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ORIGINAL ARTICLE

Increased Oxidatively Modified Forms of Albumin in Association with Decreased Total Antioxidant Activity in Different Types of Hypertensive Disorders of Pregnancy

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Abstract Protein oxidation has been demonstrated in preeclampsia, but this finding has not been established in other hypertensive disorders in pregnancy (HDP). The present study comparatively evaluated ischemia modified albumin (IMA) and advanced oxidation protein products (AOPP) in different HDP and investigated their association with total antioxidant activity (AOA) and total thiols. There was a significant increase in AOPP and IMA, a significant decrease in AOA, total thiols and albumin in every HDP compared to controls. Among HDP groups, eclampsia patients showed more significant change in each of the parameter. IMA and AOPP were negatively associated with AOA in every HDP and with total thiols only in eclampsia. The present study supports the hypothesis of oxidative stress, as evidenced by increased protein oxidation, decreased antioxidant status and significant negative association between protein oxidation and AOA in every HDP. The imbalance of prooxidants and antioxidants was further augmented in eclampsia.

Keywords Protein oxidation · Hypertensive disorders of pregnancy · Ischemia modified albumin · Advanced oxidation protein products · Total antioxidant activity · Total thiols

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Abbreviations

AOPP	Advanced oxidation protein products
IMA	Ischemia modified albumin
AOA	Total antioxidant activity
ROS	Reactive oxygen species
HDP	Hypertensive disorders in pregnancy

Introduction

Hypertensive disorders of pregnancy (HDP) represent a group of disorders associated with increased maternal and fetal mortality and morbidity [1]. Increased free radical activity and oxidative stress may contribute to the pathophysiology of hypertension in pregnancy [2, 3]. Reactive oxygen species (ROS) can react with most of the macromolecules, but proteins are likely to be the main targets owing to their relative high abundance, crucial requirement for cell function and rapid reaction rates with both radicals and other oxidants. Albumin represents a powerful circulating antioxidant that accounts for almost all of the excess plasma protein oxidation [4, 5]. The shift towards oxidatively modified proteins replacing the lipid peroxidation products in demonstration of oxidative stress could be due to relative early formation, longer half life and greater stability of these molecules [6, 7].

Advanced oxidation protein products (AOPP), an oxidatively modified form of proteins (primarily albumin) by ROS, can directly modify amino acid residues generating dityrosine residues, carbonyl groups, disulfide bridges, and cross-links. These highly reactive agents cause alterations in structure and function of albumin, giving rise to attrition in its antioxidant properties [8]. Alteration in

protein oxidation indicator, AOPP without any change in lipid peroxidation product has been reported in preeclampsia [9].

The structure of albumin is subjected to modifications by ROS with reduced ability of its binding capacity to transition metals to form ischemia modified albumin (IMA), which forms the basis of its estimation in biological fluids and a sensitive marker in conditions of oxidative stress related to ischemia reperfusion [10–12]. We have earlier shown that IMA in serum was in accordance with the severity of preeclampsia [13]. Although there are reports on protein oxidation parameters such as IMA and AOPP in HDP, most of the studies have focused on preeclampsia with no reports so far on their analysis in other hypertensive disorders such as gestational hypertension and chronic hypertension [14–16]. The present study was taken up to comparatively evaluate two modified forms of plasma albumin, IMA and AOPP in different types of HDP. We also evaluated their association with determinants of total antioxidant capacity, total antioxidant activity (AOA) and total thiols in these groups of disorders.

Methodology

Study Population

The protocol of the study was approved by the institutional ethical committee. Informed written consent was obtained. Those willing to give consent to participate were included in the study. Patients with HDP were classified based on the hypertensive disorder into 4 groups according to Davey [17].

Chronic hypertension was diagnosed based on elevated blood pressure (n = 40, systolic ≥140 mmHg or diastolic ≥90 mmHg) on two occasions 6 h apart that was present prior to pregnancy. Gestational hypertension was diagnosed based on elevated blood pressure (n = 40, systolic ≥140 or diastolic ≥90 mmHg) after 20 weeks without presence of protein in the urine. Preeclampsia was diagnosed by elevated blood pressure after 20 weeks of gestation (n = 40, systolic ≥140 or diastolic ≥90 mmHg) plus proteinuria (>0.3 g/24 h). Presence of eclampsia (n = 40) was based on the new onset of grandmal seizures in a woman with preeclampsia.

