Original Article

Elevated Serum Protein Carbonyl and Reduced Antioxidant Capacity in Women with Preeclampsia

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Abstract

Objectives: To determine the level of oxidative stress in preeclampsia by estimation of serum protein carbonyl content and antioxidants capacity in preeclampsia.

Methodology: This comparative cross-sectional study was conducted at dept. of Obs & District Teaching Hospital of D.I.Khan, KPK from March 2018 to January 2019. Forty clinically diagnosed preeclampsia women and 40 normal pregnant women aged between 27-35 years of the same gestational age were enrolled. The serum protein carbonyl content was determined through 2, 4-dinitrophenylhydrazone (DNPH) assay and serum antioxidant capacity was estimated through Ferric Reducing Ability of Plasma (FRAP) assay. Statistical analysis was performed through T- test/ T-statistics.

Results: Serum protein carbonyl content was significantly elevated ($p<0.0001$) in preeclamptic women as compared to normal pregnant women while serum antioxidant capacity (FRAP value) was significantly decreased ($p<0.0001$) in preeclampsia women as compared to the normal pregnant women.

Conclusion: Increased serum protein carbonyl content and reduced antioxidant capacity depict high levels of oxidative stress in women with preeclampsia that may play an important role in the initiation of endothelial dysfunction and expression of preeclampsia. Regular intake of adjuvant antioxidants supplementation can minimize the further progression of preeclampsia.

Keywords: Preeclampsia, Protein carbonyl, DNPH assay, Antioxidant capacity, FRAP Value

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Introduction

In the human body, there is a balance between the production of free radicals and antioxidant defense mechanism. When this balance disturbs, it may lead to the development of oxidative stress in the body.[1]

The antioxidants system of the body protects enzymes and proteins from oxidation by scavenging ROS, and helps to maintain cellular membrane integrity.[2] The certain stress full conditions usually occur in females during pregnancy, by different metabolic system derange which leads to the disturbance in the balance of anti-oxidative defense mechanism and leads to a condition called Preeclampsia. Preeclampsia is a disease with unknown etiology and is considered as a

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major cause of neonatal and maternal morbidity and mortality. Preeclampsia is a clinical condition characterized by hypertension (>140/90 mmHg), proteinuria (>300mg/day), and edema, with unknown etiology. But it is suggested that during pregnancies, basal oxygen consumption increased by several bodily organs which result in increases mitochondrial mass and reactive oxygen species (ROS) production that generate oxidative stress which leads to maternal vascular dysfunction.[3] The placenta also produces nitric oxide (NO) because it contains free radical producing macrophages.[4]

Initially, human placenta has a hypoxic environment, so it affects maternal homeostasis but when vascularization increases during placental development, an oxygen-rich environment evolved which favors the production of ROS.[5,6] In developed countries preeclampsia effects between 0.4% to 2.8% of all pregnancies, while in developing countries this number is many more and leading to per year 8,370, 000 cases worldwide. This disorder is more prevalent and associated with the highest maternal and fetal mortality and morbidity of all pregnancy intricacy with >90% of the most serious results facing in developing countries.[7] It exert immediate as well as long terms of harmful effects on the mother and the child's health. The immediate symptoms resolve after the placental removal, thus, PE is one of the most common reasons for induced preterm delivery.[8] The risk of preeclampsia markedly increases in women with previous preeclampsia, in either preexisting vascular disease or conditions associated with increased cardiovascular risk such as renal disease, hypertension, diabetes and obesity. The response of women body to oxidative stress depends on various factors i.e. hyperhomocysteinemia, low density lipoprotein, genetically determined poor resistance to oxidative stress and a dietary deficiency of antioxidants.[9]

Protein oxidation by ROS called as Protein carbonylation, play a key role in the pathophysiology of diseases and involved in regulatory physiological events as well as in tissue damage. Increased ROS generation causes oxidation of plasma protein in pregnant women which leads to preeclampsia and these oxidized proteins are known as Protein-bound carbonyls. [10]

Protein carbonyl groups are used as biomarkers of oxidative stress, because of their relatively early formation and stability than other oxidized products.[11] The mechanism of oxidative protein modifications is complex and remains incompletely defined but chemistry of the reactions that give rise to carbonyl groups have been well characterized.[12]

Protein-bound carbonyls represent a marker of global protein oxidation, generated by different ROS in blood, tissues and cells and it results in a multitude of products, arising from modification of a wide range of amino acids and hence damages the sulfur-containing, aromatic, and aliphatic amino acids.[13] Amino acids of proteins attacked by oxidants and are thus produce both protein-bound and released carbonyl groups. Because the normal structure of amino acids contains no carbonyl groups as the part of original protein.[14] These carbonyl proteins cause cellular damage to the placenta.[15]

In the current study, an attempt is made to quantify serum protein carbonyl content as a biomarker of oxidative stress in Preeclampsia women in comparison to the normal pregnant group. Serum antioxidant capacity was measured through FRAP assay (Ferric Reducing Ability of Plasma).

