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Assessment of Protein Oxidation Status and Its Correlation with Antioxidant Potential in Preeclampsia

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Abstract

Objective: The objective of the study is to assess protein oxidation status and its correlation with antioxidant potential in serum during preeclampsia (PE). **Materials and Methods:** A casecontrol study was performed on 63 pregnant subjects (mean age = 30 tential in serpreeclamptic and 32 age-matched normotensive (control) pregnant women. Serum samples were analyzed for total protein, globulin, albumin, protein carbonyls (PCO), advanced oxidation protein products (AOPP), and ferric reducing antioxidant potential. **Results:** Compared to the control pregnant subjects, a compromised serum protein pattern, elevated PCO ($P < 0.01$), and AOPP ($P < 0.02$) were observed in PE pregnant women that were correlated significantly with the total antioxidant potential of serum. **Conclusion:** The results of the study suggest that PE pregnancies are susceptible to protein oxidation which may be due to diminished antioxidant potential during PE. The study concludes that oxidative modulation of proteins may be one of the major causative factors in complicating the maternal and fetal conditions during PE.

Keywords: Biomarkers, Oxidative stress, Preeclampsia, Protein oxidation

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Introduction

Preeclampsia (PE) is a pregnancy-associated hypertensive disorder that adversely affects maternal and fetal health and pregnancy outcome. Hypertensive disorders of pregnancy are responsible for 7–8% of maternal mortality and PE is one of the most common hypertensive disorders of pregnancy and is reported to complicate 2–8% of pregnancies worldwide.[1] Although the etiology of the PE remains obscure, poor placentation is considered a vital predisposing factor. The poorly perfused placenta may elicit the generation of reactive oxygen species (ROS) leading to the prevalence of oxidative stress.[2-4]

Proteins are the major target of the oxidative stressors, their oxidation results in alterations in their structure followed by functions.[5-8] Oxidative damage to proteins precedes the oxidative modifications of lipids and may contribute to severe micro and macrovascular complications. The present study reports the extent of oxidative burden in PE pregnancies measured in terms of authentic and stable biomarkers of protein oxidation. We also correlate the oxidation of proteins with the total serum antioxidant potential in PE patients.

Materials and Methods

Collection of Sample

The casecontrol study was conducted on 63 pregnant women with a mean age of 30 pregnancies measured in terms of authentic and stable biomarkers of protein oxidation. We also correlaJeevan Jyoti Hospital, Prayagraj, India. The study was performed after approval of institutional Ethics Committee of SHUATS, Prayagraj, India (Reg no: IEC/SHUATS/2019/D/01). All the participants were informed about the study procedure and had provided their consent for the study. PE ($n = 31$) was determined by proteinuria conc. 100 mg/dL or more in 4 h apart urine samples or r1+ in dipstick urinalysis accompanied by at least 140/90 mm Hg blood pressure (BP) on two different occasions at least 6 h apart. Mean systolic and diastolic blood pressures were 151.3 ± 3 mmHg; 100.0 ± 1.82 mmHg and 111.2 ± 4.94 mmHg; 70.83 ± 3.41 mmHg respectively in select PE and normal pregnancies.

Age, gravida, parity, and ethnicity matched controls ($n = 32$) who had normal BP, no proteinuria and did not show any history of glycemic imbalance, hyperthyroidism, diabetes, and other serious pathologies were selected and categorized as normal pregnant (NP). Blood samples were obtained by venipuncture and divided into EDTA, fluoride, and plain vacutainers. All the samples were centrifuged for 10 min at 3000 30gmin and tests were performed immediately.

Measurement of Total Protein, Albumin, and Globulin Content

Total protein, albumin, and globulin levels were measured to evaluate their concentration in serum samples. All measurements were performed on biochemical analyzer Erba EM200 (TRANSASIA BIOMEDICAL LTD, ERBA MANHEIM, GERMANY) using TRANSASIA commercial kits.

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Estimation of Advanced Oxidation Protein Products (AOPP)

Serum AOPP was determined according to the procedure of Witko-Sarsat *et al*., 1996.[9] 2.0 mL of serum sample in phosphate-buffered saline (PBS)) as test, chloramines-T solution (0–100 µmol/L) for calibration and PBS as blank were applied.10 µL of 1.16 M potassium iodide and 20 µL of acetic acid were added and absorbance was measured at 340 nm. Concentration of AOPP was expressed as µmol/L of chloramine-T equivalents.

Determination of Protein Carbonyls (PCO)

PCO in serum were determined as described by Levine *et al.* 1990.[10] Serum samples were divided into test and control. Briefly, 4.0 mL of 10 mM 2, 4-dinitrophenylhydraziine in 2 M HCl was added to the test sample and 4.0 mL of 2 M HCl was added to the control. The contents were incubated in the dark for 1 h at 37 37etrophenylhydraziineacid were added and abs% TCA was added to both the tubes and the mixture was left on ice for 10 min followed by centrifugation at 3,500 rpm for 20 min. The supernatant was aspirated and discarded. The protein pellets obtained were washed 3 times with ethanol: Ethyl acetate (1:1; v/v) solution and were dissolved in 6 M guanidine hydrochloride. The tubes were again subjected to centrifugation for the removal of insoluble material. PCO in supernatant was measured at 370 nm by reading the samples against control and calculated using an absorption coefficient (e) of 22,000 M^{-1} cm^{-1}. Data were expressed in nmol/L of serum.

