Plasma Neutrophil Elastase, α_1 -Antitrypsin, α_2 -Macroglobulin and Neutrophil Elastase– α_1 -Antitrypsin Complex Levels in patients with Dengue Fever

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Abstract Dengue fever (DF) is characterized by systemic inflammatory response including neutrophil activation leading to uncontrolled elastase activity. This study was aimed to measure the activity of plasma neutrophil elastase (NE), its endogenous inhibitors α_1 -antitrypsin (α_1 -AT) and α_2 -macroglobulin (α_2 -MG) and elastase in complex with α_1 -AT (NE– α_1 -AT complex) in DF. 50 dengue patients [39 DF and 11 dengue hemorrhagic fever (DHF)] and 52 healthy subjects were included in the study. NE was measured using *N*-succinyl-tri-alanine-*p*-nitroanilide as substrate. α_1 -AT, α_2 -MG and NE– α_1 -AT complex were estimated by ELISA. The result analysis indicated that the dengue patients had significantly higher elastase activity with significantly reduced inhibitor levels compared to controls. Between DF and DHF patients, DHF group had significantly higher elastase activity. In conclusion, significantly elevated NE and reduced inhibitors level in dengue fever indicate these parameters could be of significance in DF particularly for the assessment of progression of inflammatory processes.

Keywords Dengue fever · Neutrophil elastase · α_1 -Antitrypsin · α_2 -Macroglobulin · NE– α_1 -AT complex

Introduction

Dengue fever (DF) is an acute infectious disease of viral etiology with an estimated global incidence of close to 400 million per year [1, 2]. In response to any infection or tissue injury, the body initiates a series of cellular events including recruitment of neutrophils to the site of injury. The outcome of these events is an induction of a localized inflammatory response that allows cells to move out of the vessels to the site of infection/injury to repair cellular damage and restrict the replication of a pathogen. During dengue virus (DENV) infection, this normal process is affected. Studies demonstrate that DENV can be considered as an infectious trigger of an acute vascular disorder associated with inflammatory response [3].

The pathogenesis of DENV infection has been linked to the ability of the DENV to produce various inflammatory mediators such as neutrophils, plasma cascade systems and cytokines [1]. Neutrophils are known to have a crucial role in the pathogenesis of DENV infection as neutropenia is one of the most salient clinical features of dengue infection [4]. Elevated production of IL-8, a chemoattractant cytokine with potential pro-inflammatory effects in DENV infection [1, 3, 5] is known to activate and degranulate neutrophils [1, 6]. It is expected during DENV infection degranulation of neutrophils could release NE and consequent endothelial damage [7, 8]. Therefore it was considered worthwhile to study the levels of elastase and its endogenous inhibitors as in inflammatory states like DENV infection where large numbers of polymorphonuclear leukocytes are infiltrated and activated.

Thus this study was aimed to determine the levels of plasma elastase activity, its two inhibitors (antiproteases):

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alpha₁-antitrypsin (α_1 -AT) and alpha₂-macroglobulin (α_2 -MG) and elastase in complex with α_1 -AT (NE- α_1 -AT complex) in DENV infection to associate with inflammatory processes.

Materials and Methods

Study Population

The present study is a case control study carried out in the Department of Biochemistry, Sri Devaraj Urs Medical College, the constituent college of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka. A total of 50 dengue patients admitted in the Department of Medicine of R. L. Jalappa Hospital and Research Centre, the teaching hospital of the medical college were included in the study; of them 39 were dengue fever (DF) and 11 were dengue hemorrhagic fever (DHF) patients. Dengue was confirmed by non-structural 1 antigen (NS1 antigen). Samples from dengue confirmed patients were obtained on day 5 to day 7 after onset of fever. 52 age and gender matched normal healthy individuals without fever were selected as controls. Every enrolled individual gave their informed written consent to participate in the study. The study protocol was approved by Institutional Ethical Committee.

