Quantitative Profiling of Plasma Protein Fractions in Preeclamptic and Normotensive Pregnant Women

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ABSTRACT

Present study was aimed to investigate electrophoretically separated plasma protein profile of preeclamptic (n=30) and normotensive pregnant women (n=30). The plasma proteins were determined through SDS-PAGE in distinct bands. The protein fractions were quantified using Total Lab Quant software (version 1.0.8) and band percentages of the fractions were calculated and analyzed statistically by Student t-test using Graph Pad Prism (version 5.0). Twelve protein fractions were detected ranging from 250-15 kilodalton (kDa). Significant elevation (p=0.0001) was observed in 250, 173, 131 and 114kDa protein fractions whereas, significant reduction (p=0.0001) in 76 and 66kDa protein fractions was observed when compared in preeclamptic and normotensive pregnant women. However, no significant variations were observed in 90, 59, 51, 25, 18 and 15kDa protein fractions in both the groups. Plasma protein profile, suggests that these six protein fractions have potent linkages to the biology of preeclampsia.

Key words: Preeclampsia; Electrophoresis; Proteins; SDS-PAGE

INTRODUCTION

Preeclampsia (PE) is a relatively common hypertensive disorder of pregnancy that occurs on or after the 20th week of gestation in previously healthy women and is defined as the occurrence of hypertension and significant proteinuria (Eiland et al., 2012). While the etiology is unclear, the placenta is the origin of PE and it releases factors into maternal circulation to induce systemic endothelial dysfunction (Wong & Cox, 2014).

Researchers have discovered that PE is a multi-systemic disorder with complex pathophysiological changes, such as inflammatory response, endothelial dysfunction, activated coagulation system and metabolic changes (Roberts & Lain, 2002). Babies born to preeclamptic mothers are also affected; one third are born preterm, 20% are growth restricted, and evidence indicates that there may be an increase of three- to ten-fold in perinatal deaths (Sibai, 2005; Ferrazzani, et al., 2011).

Fifty one proteins have been identified that are differentially expressed between severe PE women and healthy pregnant women during the third trimester (Liu et al., 2011). HDL associated proteins, SERPINF1, alpha-2-HS-glycoprotein, transthyretin and Retinol binding protein 4, have also been identified and differentially expressed between PE and normal pregnant women (Potter & Nestel, 1979). Fibronectin was found to be up-regulated in the sera of pregnant women complicated with PE, which is known as important biomarker for the evaluation of endothelial damage in PE (Shaarawy & Didy, 1996). In pregnancy with PE, the free iron and ceruloplasmin concentrations were statistically significantly high as compared to normal pregnant ones (Hameed and Wasan, 2013). Serum C-reactive protein (CRP) levels in mild and severe PE were distinctly higher than those of normal pregnant women in third trimester of pregnancy. Albumin was found to be lower in women with severe PE (Benoit & Rey, 2011). When PE is accompanied by proteinuria there is a remarkable fall in albumin and an increase in alpha (2) macroglobulin level (Horne et al., 1970). Studies have shown that the expression levels of these proteins are increased or decreased according to the disorder and its degree in PE (Baumann et al., 2007).

Currently preeclampsia is diagnosed from clinical observations that occur late in the disease process. Unknown factors possibly released by the preeclamptic placenta into the maternal circulation have been associated with the pathophysiological changes that are characteristic of the disorder. It is generally believed that the pathophysiological changes occurring in preeclampsia may result from the abnormal expression of some proteins.

Aim of the present study was to assess the effects of PE on plasma protein profile of preeclamptic women and comparing with those of normotensive pregnant women by comparing SDS-PAGE technique. For this purpose, high and low molecular weight protein profiles were brought under observation to relate the fluctuations in
different protein fractions with various health risks in preeclamptic women.

**MATERIALS AND METHODS**

Blood samples (5 ml) of preeclamptic patients (n=30) were collected from Jinnah and General Hospitals of city and age matched healthy normotensive pregnant females from local population. The average age of subjects was 18-40 years and they were between 29–40 weeks of gestation. Written informed consent was taken from each subject prior to the study. Each woman was interviewed (history-taking) and examined for the signs and symptoms of preeclampsia.

Polyacrylamide gel of 12 % and 6% were prepared, for analysis of low and high molecular weight protein fractions, respectively. Protein concentrations were determined by Bradford Assay (Bradford, 1976). Plasma samples were diluted by distilled water and loading dye and then proteins were denatured by heating for two minutes in the boiling water bath before loading onto the gel. Protein marker and equal concentration of each of the plasma samples were loaded in separate wells. The gel was electrophoresed at a current supply of 30 mA and 200 V, in a cooling chamber maintained at 4°C until the dye reached the lower end of the gel. After overnight fixation, gel was stained with coomassie blue for 1 hour with constant agitation and destained afterwards until clear background appeared and protein fractions became visible in the forms of blue colored bands.