Methodology

The screening for preeclampsia was performed in pregnant women of the third trimester attended Obstetrics & Gynecology Departments of District Teaching Hospital of D.I.Khan, KPK from March 2018 to January 2019. Clinically diagnosed 40 preeclampsia women and 40 normotensive pregnant women as controls were selected for the study. The sample size calculation for the study was performed according to the method of Charan and Kantharia.[16] The cumulative error was kept at 0.05. Preeclampsia subjects were matched to control for gestational age, maternal age and parity. Inclusion criteria comprise of (a) clinically diagnosed pregnant women with preeclampsia, (b) non-smoker, (c) singleton or multiple gestation, (d) non-diabetic, (e) do not have any infection. Exclusion criteria: (a) chronic hypertension (b) normotensive. This comparative cross-sectional study was conducted according to the ethical guidelines and institutional approval of board of study of Gomal Medical College.

Five ml of peripheral blood samples were drawn through venipuncture into blood collection tubes from preeclampsia and normal pregnant women respectively. The blood was allowed to clot for 30 minutes thereafter, centrifugation was performed for 15 min at 4000rpm, and serum was separated and stored at -8^{0}C till further analysis. Each sample was labeled with a patient identification code.

The serum antioxidant capacity was measured by using the 'ferric reducing ability of plasma' (FRAP) assay as described by Benzie and Strain.[17] The principle of this method is based upon the reduction of the ferric-tripyridyltriazine complex to the ferrous form, blue color develops, and absorbance change is measured at 593 nm.

Briefly, FRAP working reagent was prepared as follows: 300 mmol/l acetate buffer pH 3.6, 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) solution in 40 mM HCl and 20 mmol/l $FeCl_3.6H_2O$ were mixed in ratio10:1:1 respectively, and kept in water bath at 37^0C. All samples run at least in triplicate. The 100 µl of serum was mixed with 900 µl of FRAP reagent and absorbance was measured at 593nm in 0 minute and after 4 minutes of incubation at 37°C. The sample blank was prepared by mixing the 100 µl of water in FRAP reagent. FRAP value was calculated by the equation:

$$FRAP\,\mu MoleValue = \frac{0\text{ - to }4\text{ - min }\Delta A_{593}\text{ nm test sample}}{0\text{ - to }4\text{ - min }\Delta A_{593}\text{ nm test standard}} \times FRAP_{std}\,\mu M$$

The DNPH assay was used to determine serum protein carbonyl spectrophotometrically. The 2,4-dinitrophenylhydrazone (DNPH) react with serum protein carbonyl to produce a Schiff base that afterward produces a corresponding hydrazone which is measured spectrophotometrically.[18]

The serum sample was diluted with phosphate–buffered saline in (1: 10) to make total proteins final concentration less than 10 mg/ml. The diluted serum (200µl) was mixed with (800 µl) of 10 mM DNPH in 2.5 M HCl. In the second test tube sample control was made by mixing the equal volume of diluted serum with (800 µl) of 2.5M HCl. Afterward; incubation of both tubes was performed in a dark environment, at room temperature for one hour. The reaction mixture was vortexed with an interval of 15 minutes. The (1 ml) of trichloroacetic acid (20% w/v) was added in reaction mixture and incubated on ice for five minutes. The centrifugation of both tubes were performed at 10,000 × g for 10 minutes at 4° C, the supernatant was discarded and a second wash with 10% trichloroacetic acid (4ml) was performed. The resultant protein pellets were broken mechanically and three times washed with (1 ml) of ethanol: ethyl acetate (1:1 v/v) to remove free DNPH and lipid contaminants and again centrifuged at 10,000 × g for 10 min at 4°C. Finally, the re-suspension of protein pellet was done in (500µl) of 6 M guanidine hydrochloride and centrifuged at 10,000 × g for 5 minutes at 4°C. The absorbance of the supernatant was done at 370 nm using control as a blank. The concentration (nmol/ml) of protein carbonyl was calculated from the equation.

Protein carbonyl concentration (nmol/ml) = [A370 / 0.011 µM-1] [500 µl/200 µ]

To test the differences between groups statistics was applied in excel. Data were expressed as mean, standard deviation (SD). For statistical analysis, student t-test was used to calculate mean values, standard deviation, compared the results of two groups for checking the significance. The values of P <0.05 were taken as significant.

