Original Research Article

Predictive value of CRP as a marker of severe dengue

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Abstract

Context: The cornerstone of management of patients with dengue and prevention of dengue-related mortality is early recognition of severe cases and timely management. C-reactive protein (CRP) is an important marker of inflammation claimed to be useful as a single measurement parameter of severity of dengue infection. Studies in paediatric population using CRP as the sole criteria have been limited. This study attempts to assess the usefulness of CRP to identify patients who are at risk for developing severe dengue illness in places where medical resources are sparse. **Aims:** To determine whether CRP can be used as an early predictor of severe dengue illness Statistical analysis: For statistical significance of the measured CRP values, Mann Whitney U test were used. To determine the cut-off value of CRP in different dengue classification categories, ROC (Receiver operator characteristics) curve analysis was used and sensitivity and specificity of cut- off CRP was determined. **Results:** Of the 154 cases of serology positive dengue cases, 74.6% (n115) were cases of non-severe dengue, 18.8% (n29) were cases of severe shock who had presented in the early phase, and later progressed to dengue shock syndrome, 3.2%(n5) were cases of dengue haemorrhagic shock and 3.2% (n5) were cases of expanded dengue syndrome. The median value of CRP measured early in the disease process was 7.5mg/l of the severe dengue group, (17 mg/l of the expanded dengue group); higher than CRP value of 1.9mg/l of the non-severe dengue group. The P value of this median CRP between the severe dengue group and the Non severe dengue group was determined to be significant (Table -4). ROC analysis done to determine the cut off value of CRP, to differentiate severe dengue and non-severe dengue was 6 mg/dl with 100 % sensitivity and 80 % specificity with area under curve (AUC) of 0.95 which shows that severe dengue categories have a higher CRP value compared to the non-severe dengue .
Key Word: CRP, dengue, predictors of severe dengue, severe dengue,

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INTRODUCTION

Dengue is among the most significant arthropod-borne viral diseases in the world[1]. Clinically, dengue manifestation ranges from nonspecific febrile illness, dengue fever (DF), and dengue haemorrhagic fever (DHF), to the more severe form of dengue shock syndrome (DSS), according to 1997 World Health Organization (WHO) classification[2]. Limitations have been reported regarding its complexity and applicability[3,4,] and the 2012 WHO revised classification divided dengue-affected patients into two categories: Non severe (with and without warning signs) and severe dengue[5]. The cornerstone of management of patients with dengue and prevention of dengue-related mortality is early recognition of severe cases and timely management. CRP is an important marker of inflammation claimed to be useful as a single measurement parameter of severity of dengue infection. Studies in paediatric population using CRP as the sole criteria have been limited. This study attempts to assess the usefulness of CRP to identify patients who are at risk for developing severe dengue illness in places where medical resources are sparse. [6]

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METHODOLOGY

IEC statement -The study was approved by the institutional ethics committee of Ramaiah Medical College and informed consent for the collection of data was waived off.

Study design: Retrospective study with purposive sampling to match inclusion and exclusion criteria over a period of one year.

Inclusion criteria

- Children aged 0-16 years with lab confirmed serology of dengue fever admitted to our hospital, between 2017 June and 2018 May were included in this study.
- Dengue fever confirmed by serological evidence of dengue fever (with NS positive with IgM positive, IgM positive with IgG positive or only IgM positive was considered for diagnosis.

Exclusion criteria

- Patients having concurrent bacteraemia - (elevated total counts, blood culture positive , for bacterial infection, urine routine suggestive of infection , urine culture positive for bacterial infection)
- Clinically diagnosed cases of dengue with superimposed bacterial infection.
- Patients started on antibiotics / antimalarial for suspicion of bacterial infection / malaria respectively.
- Peripheral smear positive for malaria.
- Patients developing secondary infections during hospital stay.
- Patients having chronic inflammatory conditions such as juvenile rheumatoid arthritis.

Sample size: Sample size - 140 pts. Sample size was calculated with 95 % confidence interval and absolute precision of 7 % considering ~25 % of dengue patients admitted in our hospital to be affected with severe dengue based on previous hospital statistics, with the predicted value of CRP being more than 6mg/L. Study was a retrospective study with purposive sampling process to target the required sample size over a period of one year.

Laboratory methods

1. Children were diagnosed with based on serology. Rapid card test was used to detect Dengue NS1 antigen and differential detection of IgG and IgM antibodies in human plasma/serum and confirmation was done using Dengue IgM capture ELISA.
2. CRP was determined in patients using quantitative assay immunoturbimetric analysis with latex particles coated with anti -CRP antibodies with a lower range of detection of 0.3 mg/

Statistical methods

For statistical significance of the measured CRP values, Mann Whitney U test were used. To determine the cut-off value of CRP in different dengue classification categories, ROC (Receiver operator characteristics) curve analysis was used and sensitivity and specificity of cut-off CRP was determined.