Patients with acute and chronic infections, T2DM, cardiac diseases, malignancy, stroke, chronic obstructive lung disease, liver disorders, acute renal failures, history of smoking were excluded from the study.

Sample Collection

6 ml of fasting venous blood was collected from all the subjects in tubes containing sodium fluoride (for FBS estimation), EDTA (for hematologic studies), Sodium Heparin (for elastase, α_1 -AT, α_2 -MG and NE- α_1 -AT complex estimation). For investigations like CRP, renal function and liver function tests blood was collected in tubes without anticoagulant. Blood samples were centrifuged within 2 h of collection. After centrifugation, serum and plasma were separated and aliquots were stored at -70°C until assayed.

Methods

Basic blood chemistry measurements were done by standard methods using Vitros 250 Dry chemistry analyzer (Johnson & Johnson). Complete Blood Count was performed by Beckman-Coulter, automatic blood cell counter. Serum C-reactive protein (CRP) estimation was done by rapid latex slide tests. Plasma elastase was estimated using succinyl tri-L-alanyl-p-nitroanilide (STANA, SIGMA) as

substrate at 410 nm as per the procedure described by Beith et al. [9]. Plasma α_1 -AT and α_2 -MG were analyzed using Enzyme Linked Immunosorbent Assay, Immunology Consultants Laboratory, Inc, USA. NE- α_1 -AT complex was quantified by ELISA (Calbiochem).

Statistical Analysis

The results were analyzed by SPSS software version 22 (licensed version) for statistical significance. The results are expressed as mean $\pm$ SD. All variables were checked for normal distribution by Shapiro–Wilk test. Differences between groups were analyzed using Student's unpaired *t* test for normally distributed parameters and Mann–Whitney *U* test for not-normally distributed parameters. *p* value <0.05 was considered statistically significant and <0.001 as highly significant.

Results

Baseline characteristics of study groups are shown in Table 1. The mean age was 48 years. Majority were males, 61.5% and females were 38.5% in control group and in dengue group 60% were males and 40% were females. There were significant changes in hemodynamic parameters between control and patient group. Significant decrease in platelet count (272.23 ± 61.24 – $71.94 \pm 39.17 \times 10^3/\text{l}$, $p < 0.001$), total WBC count (7.04 ± 1.02 – $4.38 \pm 1.81 \times 10^3/\text{l}$, $p < 0.001$), neutrophils (51.27 ± 7.37 – $40.68 \pm 12.37\%$, $p < 0.001$) and lymphocytes (37.86 ± 6.62 – 33.99 ± 9.20 , $p < 0.05$) observed in dengue patients whereas we found significant increase in monocytes in dengue patients as compared to controls (5.01 ± 1 – 14.24 ± 6.09 , $p < 0.001$).

Plasma levels of elastase activity, α_1 -AT, α_2 -MG and NE- α_1 -AT complex in the study groups are presented in the Table 2. Plasma elastase activity in patients with DENV infection were significantly higher than in healthy subjects ($p < 0.001$) whereas plasma levels of inhibitors α_1 -AT and α_2 -MG were significantly lower in dengue patients as compared to healthy control group ($p < 0.001$). When plasma NE- α_1 -AT complex was estimated in the two group, dengue patients had higher concentration but the results were not statistically significant ($p = 0.068$).

When elastase activity was compared between DF and DHF patients, DHF patients had higher activity of 1.044 ± 0.17 U/ml/min compared to DF group with elastase activity of 0.70 ± 0.15 U/ml/min (Fig. 1). However there was no significant difference in the levels of α_1 -AT, α_2 -MG and NE- α_1 -AT complex.