Estimation of Antioxidant Potential

The antioxidant potential of serum was measured in terms of ferric reducing antioxidant potential (FRAP) values, as described by Benzie and Strain, 1996.[11] 2 mL of FRAP working reagent (10 volumes of 300 mM acetate buffer, pH 3.6, plus 1 volume of 2,4,6-tripirydyl-S-triazine in 40 mM Hcl, plus 1 volume of 20 mM $FeCl_3.6H_2O$) and 900 lume of 20 mM FeCleClIClmM FeClf 300 mM acetate buffer, pH 3.6, plus 1 volume otential (FRAP) values, as described mixture was measured at 593 nm. FRAP values are represented in µs d/L of serum.

Statistical Analysis

Students *t*-test was performed to determine significance between normal and and each diseased group using GraphPad Prism 5.01 for Windows (Graphpad Software Inc., San Diego, CA). The results are reported as mean ± standard error of mean (SEM). The difference in values were considered to be significant when $p < 0.05$.

Results

PE pregnant women showed elevated PCO and AOPP levels in comparison to the NP with significant levels ($P < 0.01$ and $P < 0.02$, respectively; Figures 1a and 2a). Elevation in PCO and AOPP levels was inversely correlated with the total serum antioxidant potential measured in terms of FRAP [Figures 1b and 2b]. Albumin, globulin, and total serum protein levels in PE group were reduced in comparison to NP, however, the reduction was insignificant ($P > 0.05$) [Table 1].

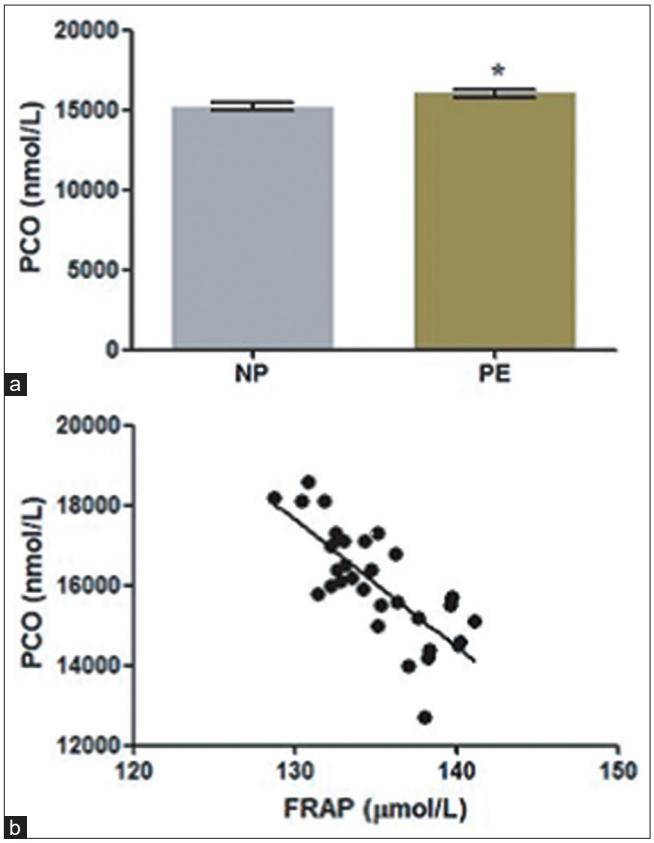

Figure 1: (a) Comparative protein carbonyls (PCO) content in normal normal pregnant (NP) and preeclamptic (PE) pregnancies; $p < 0.01$ in comparison to NP. Values are mean sed Renal and Hepatic Functions andb) PCO level plotted as a function of ferric reducing antioxidant potential values in PE patients; $P < 0.0001$, $r^2 = 0.58$

Discussion

The implementation of oxidative stress in PE has been duly reported, however oxidative damage to proteins has not been systematically characterized. Evidences suggest that the placental mitochondria are a major source of ROS in PE. ROS targets proteins and modifies amino acids: proline, histidine, arginine, and generates carbonyls moieties.[12,13] The carbonyls can be formed by amino acid side chains oxidation or by glycation and glycoxidation reactions. In fact, the generation of PCO derivatives can also occur through oxidative denaturation of proteins tthrough throudation pathway.[14] PCO are the authentic and potent biomarkers of oxidative protein damage because of their early formation relative stability. Elevated PCO is reported to be associated with several diseases.[15,16] In the present study, observed increased level of PCO in PE group compared to NP group is indicative of an enhanced protein oxidation in this pathological pregnancy. The results are in accordance with the other studies performed in different tissues in PE[17-20] and are corroborated by studies by Negi *et al*. and Bharadwaj *et al*., that have reported increased protein carbonylation in preeclamptic cases which is further exacerbated in eclamptic women when compared with normal pregnancy.[21,22]