Detailed description of the procedure

This study was done by retrospective observation of case records of patients selected by purposive sampling, admitted to our hospital between June 2017 and May 2018. During this period 192 dengue serology positive and those meeting the exclusion criteria were analysed. Children with history of use of antibiotics, prior hospital admission, evidence of bacteraemia and malaria were scrutinised and excluded from the study. CRP was sent only in 154 cases based on physician's discretion. Our sample size was extended to 154 from the calculated minimum sample size of 140 pts. Sample size was calculated with 95 % confidence interval and absolute precision of 7 % considering ~25 % of dengue patients admitted in our hospital to be affected with severe dengue based on previous hospital statistics, with the predicted value of CRP being more than 6mg/L. The cases were categorised based on clinical, laboratory observations and final outcome of the disease as per the 2009 WHO classification into severe dengue and non-severe dengue as well as the 1997 classification into DSS, DHF without shock and DF cases for comparison. The data thus obtained was entered in a pre-designed proforma CRP done in these patients at admission were noted and correlated with final outcome of the disease, and analysed using IBM SPSS version 20. Mann Whitney U test as used to assess for significant statistical difference of CRP between various dengue categories and ROC analysis was used to determine the cut off of CRP to differentiate different dengue classification and to measure sensitivity and specificity of the determined value.

OBSERVATIONS AND RESULTS

Table 1: Patient characteristic

Males N (%)	121(63)
Female N (%)	21(37)
Fever, N (%)	189(99)
Abdominal pain, N (%)	61(31.9)
Vomiting N (%)	89 (46.6)
Rashes, N (%)	12(6.3)
Epistaxis, N (%)	6(3.1)
Gastrointestinal bleeding N (%)	14(7.3%)
Headache, N (%)	13(6.8)
Myalgia, N (%)	16(8.4)

Convulsions N (%)	5(2.6)
Cough, N (%)	23(12)
Diarrhoea, N (%)	15(7.9)
Throat pain N (%)	5(2.6)
Puffiness of face, N (%)	2(1)
Fatality, N (%)	2(1)

Table 2: Age distribution

Age in years	n	Percent %
,<1	18	9.4
1-5	42	21.9
6-10	68	35.4
11-16	64	33.3
Total	192	100.0

Table 3: Diagnosis Classified according to WHO 2009

Diagnosis	n	Percent %
Severe dengue	39	23.4
Non severe dengue	115	76.6
Total	154	100.0

Table 4: Median CRP value WHO classification 2009 and 2012

	n	Median CRP mg/l	
Severe dengue	39	7.5	p value 0.000
Non severe dengue	115	1.9	

Table 5: Median CRP value WHO classification 1997

Diagnosis	N	Median crp mg/l	Range	
Dengue shock syndrome	34	5.2	0- 175	$P^1<$ 0.008 $P^2<$ 0.009 $P^3<$ 0.08
Dengue haemorrhagic shock	5	11.6	0.9- 39.4	
Dengue fever	115	1.9	0- 185	
Total	154			

P^1 - CRP values between DSS and DF

P^2- CRP values between DSS and DHF. P^3- CRP values between DF and DHF

Patient characteristics and age distribution: The final study sample included 154 cases of dengue of which 62.5 % (n120) pts. were boys and 37.5 % (n72) patients were girls (table 1). Majority of these patients were children in the age group of 6-10yrs (35.4 %, n68) (table 2).

Dengue categories: Using operational definitions of dengue of 2009 WHO classification 23.4% (n39) were case of severe dengue (SD), and 76.6% (n115) were cases of non-severe dengue (NSD) (table -3). A better performance of the revised classification, 2009 to the 1997 classification has already been studied with respect to effective screening and patient management. However CRP has not been used a sole criterion for predicting severe dengue cases using both these classification. Hence cases were classified as per both the classifications to bring out differences between the two classifications. As per the 1997 classification and definitions 34 patients were diagnosed as Dengue shock syndrome (DSS), 5 patients as Dengue haemorrhagic syndrome (DHF) without shock and 115 cases as dengue fever (DF) (table -5)

Correlation of dengue categories with CRP: According to the 2009 classification, the median value of CRP of the severe dengue category was 7.5 mg/L and of the non-severe dengue group 1.9 mg/ L. This median value of CRP value of severe dengue group was found to be significantly higher (P value< 0.000) compared to the non-severe dengue group using Mann Whitney test. (table -4). As per the 1997 WHO classification, the median values of CRP of DSS/DHF without shock/DF were 5.2/11.6/l.9mg/l. The CRP values were significantly higher in the DSS compared to the DF/ DHF without shock category patients. A higher CRP in the group of patients without shock was because of inclusion of patients with expanded dengue syndrome, patients with hepatitis and minor bleeding manifestations in this category. These patients were classified as cases of severe dengue as per 2009 classification and cases of DHF without shock as per 1997 classification.

