Publications
Plasma Neutrophil Elastase, α1-Antitrypsin, α2-Macroglobulin and Neutrophil Elastase-α1-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 α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-MG) and elastase in complex with α1-AT (NE-α1-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, α1-AT, α2-MG and NE-α1-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 · α1-Antitrypsin · α2-Macroglobulin · NE-α1-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 repel 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 chemotaxic 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):
alpha1-antitrypsin ($\alpha_1$-AT) and alpha2-macroglobulin ($\alpha_2$-MG) and elastase in complex with $\alpha_1$-AT (NE-$\alpha_1$-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 hemostatic studies), Sodium Heparin (for elastase, $\alpha_1$-AT, $\alpha_2$-MG and NE-$\alpha_1$-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 $-70^\circ$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-L-p-nitroanilide (STANA, SIGMA) as substrate at 410 nm as per the procedure described by Beith et al. [9]. Plasma $\alpha_1$-AT and $\alpha_2$-MG were analyzed using Enzyme Linked Immunosorbent Assay, Immunology Consultants Laboratory, Inc. USA. NE-$\alpha_1$-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 ± SD. All variables were checked for normal distribution by Shapiro-Wilk test. Differences between groups were analyzed using Student’s unpaired i 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 hemostatic parameters between control and patient group. Significant decrease in platelet count (272.23 ± 61.24–71.94 ± 39.17 x 10^6/l, $p < 0.001$), total WBC count (7.04 ± 1.02–4.38 ± 1.81 x 10^3/l, $p < 0.001$), neutrophils (51.27 ± 7.37–40.68 ± 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 ± 6.09, $p < 0.001$).

Plasma levels of elastase activity, $\alpha_2$-AT, $\alpha_2$-MG and NE-$\alpha_1$-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 $\alpha_1$-AT and $\alpha_2$-MG were significantly lower in dengue patients as compared to healthy control group ($p < 0.001$). When plasma NE-$\alpha_1$-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 $\alpha_1$-AT, $\alpha_2$-MG and NE-$\alpha_1$-AT complex.
Table 1: Basic characteristics of study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 52)</th>
<th>Cases (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.48 ± 6.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.50 ± 4.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.986</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>32/20 (61.5%/38.5%)</td>
<td>30/20 (60%/40%)</td>
<td>-</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>42.27 ± 3.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.01 ± 5.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.407</td>
</tr>
<tr>
<td>PL count (10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td>272.23 ± 61.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.94 ± 39.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Total WBCs (10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td>7.04 ± 1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.38 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>51.27 ± 7.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.68 ± 12.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>36.86 ± 6.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.99 ± 9.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>5.01 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.24 ± 6.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

<sup>a</sup> Student’s unpaired t test and <sup>b</sup> Mann-Whitney U test were used. Values are expressed as mean ± SD.

Table 2: Comparison of plasma NE activity, α<sub>1</sub>-AT, α<sub>2</sub>-MG and NE-α<sub>2</sub>-AT complex between cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 52)</th>
<th>Cases (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastase activity (Units/ml)</td>
<td>0.35 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-AT (mg/dl)</td>
<td>123.35 ± 25.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166.25 ± 7.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt;-MG (mg/dl)</td>
<td>209.42 ± 31.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.75 ± 24.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NE-α&lt;sub&gt;2&lt;/sub&gt;-AT complex (ng/ml)</td>
<td>214.89 ± 13.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>238.38 ± 62.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.068</td>
</tr>
</tbody>
</table>

<sup>a</sup> Student’s unpaired t test and <sup>b</sup> Mann-Whitney U test were used. Values are expressed as mean ± SD.

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 chemotactic 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 sequelae 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 Jaffé et al. [1].

Though an increased levels of antiproteases (α<sub>1</sub>-AT and α<sub>2</sub>-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 has been shown to destroy α<sub>1</sub>-AT and α<sub>2</sub>-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.

Alpha<sub>1</sub>-proteinase inhibitor binds with elastase at a 1:1 molar ratio and forms an 80KD elastase-α<sub>1</sub>-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.

