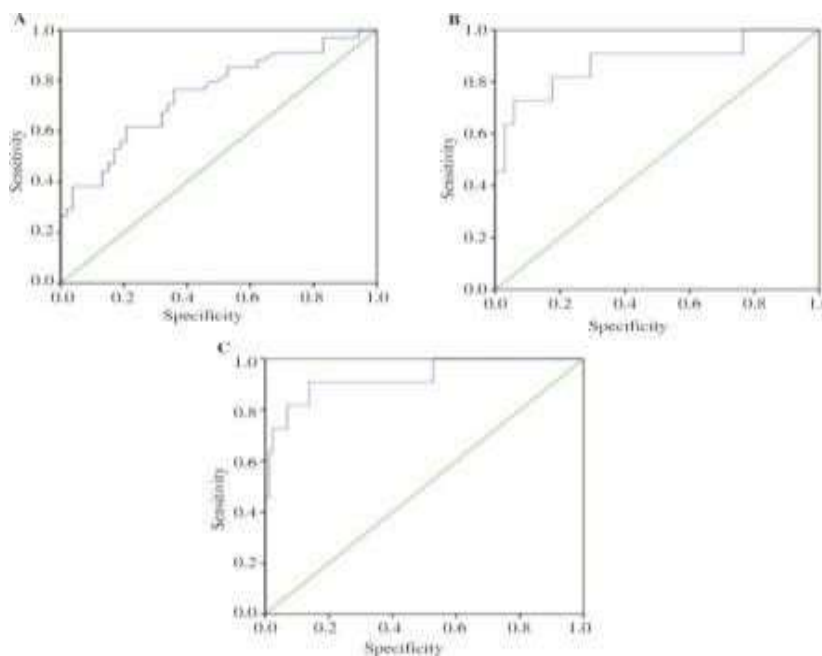

Figure 2.

Receiver-operating characteristic (ROC) curve analysis for dengue (A) without warning signs versus dengue with warning signs, (B) with warning signs *versus* severe dengue, and (C) non-severe dengue *versus* severe dengue.

DISCUSSION
Dengue is an acute infection with majority of the cases either asymptomatic or have diverse clinical signs and symptoms. Dengue has been declared to be hyper

endemic in India by WHO and cyclical epidemics of dengue is increasing every year.[9] The mean age of patients with dengue fever in our study was approximately 40 years. A male preponderance of dengue (75%) was observed in this study. in a study in India with 60% males being affected with dengue. Similar finding was noted by Garg *et al.* in Indian population.[12] Higher dengue in males could be due to increased exposure to outdoor activities or at workplace.

According to another theory, female immune responses are probably more competent than males, leading to higher cytokine production and thus making them more immune to dengue infection than males.

C-reactive protein is an acute phase reactant produced by liver in response to any infection or inflammation. High CRP levels are found to be associated with both bacterial and viral infections and are mainly used as a biomarker for bacterial infections. CRP concentrations normally increases from 10 to 40 mg/L in viral infection unless there is an associated complication or if it is present as a severe disease and more than 40 mg/L in acute bacterial infections[6], thereby CRP level acts as a strong predictor for infectious disease in general practice. In our study, the median CRP level was high in severe dengue group *i.e.* 96.2 mg/L (38.9, 124.0) compared to the non-severe dengue group *i.e.* 5.3mg/L (1.6, 8.3). This was in harmony with the study done by Chen *et al*.[6], where mean CRP level was found to be significantly higher in DSS (median CRP>100 mg/L) and severe dengue compared to non-severe DF (median CRP>30 mg/L). In 2004, a study conducted by Bodinayake *et al*. showed that there were more chances of bleeding, thrombocytopenia, and fluid accumulation in patients with elevated CRP levels although the results were not statistically significant due to small number of patients included in the study. In the present study, CRP cutoff value of 21.6 mg/L had excellent sensitivity (100%) and specificity (81.6%) for predicting severe dengue infection. A similar study done by Chen *et al*. demonstrated CRP cutoff value of 30.1 mg/L had 100% sensitivity in predicting dengue shock syndrome.[6] Conversely, Kutsuna *et al*. reported low serum CRP level (mean value of 5.1) as a predictor of non-severe DF and useful in distinguishing dengue from malaria. However, they could not detect the CRP level in severe DHF/DSS and severe dengue cases, indicating CRP level may be associated with dengue severity. Furthermore, Pan *et al*. concluded that markedly increased CRP levels are not common in pediatric dengue patients as they observed a highest CRP level of 12.7 mg/L in the dengue group.[11] Whether age of dengue patients has any correlation with CRP level enhancement needs to be determined. CRP is an acute phase protein, which means its level in blood increases quickly and robustly in response to various inflammatory states and thus high serum CRP indicates tissue damage.[13] In fact, majority of the patients with very high CRP typically show organ dysfunction including acute kidney injury, pancreatitis, hepatitis, myocarditis.[18] First, we've now not included pediatric populace. Second, the sample length is small which resulted in handiest few cases of severe dengue to examine with the alternative organization. Third, we have no longer categorized sufferers with dengue based on distinct levels of illness. Fourth, this look at became confined to a small geographic place. Further potential studies with extended sample size and patients from special age organizations and exclusive geographical locations need to be performed for obtaining a greater generalized result. Laboratory finding including leukopenia, thrombocytopenia, and increased liver enzymes are commonly detected in dengue patients. The current study detected considerably high CRP level in severe dengue patients compared to non-severe dengue patients with or without warning signs.

Therefore, this study helps to establish the importance of CRP test as a simple, reliable tool for case identification of dengue based on severity, thus aiding the clinicians to triage patients requiring hospitalization. This could be potentially useful in developing countries where the resources are sparse while the burden of the disease is high.

Conflict of interest statement: We declare that we have no conflict of interest.

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