Observed elevated AOPP in our study further corroborates our understanding of increased protein oxidative damage in preeclamptic maternal system as compared to uncomplicated pregnancies. AOPP are the final products of oxidant-mediated

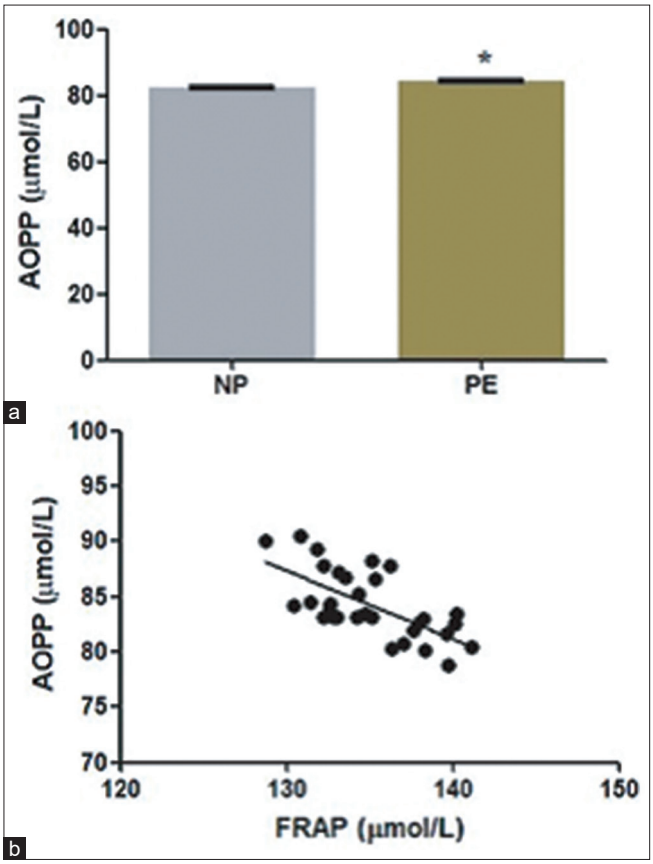

Figure 2: (a) Comparative advanced oxidative protein products (AOPP) levels in normal (NP) and preeclamptic (PE) pregnancies. $P < 0.02$ in comparison to NP. Values are mean son to NP. Values are mean cts (AOPb) AOPP level plotted as a function of ferric reducing antioxidant potential values in PE patients; $P < 0.0001$, $r^2 = 0.44$

Table 1: Comparative analysis of serum total protein, albumin, and globulin in normal and preeclamptic pregnancies. Data are represented as mean±SEM; *P* values shown are calculated between normal pregnancy and preeclampsia group

Parameters	*Normal Pregnancy*	*Preeclampsia*	*P-value*
Total protein (g/dL)	6.6±0.03	6.5±0.04	0.1
Albumin (g/dL)	3.9±0.03	3.8±0.03	0.2
Globulin (g/dL)	2.7±0.01	2.6±0.02	0.2

damage of various proteins and have been established as authentic biomarker of protein oxidation.[5,8] A study by D study *et al*. has demonstrated similar findings and reported an increased protein oxidation in terms of AOPP and ischemia modified albumin in PE and eclampsia. The study also reported further exacerbated AOPP levels in eclampsia which is a severe form of PE.[23]

Negative correlation of AOPP and PCO concentrations with serum antioxidant potential provide evidence that compromised protein status in PE may be due to elevated oxidative stress in maternal system which complicates the pregnancy. Our statement is well supported by the studies which have documented that PE afflicted pregnancies possess lesser antioxidant potential than uncomplicated pregnancies.[22,24] Furthermore, the study by Negi *et al*. also reported a significant inverse correlation between protein oxidation and total antioxidant status.[21] Increased free radical generation and decreased antioxidant capacity have been reported to be involved in endothelial dysfunction in PE, which is further implicated in the pathogenesis of the disease.[25]

Recent studies have reported misfolded proteins in the urine, plasma and placenta of the maternal PE[26,27] however the reason behind this misfolding is not well explained or unclear. We hypothesize that oxidative modification may be linked to the misfolding of urinary proteins since oxidative damage weakens protein stability and has been established as a risk factor for protein misfolding.[28,29]

Determination of protein fractions is of great assistance in providing a clearer picture and understanding of the disease processes. Decrease in serum albumin, globulin, and total protein in PE pregnancies coordinates with the proteinuria state in PE due to increased capillary permeability and endothelial damage and thus loss of proteins.[6] The results of the study add protein oxidation to the spectrum of abnormalities in PE pregnancy along with compromised protein pattern which may serve as indicative factor of PE.

Conclusion

Investigations of specific biomarkers are badly needed for early detection and timely management of PE in order to prevent the complications that it can cause during pregnancy and the outcome. Studied protein oxidation biomarkers may be some step toward said necessity. The study reports a compromised serum protein pattern in PE pregnancies.

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