Normal pregnant women (n = 40) without any complications, matched for maternal and gestational age were selected as controls. Patients with any other complications other than HDP such as diabetes mellitus, heart diseases, severe anemia and renal diseases were excluded from the study.

Materials and Methods

Peripheral blood sample was collected from each patient with disposable syringes through venipuncture into EDTA vacutainers. The blood samples were centrifuged at 3000 rpm for 10 min, to separate the plasma, which was aliquoted into microfuge tubes and immediately stored at −20 °C until analysis was done (within <6 months, thawed once). The blood samples were collected prior to their antihypertensive treatment except for chronic hypertension patients in whom the blood was drawn after the antihypertensive therapy.

IMA was estimated by Bar-Or et al. method [18]. Briefly, cobalt chloride was added to the sample and mixed. The mixture was incubated for 10 min to ensure cobalt albumin binding.

Dithiothreitol was added as coloring agent and mixed. After a 2-min incubation period, sodium chloride was added. The absorbance of assay mixtures was read at 470 nm in spectrophotometer. (Thermo Scientific, Madison, WI). The blank was prepared similarly with the exclusion of dithiothreitol. The results were expressed as absorbance units (ABSU).

AOPP was estimated by Witko-Sarsat's method [19]. Briefly, plasma was diluted at 1:50 ratio with PBS. 1.16 M potassium iodide and 20 µl absolute acetic acid were added and absorbance was immediately measured at 340 nm. Chloramine T was used at 0–100 µmol/L concentration range for calibration. Results were expressed as µmol/L of chloramine T equivalents.

Assay for total thiols was according to GL Ellman's procedure [20]. The sample was mixed with Tris EDTA buffer (0.25 mol/L Tris –0.02 mol/L EDTA). DTNB reagent (5′dithiobis 2-nitrobenzoic acid) was added to it and the final volume was made up to 4 ml with absolute methanol and kept for 15-20 min. Blanks were run for each sample, prepared as above, with the exception that there was no DTNB in the methanol. Absorbance was read at 412 nm in a spectrophotometer and subtracted from blank. The results were reported as micromoles per liter.

Plasma albumin by bromocresol green method [21]. Bromocresol green reagent was added to the sample and mixed, followed by incubation at room temperature for 10 min. The absorbance was read at 630 nm against a reagent blank, using spectrophotometer. The albumin concentration was expressed in g/L.

Total antioxidant activity (AOA) was estimated by Koracevic et al. by method [22]. To the sample, sodium phosphate buffer, Fe–EDTA complex and H_2O_2 were added and incubated at 37 °C for 1 h. Acetic acid and thiobarbituric acid were added to the test tubes, followed by incubation for 10 min at 100 °C and cooled. Absorbance

was measured at 532 nm. In the sample blank Fe–EDTA mixture and H_2O_2 were added after 20 % acetic acid. Reagent blank contained all the similar reagents except that plasma was replaced with phosphate buffer. Standards containing 1 mmol/L uric acid were used for calibration. The values were expressed as mmol/L.

Statistical Analysis

Data was analyzed with the statistical package, SPSS-19. Mean and standard deviation were used to assess the level of various parameters. The significance of difference in the mean was calculated using one way ANOVA. Pearson's regression analysis was applied to find the association of protein oxidation markers with antioxidant parameters. p value <0.05 was considered statistically significant.

Results

Table 1 shows the demographic and clinical data of the hypertensive groups and normal pregnant controls. Pregnant women with hypertensive disorders and control groups were of similar age groups. However, there was a significant difference in blood pressure, gestational age at delivery and birth weight in the hypertensive groups on comparison with the controls.

Table 2 shows the comparison of protein oxidation parameters in the different groups. Plasma AOPP and IMA were elevated significantly in all the hypertensive groups, compared to controls ($p < 0.001$ for chronic hypertension and gestational hypertension vs. controls, $p < 0.0001$ for preeclampsia and eclampsia vs. controls). When the hypertensive groups were compared, the significance of elevation of AOPP and IMA was highest in eclampsia ($p < 0.001$ when compared to chronic hypertension, gestational hypertension and preeclampsia) followed by the preeclampsia group (for AOPP, $p < 0.001$ when compared to gestational hypertension, $p < 0.01$ compared to chronic hypertension, for IMA $p < 0.01$ when compared to gestational hypertension, and chronic hypertension). Plasma AOPP was increased significantly in chronic hypertension when compared to gestational hypertension ($p < 0.05$), whereas the increase in IMA was not significant ($p > 0.05$).