Cut off value of CRP: To determine a cut value of CRP to differentiate severe dengue from less severe forms (as per 2009 classification), ROC curve analysis was performed. The area under curve (AUC) under ROC analysis between severe dengue and non-severe dengue as per 2009 classification was 0.958 with 100 % sensitivity and 80% specificity with a cut off vale of CRP 6 mg/l to differentiate severe dengue and non-severe dengue. The ROC curve analysis was also performed to determine cut off values of CRP (for different categories of dengue as per the 1997 classification), between i) DF and DHF/DSS and ii) DF/ non shock DHF and DSS. AUC between DF AND DHF/DSS patients was 0.59 which was insufficient to differentiate these category of patients using CRP in contrast AUC between DF/non shock DHF and DSS was 0.86, corresponding to a sensitivity of 84% and specificity of 87% with an cut off value of CRP of 6.2mg/l which could help differentiate DSS from less severe forms of dengue (DF/non shock dengue)

DISCUSSION

The severity of dengue as attributed to its inflammatory response is mainly due to the release of IL substance esp.

the IL -6. CRP is a surrogate but cost effective marker of IL-6 which has been found to be high in dengue shock syndromes. This formed the basis of our study. Also CRP, Tumour Necrosis factor alpha (TNF ALPHA), IL-6 and Nitric Oxide implicated in dengue infection are important source of inflammatory markers in DEN-4 AND DEN -2 infections.[7,8] CRP concentrations may increase up to 1000 fold in certain acute inflammatory event. Studies of raised CRP in CRP in dengue support this claim in stage II and IV of dengue shock syndrome but there only few studies to prove an rise in CRP in early phases of dengue to predict a severe disease course in the later phases of the illness as per new definitions of severe disease.[9] As per the pathogenesis in dengue illness, severe forms of illness are due to severe inflammation which can be measured indirectly by inflammatory markers and hence predict severe case progression in the early phases.[10,11] In our study CRP done at admission in patients with early dengue (based on physician's discretion) were correlated to the outcome of the illness to DF, DF with warning signs, DHF and severe dengue. As per the 2009 WHO classification, dengue is classified as dengue without warning signs, dengue with warning signs and severe dengue. Patients with nausea, vomiting, rash, aches and pain, leucopoenia and positive tourniquet test were labelled as dengue without warning signs. Patients with abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation ascites, pleural effusion), mucosal bleeding, lethargy, restlessness, liver enlargement > 2 cm. laboratory arameters raised haematocrit concurrent with a decrease in platelet count were diagnosed as dengue with warning signs. Patients with shock, fluid accumulation with respiratory distress, severe bleeding as evaluated by clinician, severe organ involvement, liver AST or ALT $\geq$1000, impaired consciousness, failure of heart and other organs were categorised as severe dengue. Expanded dengue syndrome was included as a subclass of dengue shock syndrome. As per the 1997 WHO classification patients were classified as DF, non-shock DHF and DSS. Patients were categorised as DSS if there was circulatory failure associated with evidence with DHF. Patients with fever, thrombocytopenia, bleeding, and evidence of plasma leakage were characterised as DHF. Child with blood culture positivity for bacterial infection , patients having concurrent bacteraemia - (elevated total counts, urine routine suggestive of infection , urine culture positive for bacterial infection), clinically diagnosed cases of dengue with superimposed bacterial infection, patients started on antibiotics / antimalarial for suspicion of bacterial infection / malaria respectively, peripheral smear positive for malaria, patients developing secondary infections during hospital stay and patients having chronic inflammatory conditions such as juvenile rheumatoid arthritis were excluded from the study. Our analysis of the data demonstrated a CRP cut off of 6mg/l to differentiate severe dengue from non-severe dengue with good sensitivity. This finding was similar to a study published by Sandhya *et al*[12] where a positive correlation of increased CRP was seen in severe dengue cases. This was also the finding in adults in a study done by Chen *et al*[6] where such DSS had elevated CRP compared to the non-severe forms. Hence using this CRP level appropriate management can be initiated in patients with higher risk of progressing to severe forms of the disease. Organ dysfunction such as hepatitis and expanded dengue syndrome had very high CRP values. The more severe the hepatitis, higher the CRP at presentation. The exclusion criteria were adhered to select the case to exclude super added bacterial infection aetiologies of raised CRP.

The limitation of our study were

1. Larger sample could have provided a larger number of severe dengue category patients which could have increased the statistical power of the study.
2. Case to case controlled match of non-severe to severe dengue on basis of age, could be further attempted considering different levels of immunological response in different age groups and children.
3. Nevertheless the limitations our study, our study undisputedly established the fact that higher CRP values at admission need strict monitoring and management to guide differentiate mild and severe forms of disease. CRP may prove to be an effective single early marker for dengue severity and help triage patients in medical resource sparse countries where also burden of dengue is high.
4. Based on the results of this study a further prospective study involving a larger sample size with equal distribution of patients in different dengue categories could be planned to increase the statistical power of the study. Also use of Hs CRP (High sensitivity CRP) could prove to be a better marker of inflammation.

CONCLUSIONS

CRP being an inflammatory marker could serve as an important economical early differentia to identify severe disease especially in resource poor developing countries as well in peripheries to carefully monitor patients with dengue for better outcomes in illness. CRP could serve as a single early predictor of dengue shock syndrome/severe dengue and prompt early referral to higher centres for management.

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