References
Correlation of Plasma Neutrophil Elastase Activity and Endogenous Protease Inhibitor Levels with the Severity of Pre-eclampsia

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ABSTRACT

Introduction: Pre-eclampsia (PE) is a common maternal syndrome characterized by severe systemic inflammatory response including neutrophil activation leading to uncontrolled activity of elastase. The excessive activity of elastase would lead to destruction of the integrity of endothelial cells and could exacerbate the pathophysiological symptoms in PE. Thus, assessment of NE activity and its control mechanisms would be of relevance in the determination of severity of PE.

Aim: To correlate the activity of plasma NE and its endogenous inhibitors \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-AT) and \( \alpha_1 \)-macroglobulin (\( \alpha_1 \)-MG) with severity of PE.

Materials and Methods: A comparative study was conducted between normotensive pregnant (n=60) and pre-eclamptic (n=50) women. Serum C-Reactive Protein (CRP) was estimated by rapid latex slide and uric acid by uricase method. Plasma elastase was estimated using succinyl tril-L-lysine-p-nitroanilide as substrate. Plasma \( \alpha_1 \)-AT, \( \alpha_1 \)-MG and NE- \( \alpha_1 \)-AT complex were quantified by ELISA. ANOVA and Pearson’s correlation tests were used to analyze the data. The results were expressed as mean±SD and p-value <0.001 was considered statistically highly significant.

Results: The activity of elastase was increased significantly in severe PE (0.62±0.08) in comparison to normal (0.35±0.10) and mild pre-eclamptic subjects (0.37±0.03). The values of \( \alpha_1 \)-AT were significantly less in mild (83.4±25.08) and severe PE (69.94±20.39) in comparison to normal (110.26±42.39). There was a significant rise in the levels of \( \alpha_1 \)-MG in severe PE. However, the complex estimation did not evince any significant changes.

Conclusion: The results of the present study indicate a significantly elevated elastase activity, \( \alpha_1 \)-MG levels and decreased \( \alpha_1 \)-AT in severe PE patients. The correlation analyses of PE severity parameters with NE, \( \alpha_1 \)-AT and \( \alpha_1 \)-MG further support the roles of these molecules in the assessment of severity of PE.

Keywords: \( \alpha_1 \)-antitrypsin, \( \alpha_1 \)-macroglobulin, NE- \( \alpha_1 \)-AT complex

INTRODUCTION

PE is a major cause of maternal and neonatal morbidity and mortality. It is a multisystem disorder which is characterized by vasoconstriction [1], leukocyte activation [2], enhanced inflammatory response [3] and oxidative stress [4]. The causes for the development of PE are still unclear and are a topic of active investigation. The pathological lesions of decidua vessels in PE have similarity to atherothrombotic lesions of arteries [5]. Neutrophils have been implicated in the pathogenesis of atherothrombotic changes and endothelial dysfunction through release of variety of substances. Elastase is one of such molecules released from neutrophils and is an established marker for neutrophil activation [6–8]. Neutrophil Elastase (NE), a serine protease stored in the primary granules of neutrophils, is capable of degrading various extracellular matrix proteins such as elastin, collagen, fibrinogen and proteoglycans [9]. Therefore, it can cause vascular basement membrane damage and can facilitate tissue infiltration of neutrophils. Activation of neutrophils is implicated in PE and consequently contributes to vascular basement membrane damage leading to edema and proteinuria [10], a usual observation in PE. A positive correlation has been demonstrated between von Willebrand Factor (a marker of endothelial damage) and NE by Green IA et al., indicating that neutrophil activation could contribute to endothelial damage and dysfunction in PE [11].

Thus, uncontrolled neutrophil activation can lead to destruction of the integrity of endothelial cells and could exacerbate the pathophysiological symptoms in PE. It is well established that PE is manifested as mild, moderate and severe forms in pregnant women but it is unclear what exaggerates the symptoms and the severity.

This study is an attempt in this direction to correlate the activity of neutrophil elastase and its endogenous inhibitors \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-AT) and \( \alpha_1 \)-macroglobulin (\( \alpha_1 \)-MG) with severity of PE.

MATERIALS AND METHODS

The present study is a comparative study conducted during the period of October 2016 to April 2016. The subjects of this study were the pregnant women attending or admitted in the Department of Obstetrics and Gynecology, PL. Jhalawar Hospital and Research Center, the teaching hospital of Sri Dwaraj Urs Medical College (SDUMC) and the Biochemical evaluation was carried out in the Department of Biochemistry of SDUMC, a constituent college of Sri Dwaraj Urs Academy of Higher Education and Research, Kolli, Karnataka, India. Every enrolled pregnant woman gave their informed written consent to participate in the study. This study was performed after obtaining Institutional Ethical Committee approval and the study complied with the Helsinki Declaration.