Stained gels were scanned for quantification. Total Lab Quant was used for the quantification of separated protein fractions. It provided the data of molecular weight and density covered by each fraction. The band percentage shown by each protein fraction was recorded. The data were analyzed using Student t-test and employed in finding the augmentation or decrease and appearance or disappearance of particular protein fractions for comparison between the control and preeclamptic subjects.

**RESULTS**

Variations in the plasma protein fractions of preeclamptic patients were considerable when compared to control subjects. An overall comparison of plasma protein profiles of both control and patient groups indicated the expression of twelve protein fractions ranging between 250-15kDa (Table 1). Protein fractions of 250, 173, 131 and 114kDa showed significant increase (p=0.0001), whereas 76 and 66kDa protein fractions indicated a significant decrease (p=0.0001) in preeclamptic patients compared to control subjects. However, no significant variations were observed in 90, 59, 51, 25, 18 and 15kDa protein fractions in both the groups (Table I, Fig. 1 and 2).

**Fig. 1:** Photographs showing electrophoretically resolved low molecular weight plasma protein fractions in distinct bands in control subjects (C) and preeclamptic group (PE). M= Protein markers with known molecular weight in kilodaltons.

**Fig. 2:** Photograph showing electrophoretically resolved high molecular weight plasma protein fractions in distinct bands in control subjects (C) and preeclamptic group (PE). M= Protein markers with known molecular weight in kilodaltons.
Table I: Average percent raw volumes exhibited by different protein fractions in normotensive and preeclamptic patients. Values are Mean ± SEM.

<table>
<thead>
<tr>
<th>Molecular weight of proteins (kDa)</th>
<th>Average band percentage of protein fractions</th>
<th>Percentage difference of protein fractions in Preeclamptic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Group</td>
<td>Preeclamptic Group</td>
</tr>
<tr>
<td>250</td>
<td>3.34 ± 0.13</td>
<td>5.13 ± 0.21</td>
</tr>
<tr>
<td>173</td>
<td>7.15 ± 0.19</td>
<td>13.09 ± 0.55</td>
</tr>
<tr>
<td>131</td>
<td>5.01 ± 0.08</td>
<td>6.65 ± 0.27</td>
</tr>
<tr>
<td>114</td>
<td>3.73 ± 0.12</td>
<td>5.89 ± 0.18</td>
</tr>
<tr>
<td>90</td>
<td>3.88 ± 0.10</td>
<td>3.61 ± 0.12</td>
</tr>
<tr>
<td>76</td>
<td>20.65 ± 0.69</td>
<td>13.72 ± 0.57</td>
</tr>
<tr>
<td>66</td>
<td>35.97 ± 0.29</td>
<td>26.73 ± 0.87</td>
</tr>
<tr>
<td>59</td>
<td>7.56 ± 0.26</td>
<td>8.46 ± 0.39</td>
</tr>
<tr>
<td>51</td>
<td>7.26 ± 0.27</td>
<td>7.79 ± 0.38</td>
</tr>
<tr>
<td>25</td>
<td>15.22 ± 0.41</td>
<td>14.56 ± 0.34</td>
</tr>
<tr>
<td>18</td>
<td>1.94 ± 0.12</td>
<td>2.05 ± 0.14</td>
</tr>
<tr>
<td>15</td>
<td>1.31 ± 0.09</td>
<td>1.25 ± 0.11</td>
</tr>
</tbody>
</table>

↓ Decrease, ↑ Increase, *** Significant at p<0.001 SEM: standard error of estimation

**DISCUSSION**

In present investigation, the protein profile of normotensive control and preeclamptic patients was studied by SDS-PAGE. The protein fractions ranged between 250-15kDa. The various protein fractions observed included 250 kDa fibronectin (Yang et al., 2009), 173 kDa α-2-macroglobulin (Kim et al., 2013), 131 kDa ceruloplasmin (Castellani et al., 1999), 114 kDa C-reactive protein (Jinbo et al., 1998), 76 kDa transferrin (Jiang et al., 1998) and 66 kDa albumin (Allen et al., 2006). Significant elevation in 250, 173, 131 and 114kDa protein fractions was observed. Whereas, in 76 and 66kDa protein fractions, significantly reduced expression was observed.

Protein fraction of 250kDa reported as fibronectin was found to be increased by 53.39% in preeclamptic patients as compared to controls. Fibronectin plasma levels increase after major trauma resulting in vascular tissue damage, after inflammation, and in diseases such as ischaemic heart disease, stroke and atherosclerosis, (Peters et al., 2003; Claudepierre et al., 1999). Liu et al. (2011) reported that up-regulation of fibronectin is known as an important biomarker for the estimation of endothelial damage in preeclampsia (Shaarawy & Didy, 1996). Fibronectin up-regulation also indicates atherogenesis of the arteriole.