Results

The baseline characteristics of Preeclampsia subjects and controls are given in Table I. There was no significant difference between mean age and height of preeclampsia subjects and control. While a significant difference in means weight, gestation, systolic and diastolic blood pressure and proteinuria of both subjects and control were observed. (Table I) FRAP assay results revealed that FRAP value was significantly reduced (p<0.0001) in preeclampsia women as compared to normal pregnant women. (Table II)

Mean values of serum protein carbonyl content (nmol/ml) in preeclampsia women was significantly elevated (p<0.0001) as compared to normal pregnant women. (Table II)

The FRAP Assay was performed on serum samples of both, preeclampsia and control group. The study results

Table I: Baseline characteristics of Preeclampsia subjects and controls

Variables	Preeclampsia subjects Mean±SD	Control Mean±SD	p-value
Age	30.25 ± 2.38	29.85 ±2.21	0.21
BMI	27.79± 2.85	24.72± 2.52	0.001
Gestation week	31.35±1.71	31.17±1.61	0.31
Systolic (mmHg)	152.75±7.24	114.70±2.50	<0.001
Diastolic (mmHg)	92.87±7.24	76.37±5.43	<0.001
Proteinuria	2.67±0.19	0.21±0.04	<0.001

Table II: FRAP Value and Protein carbonyl levels of Preeclampsia subjects and controls

Variables	Preeclampsia subjects Mean±SD	Control Mean±SD	p Value
FRAP Value (µmol)	284.97±9.58	518.90 ±10.18	<0.001
Protein Carbonyl concentration (nmol/ml)	192.67±6.82	62.22±5.34	<0.001

showed reduced FRAP value 259.49±19.50 in preeclampsia women which indicated reduced serum antioxidants capacity while FRAP value 536.40 ±20.30 was significantly high in normal healthy women. (Table II)

Discussion

In the current investigative study, we have evaluated the serum antioxidant level and protein carbonyl content in clinically diagnosed preeclampsia women by using normotensive pregnant women as control group. We found serum protein carbonyl levels was significantly elevated in preeclampsia, while antioxidants level was reduced significantly. A similar finding has been reported in another study, which performed FRAP assay in preeclampsia women and reported significantly reduced FRAP activity in preeclampsia compared with normal pregnant women.[19]

Likewise, another study enrolled women with mild and severe preeclampsia and normotensive pregnant women as control and reported protein carbonyls were 7.8+2.6 nmol/10 mg protein in the normal case, 11.7+2.9 nmol/10 mg protein in mild preeclampsia and 14.9+2.7 nmol/10 mg protein in severe preeclampsia indicated a high level of protein carbonyl content in preeclampsia.[18] The previous investigative study on plasma protein carbonyls content was conducted on 47 preeclamptic, 45 healthy pregnant, and 22 healthy non-pregnant women, and protein carbonyl content was evaluated through a sensitive ELISA-method and concluded that preeclamptic women had higher plasma protein carbonyl levels than healthy pregnant women ($P < 0.0001$).[20]

Another study demonstrated plasma protein carbonyl in preeclamptic women (7.72 ± 4.10 nmol/mg) as compared to normal pregnant women controls (2.85 ± 3.06 nmol/mg). Few other studies have also documented significantly elevated oxidative stress marks in preeclamptic women.[21] Recent study has evaluated the relationship between urinary protein carbonyl content and protein misfolding in preeclampsia women. They found significant elevation in urinary protein carbonyl level and a weak correlation with urinary protein misfolding.[22]

In preeclamptic pregnancies, increase protein carbonyl has been documented in the umbilical cord blood, which attributed to excessive generation of reactive oxygen species.[23]

In the placentas of preeclamptic patients, an increase in the level of oxidative stress biomarkers such as lipid peroxidation and protein carbonyls leads to pathological placental conditions. That may be due to excessive production of free radicals and a decrease in enzymatic and non-enzymatic antioxidant defense mechanisms, which ultimately leads to production of high oxidative stress in maternal body. Carbonyl content is also significant clinically, if preeclamptic patients have HELLP syndrome.[23] The findings of current investigative study clearly demonstrates the high level of oxidative stress in preeclampsia women.

Conclusion

The outcome of the current study revealed significant lower serum antioxidant level increases the protein carbonyl content in preeclampsia patients, which indicating high oxidative stress in preeclamptic women. It is suggested that the preeclamptic women should use anti-oxidant supplements to cope with preeclamptia-induced oxidative stress.

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