A total of 50 pregnant normotensive women and 50 pre-eclamptic pregnant women (27 mild and 23 severe cases), were included in the study. All the women were in the age group of 19-36 years and were over 20 weeks of gestation. Normal pregnancy was diagnosed on the basis of clinical and ultrasound evaluation and all of them presented a normal course and outcome of pregnancy. The pre-eclamptic patients were diagnosed by the presence of hypertension (≥140 mm Hg systolic BP and ≥90 mm Hg diastolic BP) on two occasions
with 4-6 hours apart, proteinuria (≥ 1+ by urine dipstick method) with or without pathological oedema. PE was considered as severe, if the subjects had at least two of the following: ≥ 10 mmHg diastolic BP; ≥ 10 mmHg systolic BP; dipstick proteinuria of 3+ or more. All the other cases were considered as mild PE. All patients with any infection, twins, history of pregestational diabetes, gestational diabetes mellitus, renal disease, liver disease, cardiovascular disease and hypertension were excluded from the study.

Almost 6 ml of blood was collected from an antecubital vein from all the subjects in tubes containing EDTA (for haematologic studies); Sodium heparin (for NE, α1-AT, α1-MG and NE, α1-AT, ASc,complex estimation) and in tubes without anticoagulant (for CRP estimation). Blood samples were centrifuged within two hours of collection. After centrifugation, serum and plasma were separated and aliquots were stored at −70°C until assayed. Samples were thawed at room temperature, vortexed and centrifuged before analysis.

Complete blood count was performed by Beckman-Coulter, an automatic blood cell counter. Serum C - Reactive Protein (CRP) estimation was done by rapid latex slide tests. Serum uric acid was estimated by uricase method [12] using Dry Chemistry Vitros 250 Johnson and Johnson analyzer. Estimation of plasma elastase was done using succinyl-thi-L-3,5-dinitrobenzoic acid (STANA, from SIGMA) as substrate at 410 nm as per the procedure described by Schiff et al. [13]. Plasma α1-AT and α1-MG were analyzed using ELISA kit purchased from Immunology Consultants Laboratory, Inc., USA. NE, α1-AT complex was quantified by ELISA (CibaCorporation).

**STATISTICAL ANALYSIS**

The data were statistically analyzed by SPSS software version 22.0. The results were expressed as mean±SD. For statistical differences in means between the groups ANOVA (Analysis of variance) was used. Pearson’s correlation coefficient test was used to analyze the correlation of severity parameters (BP and proteinuria) with elastase and its inhibitors. A p-value <0.001 was considered highly significant.

**RESULTS**

The baseline physical and biochemical characteristics of the normal, mild and severe pre-eclamptic subjects are depicted in [Table/Fig-1]. The gestational age was in the range of 34 to 37 weeks for normal, and 31 to 36 weeks for mild to severe PE subjects. The blood pressure was elevated significantly in the case of mild and severe cases of PE in comparison to normal. The blood pressure was also significantly higher in severe PE compared to mild PE.

The data on proteinuria was suggestive of PE as per the criteria defined. Serum uric acid showed significant rise in PE group (mild 7.53±1.36; severe 8.16±1.57) compared to controls (4.83±1.30). When serum CRP was compared, mild (12.44±1.40) and severe (14.26±13.98) pre-eclamptic women presented significantly higher CRP levels as compared to normo-tensive pregnant women.

The data on NE, α1-AT, α1-MG and NE-α1-AT complex are presented in [Table/Fig-2]. The activity of neutrophil elastase was increased two fold in severe PE (0.62±0.08) in comparison to normal (0.35±0.10) and mild pre-eclamptic subjects (0.37±0.03) and was statistically highly significant. The values of α1-AT have been on the decline and were statistically less in mild and severe PE in comparison to normal; a 60% reduction in severe and 40% reduction in mild. There was a significant rise in the levels of α1-MG in severe pre-eclamptic women. However, the complex estimation did not evince any significant changes indicating normal balance and did not contribute to analytic value. Significant association between elastase activity and disease severity is depicted in [Table/Fig-3].