Protein fraction of 173kDa reported as α-2-macroglobulin was found to be increased by 83.13% in preeclamptic patients as compared to controls. α-2-macroglobulin (A2M) is a protease inhibitor in mammals (Armstrong, 2006). When preeclampsia is accompanied by proteinuria then there is a distinct decrease in albumin and an increase in α2-macroglobulin (Horne et al., 1970).

Protein fraction of 131kDa reported as ceruloplasmin, was found to be increased by 32.80% in preeclamptic patients as compared to controls. Ceruloplasmin, a glycoprotein in human plasma, has shown significant elevation in mild and severe PE group as compared to control group (Shamsi et al., 2010). Ceruloplasmin is an antioxidant due to its acute phase reactant property, whose elevated concentration is observed in infection, inflammation, trauma, etc (Vassiliev et al., 2005). Aksoy et al. (2003) also found that the plasma antioxidant potential was reduced and ceruloplasmin level was increased compared to normal pregnant women. Ceruloplasmin has central roles in iron metabolism and antioxidant defense (Samokyszyn et al., 1989). Ceruloplasmin blocks the production of toxic oxygen compounds and protects cells from oxidative stress (Healy & Tipton, 2007).

Protein fraction of 114kDa was found to be increased by 57.94% in preeclamptic patients as
compared to normotensive control. C-reactive protein, a marker of tissue damage and inflammation, plays an important role in eliciting the inflammatory response that is characteristics of preeclampsia (Ustun et al., 2005). Kumru et al. (2006) observed a negative correlation between serum CRP and length and weight of the newborns in the PE group compared with the control group. CRP level is increased during inflammatory response to tissue injury or infection (Braekke et al., 2005). Erren et al. (1999) had reported that inflammatory profile was more definite when the endothelial damage was more advanced. It is well known that renal dysfunction usually occurs in PE, especially in its severe forms. In cases of renal dysfunction, endothelial dysfunction and increased levels of inflammatory markers such as CRP have been more obvious.

Protein fraction of 76 kDa in our studies was found to be lowered in preeclamptic subjects by 33.55% as compared to control. Transferrin is an iron-binding blood plasma glycoprotein that controls the level of free iron in biological fluids. In physiological and pathological conditions, important alterations of plasma serotransferrin concentration are observed (Inoue et al., 1993). Transferrin levels of the severe and mild preeclampsia groups were found to be reduced as compared to controls (Aksoy et al., 2003). A decreased plasma transferrin can occur in iron overload diseases and protein malnutrition.

The 66 kDa protein fraction was found to be declined by 25.68% in our study. In preeclampsia, hypoalbuminemia secondary to hypovolemia is caused by reduced hepatic blood flow. Thus, hypoalbuminemia can be recognized as an early mark in developing preeclampsia. Serum albumin levels may act as an indicator of the severity of preeclampsia (Gojnic et al., 2004). If serum albumin level is below 2.5 g/dL, the risks of ascites, perinatal mortality and hemolysis elevated liver enzymes low platelet (HELLP) syndrome are increased markedly (Seong et al., 2010). Most of the hypoalbuminemia cases are caused by acute and chronic inflammatory responses. When plasma proteins, especially albumin, no longer retain abundant colloid osmotic pressure to balance hydrostatic pressure, edema develops. Serum albumin level serves as an important predictive indicator and decreased serum albumin levels associate with an increased risk of morbidity and mortality. Moreover, decreased albumin level is frequently obvious in patients with cancer, liver ailments and sepsis (Ballmer et al., 1993; Pasanisi et al., 2001; Ruot et al., 2000).

Conclusively, up-regulation and down-regulation of different protein fractions are attributed to risk of different diseases. Such as the up-regulation of 250kDa indicates risk factor for atherogenesis of the arteriole, which results in hypoxia and ischemia in preeclampsia. Moreover, the up-regulation of protein fraction of 173kDa is the marker for nephrotic syndrome. The up-regulation of 131kDa which is a marker of allergic asthma. The up-regulation of 114kDa, in present study, is correlated positively and significantly with diastolic blood pressure and proteinuria. Maternal CRP values also correlate negatively and significantly with fetal weight at birth. The down-regulation of 76kDa protein fraction is a risk factor for bladder cancer, while the depletion of 66kDa is a marker of liver and cardiac ailments. These Six candidate protein fractions have potent linkages to the biology of preeclampsia. However, given the low power of the present 1D study, a follow-up proteomic study was conducted using the 2D technique. 2D will give us more comprehensive understanding about preeclampsia.

**REFERENCES**


