International Journal of Contemporary Pediatrics
Atukuri SR et al. Int J Contemp Pediatr. 2017 Mar;4(2):450-454
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291
DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20170687

Original Research Article

Correlation of C-reactive protein and neutrophil counts as early indicators of severe dengue in children

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Received: 25 October 2016
Revised: 20 December 2016
Accepted: 20 December 2016

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ABSTRACT

Background: Dengue has broad clinical presentation with unpredictable clinical evolution and outcome while most patients recover following a self-limiting non-severe clinical course, a small proportion progress to severe disease. As early diagnosis of dengue infection remains a challenge around the world in areas of limited resources, laboratory parameters like CRP, Neutrophil counts may serve as predictive markers to promote early diagnosis. The objectives of this study were to stratify the levels of C-reactive protein and Neutrophil counts in children with dengue fever and to determine the correlation of C-reactive protein and Neutrophil counts with the severity of dengue

Methods: This was an Observational chart based descriptive study done in all pediatric dengue children (aged 1-15 years) admitted at Father Muller medical college hospital, Mangalore during the period of June 2014 to 2016. Total sample size was 100. Data collection was done by using purposive sampling based on inclusion and exclusion criteria from case records. Controls (n = 20), children with diagnosis of viral fever were considered in the study.

Results: Out of the 100 children studied, 16% were <5 years ,33% between 6-10 years and 51% above 10 years of age. Mean CRP levels in NS1, IgM and both are 6.2, 6.9, 6.3. CRP values >5 were considered as positive. Mean CRP values in dengue fever with warning signs (DF1), without warning signs (DF2) and Severe Dengue (DF3) were 6.3, 6.2, 11.4 respectively. Absolute Neutropenia was observed in 52% of the study population of dengue (DF1) of which severe neutropenia was observed in 19% and 15% OF DF2 (with no warning signs) also showed neutropenia. CRP values are significant in the study when compared to controls and absolute neutropenia was observed in 52% of dengue with warning signs. Hence CRP and neutropenia may be helpful as early predictors of severe dengue.

Conclusions: Our study was an attempt to correlate CRP and neutropenia for early prediction of severe dengue. Mean CRP values in the population were significant statically but not as markedly elevated in other bacterial illness as the study population have no enrolled cases of severe dengue. But neutropenia (<1,500/cmm) and positive CRP (>5) may serve as predictive markers in a resource limited setting.

Keywords: C-Reactive protein, Neutrophil count, Severe dengue

INTRODUCTION

Dengue is the one of the major tropical infectious diseases. It is the most significant arthropod-borne viral diseases caused by five serotypes, approximately World Health Organization (WHO) estimates that 50-100 million dengue infections occur each year and that almost half the world's population lives in countries where dengue is endemic.[1]

Infection with one or more dengue viruses imperils an estimated 2.5 billion people living in tropical and subtropical countries, mostly in all cities.[2] In 2016, in

India 36,110 dengue cases were reported from January till September 11th with 70 deaths, in the country.[3]

Dengue has a broad clinical manifestation, ranging from non-specific febrile illness, dengue fever (DF) with and without warning signs and severe dengue, according to 2009 WHO revised classification.[4] It is a single stranded, non-segmented RNA virus, belongs to genus flavivirus, family flaviviridae. Early recognition of dengue is mostly challenging as, the initial symptoms are often non-specific, viremia may be below detectable levels and serological confirmation in dengue is late in the course of illness.[5,6] However, there are no accepted clinical guidelines for early recognition of dengue infection and no consensus to whether clinical features to distinguish dengue from other febrile illness also.[7,8]

C-reactive protein (CRP) is an acute phase protein that rise during infection phase after inflammation or any tissue injury. Clinically CRP, often utilised to distinguish between viral and bacterial infections or to assess the severity of illness or to monitor the response to therapy.[9]

Leukopenia and absolute neutropenia is also well described as a feature of dengue infection and is related to bone marrow suppression by dengue virus.[10] Mangalore being endemic for dengue with a reported incidence of 1065 cases of dengue from 2016 January till this June, 281 with confirmed ELISA test and 4 deaths.

This study is aimed to identify the early predictive markers of laboratory findings during the early stage of dengue infection which would help to validate this trail in a low resource settings. One focus of the study was to identify the early predictors of laboratory observations that could distinguish acute dengue infections at an early stage, before plasma leakage develops in patients leading to severe dengue.

METHODS

All pediatric dengue children aged from 1 year to 15 years, admitted to a tertiary care centre who was positive by either CARD or ELISA test. Study period was from June 2014- June 2016. Control group includes children with diagnosis of viral fever. Children with other infection, mixed infection of malaria and dengue, chronic diseases, on drugs like steroids, antimalarials, immunosuppressants and whose C reactive protein was not done were excluded. It was a retrospective data collection from case records. Ethical clearance obtained from the Institution ethical committee.