Correlation studies of the severity parameter; proteinuria with the levels of NE and α1-AT indicated a positive and negative picture respectively in severe form of PE [Table/Fig-4]. On the other hand,

![Graph showing statistical analysis](image)

the correlation analysis of α1-MG with proteinuria presented a negative correlation in mild and a positive correlation in severe group. However, complex correlation with the severity parameters did not present any definite correlations.

**DISCUSSION**

PE exhibits characteristics of an inflammatory disease including neutrophil activation [2,14-15]. The activation of neutrophil in PE may be due to some pro-inflammatory cytokines and chemokines released during an inflammatory responses (e.g., TNF-α, IL-6 and IL-8) [16]. Elastase activity is measured as marker of neutrophil activation in several inflammatory conditions including PE [7,8,14,15]. The complications induced by PE state are detrimental to both the mother and the foetus and have been a serious subject of investigation. Research often focuses on the changes in the biochemical parameters with no data on its onset and progress.
In the present study we have measured and compared the plasma levels of NE, α1i-AT, α1i-MG and NE-α1i-AT complex in patients with severe PE compared to normal pregnant women. In this study, the analysis of NE activity indicated a significant increase in the severe PE patients which is in agreement with previous studies (11,14). It could contribute to progressive inflammation. α1i-antitrypsin inhibits several matrix metalloproteinases (mainly NE) and thus, adequate activity of this inhibitor is critical for the maintenance of protease-antiprotease homeostasis and the prevention against proteolytic tissue damage (15). Determination of the plasma α1i-AT levels demonstrated the available level of the inhibitor capable of inhibiting intravascular proteases. Contrary to expectation of an increased level of α1i-AT in inflammation, a decreased level was observed in the study groups compared to the normal. It was highly significant in ways to overcompensation rate of elastase in the complications of PE.

It is also pertinent to note that, there was no increase in the levels of NE-α1i-AT complex in PE group consequent to the increased elastase activity. This observation could be indication of decreased synthesis of α1i-AT rather than its involvement in complex formation to control elastase activity. The reason for decreased α1i-AT is an area of concern and is suggestive that supplementation of α1i-AT would be able to minimize the destructive effects of NE on vascular tissues.

We have observed a significantly higher α1i-MG level in severe PE patients compared to normal or mild PE patients against an expected reduced α1i-MG concentration in severe PE as it is suggested to bind to elastase and get rapidly cleared from the plasma through macrophage receptors (16). Raised levels observed in this study could be attributed to renal insufficiency, a common feature in PE patients. Moreover, increased levels of this inhibitor in severe PE possibly contribute to the intravascular coagulation as α1i-MG has antithrombin activity (19) adding further to severe complications. Home CHW et al. also noted raised α1i-MG levels in PE patients compared to normal pregnant women (16). In order to understand the relation of PE severity parameters with the levels of NE, α1i-AT, α1i-MG and NE-α1i-AT complex correlation analyses were carried out. Though we have correlated these molecules with both proteinase and BP, the most meaningful and relevant correlation was observed only with proteinase and not with BP. The observation of increased activity in the activity of NE in severe PE and its positive correlation with severity marker proteinase makes the measurement of NE a dependable parameter in the determination of severity of PE. Similar picture but in a negative direction was observed in case of α1i-AT suggesting that measurement of α1i-AT along with the activity of NE could strengthen the assessment of severity of PE.

As reported by earlier studies (20-22), a significant increase in the levels of serum uric acid and CRP in PE compared to controls was observed and points to the pronounced inflammation in these patients. However, the levels did not yield any information on the severity of the PE.

LIMITATION

Since PE is a progressive disease, a follow up of mild pre-eclamptic women to assess the progression of these women to severe form would have been the better study design to emphatically conclude the role of elastase enzyme and its inhibitors in severity of PE. Moreover, urinary estimation of α1i-AT would have explained the reduced levels of α1i-AT in PE group. We do agree a larger sample size definitely would have supported the results obtained.

CONCLUSION

The present study clearly indicates an association between increased levels of NE and decreased α1i-AT with the severity of PE suggesting that both would be relevant markers for assessment of severity. Monitoring the plasma levels of these molecules thus could be of use for evaluation of the status of PE.

REFERENCES


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