Table 1 Basic characteristics of study groups

Variables	Controls (n = 52)	Cases (n = 50)	p value
Age (years)	48.48 ± 6.39 ^a	48.50 ± 4.34 ^a	0.986
Gender (male/female)	32/20 (61.5%/38.5%)	30/20 (60%/40%)	–
HCT (%)	42.27 ± 3.63 ^a	43.01 ± 5.22 ^a	0.407
PL count (10 ³ /l)	272.23 ± 61.24 ^b	71.94 ± 39.17 ^b	<0.001**
Total WBCs (10 ³ /l)	7.04 ± 1.02 ^b	4.38 ± 1.81 ^b	<0.001**
Neutrophils (%)	51.27 ± 7.37 ^a	40.68 ± 12.37 ^a	<0.001**
Lymphocytes (%)	37.86 ± 6.62 ^a	33.99 ± 9.20 ^a	<0.05*
Monocytes (%)	5.01 ± 1 ^b	14.24 ± 6.09 ^b	<0.001**

^a Student's unpaired *t* test and ^b Mann–Whitney *U* test were used. Values are expressed as mean ± SD

* Statistically significant; ** Statistically highly significant

Table 2 Comparison of plasma NE activity, α_1 -AT, α_2 -MG and NE- α_1 -AT complex between cases and controls

Parameters	Controls (n = 52)	Cases (n = 50)	p value
Elastase activity (U/ml/min)	0.35 ± 0.21 ^b	0.78 ± 0.20 ^b	<0.001*
α_1 -AT (mg/dl)	123.35 ± 25.66 ^b	66.25 ± 7.75 ^b	<0.001*
α_2 -MG (mg/dl)	209.42 ± 31.15 ^b	162.75 ± 24.16 ^b	<0.001*
NE- α_1 -AT complex (ng/ml)	214.89 ± 13.05 ^a	238.38 ± 62.47 ^a	0.068

^a Student's unpaired *t* test and ^b Mann–Whitney *U* test were used. Values are expressed as mean ± SD

* Statistically highly significant

Fig. 1 Elastase activity in DF and DHF patients

Discussion

Pathogenesis of dengue, a systemic viral infection condition, is multifactorial and complex. Host immune response is one of the factors involved in the pathogenesis of dengue infection. Clinical and in vitro observations showed that monocytes and splenic macrophages are the principal direct targets of DENV [10, 11]. Infected monocytes in turn produce high levels of several cytokines with chemoattractant activity which are known to activate and degranulate neutrophils [1, 6]. Neutropenia, an established clinical feature of DENV infection could be attributed to degranulation of neutrophils and this in turn would reflect an elevated elastase activity in dengue patients than in

normal. To obtain an insight into the levels of neutrophil elastase with the severity of dengue infection, the dengue patients were divided into DF and DHF groups. The data showed significantly higher plasma elastase activity in DHF patients suggestive of more destructive sequela in DHF patients (Fig. 1). However, a larger DHF patient group would be necessary to statistically associate the same. The finding of elevated elastase activity in dengue patients is in accordance with the study conducted by Juffrie et al. [1].

Though an increased levels of antiproteases (α_1 -AT and α_2 -MG) were expected in the patient group to counteract the elevated elastase activity as natural defense mechanism, the levels of the inhibitors were significantly decreased in dengue cases. It is known that the patients suffering from dengue exhibit an increase in oxidative stress and generate free radicals [12, 13]. Free radicals have been shown to destroy α_1 -AT and α_2 -MG and thus a possible explanation for observation of decreased levels of these inhibitors in this study may be due to the destruction of these molecules by free radicals.

Alpha₁-proteinase inhibitor binds with elastase at a 1:1 molar ratio and forms an 80KD elastase- α_1 -AT complex. The formation of complex reflects a rapid response of the organism to infection [14]. We have observed no increase in the levels of complex in dengue group consequent to the increased elastase activity. This observation indicates normal receptor-mediated clearance of complex from the plasma in DENV infection.

Conclusion

An increased elastase activity and decreased levels of elastase inhibitors in dengue patients suggest their role in the inflammatory processes and its progression in dengue infection. The present study also indicates an elevated elastase activity in dengue hemorrhagic fever in comparison to dengue fever suggesting that it could be a possible marker for assessment of severity of dengue fever.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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