Grading for the severity of dengue was noted according to WHO classification of cases, as dengue fever with and without warning signs, and severe dengue based on the clinical and laboratory observations. Neutrophil counts were considered as age specific values and absolute neutrophil count (<1500/µL) and severe neutropenia (< 500/µL).[11,12]

C-reactive protein levels (was done by immunoturbidometric method in automated Cobas 6000 analyser) and WBC counts (by Abbott Cell-Dyn 1700 automatic analyzer) were noted. Clinical details, history, investigations was entered in the predesigned proforma.

The data obtained was analysed by frequency, percentage, mean, t-test and anova test.

RESULTS

The study population includes 100 pediatric dengue cases, among which female and male constitute for 52% and 48%. Predominant age group observed in the study was between 11-15 years accounts for 51% of study population, 16% below 5 years of age, these demographic characteristics was shown in the Table 1. Mean C-reactive protein values observed in correlation with the severity of dengue in our study were 7.19±6.26, 3.23±2.41, 33.8 in DF1, DF2 and DF3. Mean C-reactive protein values correlated with type of dengue antigen, it was observed that no marked differentiation among type of dengue antigen and mean CRP.

Table 1: Classification of dengue.

Probable dengue	Warning signs**	Severe dengue
Travel to dengue endemic area	Abdominal pain or tenderness	**Severe plasma leakage leads to**
Fever with 2 of the following criteria	Persistent vomiting	Shock (DSS)
Nausea, vomiting	Clinical fluid accumulation	Fluid accumulation with respiratory distress
Rash	Mucosal bleed	**Severe bleeding**
Sches and pains	Lethargy, restlessness	As evaluated by clinician
Tourniquet test positive	Liver enlargement >2 cm	Severe organ involvement
Leukopenia	Laboratory: increase in HCT with rapid decrease in platelet count	Liver: AST or ALT >=1000
Any warning sign		CNS: impaired consciousness
Lab confirmed diagnosis		Heart and other organs

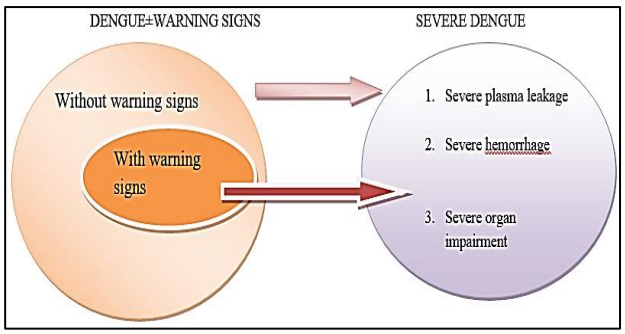

Figure 1: Dengue±warning signs and severe dengue.

Table 2: Age specific total counts and neutrophil counts.[6,7]

WBC (cells/microL)	Neutrophil count
1 year - 11.4(6-17.5)	3.5 (1.5- 8.5)
2 years - 10.6(6-17)	3.5 (1.5-8.5)
4 years - 9.1 (5.5-15.5)	3.8 (1.5-8.5)
6 years - 8.5(5-14.5)	4.3 (1.5-8)
8 years - 8.3(4.5-13.5)	4.4 (1.5-8.5)
10 years -8.1(4.5-13.5)	4.4 (1.8-8)
16 years -7.8(4.5-13.0)	4.4 (1.8-8)

Table 3: Demographic characteristic.

Age in years	No. of patients	Percentage
< 5 years	16	16%
6-10 years	33	33%
11-15 years	51	51%
Sex		
Female	52	52%
Male	48	48%
Total	**100**	**100%**

Table 4: Mean CRP values with severity of dengue.

Severity of dengue	No. of cases	Mean CRP values
DF1	69	7.19±6.26
DF2	30	3.23±2.41
DF3	1	33.8

DF1- dengue with warning signs; DF2- dengue without warning signs; DF3- severe dengue.

Table 5: Mean CRP values in types of dengue antigen.

Dengue antigen	No. of cases	Mean CRP levels
NS1 positive	76	7±6.275
Ig M positive	4	7±6.928
NS1 and Ig M positive	17	7±6.306
NS1, Ig M, Ig G positive	2	7±6.953

When compared to controls and dengue cases C-reactive protein had positive correlation in severe dengue, with significant P value (<0.0001), and non-severe dengue cases found to have no significant correlation. Neutropenia was observed in 52% of study population of dengue with warning signs (DF1).

In this study, 15% of cases of dengue (DF2) without any warning signs also had neutropenia. In correlation with neutropenia and severity of dengue with controls it was found to have significant correlation with a p value (<0.01). Severe Neutropenia was observed in 19% of study population of dengue with warning signs than compared to non-severe dengue which was 3% alone. These correlations was demonstrated in the table below.

Table 6: Statistical comparison of various dengue groups with control group, ANOVA multiple comparison.

	Control (N = 20)	DF 1 (N = 69)	DF 2 (N = 30)	DF3 (N = 1)	
Platelet	3.42±1.52	1.04±0.48	1.31±0.35	0.19	<0.0001
		P <0.01	P <0.01		
CRP	1.03±0.78	7.19±6.26	3.23±2.41	33.8	<0.0001
		<0.0001	NS	<0.01	
Neutrophil counts	5390.00±2449.04	1043.10±808.37	2232.4±1717.31	2665	<0.0001
		P<0.01	P<0.05		

Table 7: Statistical comparison of all dengue cases with control group, ANOVA multiple comparison.

	Control (N = 20)	Dengue (N = 100)	
Platelet	3.42±1.52	1.13±0.47	<0.0001
CRP	1.02±0.78	6.27±6.30	<0.0001
neutrophil counts	5390.00±2449.04	1416.11±1275.69	<0.0001

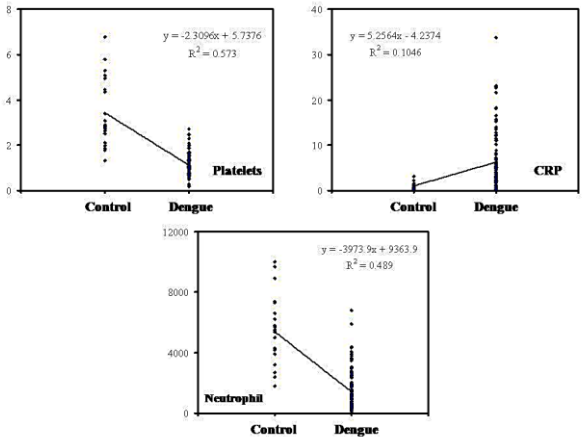

Figure 3: Association of CRP and neutrophil with dengue and control group.

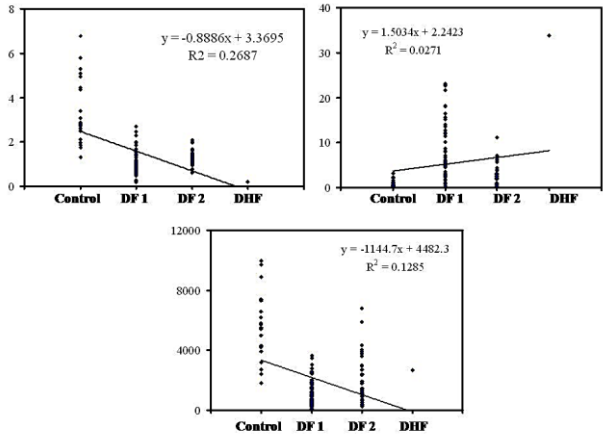

Figure 4: association of platelet, CRP and neutrophil with types of dengue and control group.

DISCUSSION

Dengue is an acute infection caused by dengue virus, where majority of cases resolve asymptomatic or may have wide spectrum of clinical signs and symptoms associated with laboratory findings. The classification of dengue according to the severity of dengue was listed in the table below. Dengue being a self-limiting infection, thorough physical examination and hematological parameters were needed to assess the severity of the disease, to reduce the mortality and morbidity especially in pediatric age group. There are very few studies done in pediatric age group.

C-reactive protein is an acute phase reactant and its level increase following any infection or inflammation. In few cases CRP is considered as non-specific biomarker of inflammation. In our study we found a positive correlation in CRP with severity of dengue (DF1) with a p value of (<0.0001) than in non-severe dengue(DF2), where no statistical significance was observed. Mean C-reactive protein in our study was 7.19±6.26 observed in severe dengue cases. Chen et al, in his study observed that increasing CRP levels with severity of dengue, mean CRP in DF (8.5), DHF I (15.2), DHF III (124.5) with significant p value (<0.0001).[14]

In contrast to our study, Kutsuna S et al concluded in their study that low CRP (mean value of 5.1) suggests Dengue fever and helpful in differentiating from malaria. Ho et al in his study observed low CRP values(<20mg/dl) as a marker for dengue.[15,16]

Leukopenia as feature of dengue infection and related to bone marrow suppression by dengue virus, in our study we observed a positive correlation with severity of dengue and neutrophil count, as 52% of cases had absolute neutropenia with a mean value of 1043.10±808.37.[7] Chadwick D et al, in his study found that leucopenia and neutropenia (mean -1320) have high positive predictive value for dengue fever and its severity.[13] Ho et al in his study concluded that leucopenia(<4000) and neutropenia may serve as predictive values.[16]

In contrast, Kalayanarooj S et al found in their study that neutrophil count (mean - 3389) was significantly higher in dengue patients than in subjects with other febrile illness.[7] Serial measurements of CRP showed increasing trends in few studies, but we have considered the one CRP value on admission, so that it could be one of the limitations. Our study population did not include many severe dengue cases, because there were no enrolled cases.

We had one severe dengue, which presented to hospital at severe stage and required ICU care, resuscitative measures and expired.

CONCLUSION

CRP and Neutropenia may be helpful parameters for diagnosis in hospitals, low resource settings and primary health centers where specific diagnostic tests were unavailable.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Atukuri SR, Nayak P. Correlation of C-reactive protein and neutrophil counts as early indicators of severe dengue in children. Int J Contemp Pediatr 2017;4:450-4.