Table 3 shows the comparison of antioxidant parameters in different hypertensive groups and control group. Antioxidant parameters significantly decreased in all hypertensive groups, compared to controls. The significant decrease in AOA compared to controls were ($p < 0.001$ vs. gestational hypertension and chronic hypertension, $p < 0.0001$ vs. preeclampsia and eclampsia) and the significant difference of total thiols compared to controls were ($p < 0.01$ vs. gestational hypertension, $p < 0.001$ vs. chronic hypertension, $p < 0.0001$ vs. preeclampsia and eclampsia). Among the hypertensive groups, eclampsia group showed more significant decrease in total thiols compared to others ($p < 0.05$ vs. preeclampsia, $p < 0.01$ vs. chronic hypertension, $p < 0.001$ vs. gestational hypertension), followed by the preeclampsia ($p < 0.01$ vs. gestational hypertension) and chronic hypertension

Table 1 Clinical characteristics in different HDP groups

Characteristics	Chronic hypertension	Gestational hypertension	Preeclampsia	Eclampsia	Pregnant controls
Age (years)	30 ± 5	27 ± 3	26 ± 7	27 ± 5	26 ± 8
SBP (mmHg)	143 ± 8$^{\beta}$	147 ± 12$^{\beta}$	153 ± 11$^{\beta}$	172 ± 14$^{\gamma}$	117 ± 11
DBP (mmHg)	93 ± 10$^{\beta}$	94 ± 11$^{\beta}$	99 ± 11$^{\beta}$	114 ± 10$^{\gamma}$	74 ± 6
Gestational age at delivery (weeks)	37 ± 3	37 ± 4	34 ± 2$^{\beta}$	32 ± 3$^{\beta}$	38 ± 2
Birthweight (g)	2998 ± 546$^{\alpha}$	3078 ± 534$^{\alpha}$	2848 ± 467$^{\beta}$	2216 ± 389$^{\gamma}$	3342 ± 379

Data expressed as mean ± SD

SBP systolic blood pressure, *DBP* diastolic blood pressure

$^{\alpha}$ $p < 0.01$ versus control; $^{\beta}$ $p < 0.001$ versus control; $^{\gamma}$ $p < 0.0001$ versus control

Table 2 Protein oxidation parameters in different HDP groups

Parameters	Chronic hypertension	Gestational hypertension	Preeclampsia	Eclampsia	Pregnant controls
AOPP (μmol/L)	111 ± 13$^{\alpha,\delta}$	107 ± 17$^{\alpha}$	121 ± 13$^{\beta,\mu,\zeta}$	138 ± 16$^{\beta,\gamma,\lambda,\zeta}$	92 ± 14
IMA (ABSU)	0.459 ± 0.139$^{\alpha}$	0.465 ± 0.154$^{\alpha}$	0.494 ± 0.175$^{\beta,\varepsilon,\mu}$	0.581 ± 0.234$^{\beta,\gamma,\lambda,\zeta}$	0.374 ± 0.114

$^{\alpha}$ $p < 0.001$ versus control; $^{\beta}$ $p < 0.0001$ versus control; $^{\lambda}$ $p < 0.001$ versus chronic hypertension; $^{\zeta}$ $p < 0.001$ versus gestational hypertension; $^{\gamma}$ $p < 0.001$ versus pre eclampsia; $^{\varepsilon}$ $p < 0.01$ versus gestational hypertension; $^{\mu}$ $p < 0.01$ versus chronic hypertension; $^{\delta}$ $p < 0.05$ versus gestational hypertension

Table 3 Antioxidant parameters in different HDP groups

Parameters	Chronic hypertension	Gestational hypertension	Preeclampsia	Eclampsia	Pregnant controls
Total thiols (μmol/L)	$99 \pm 16.2^{\alpha,\zeta}$	$108 \pm 12.9^{\delta}$	$87 \pm 19.7^{\beta,\varepsilon}$	$71 \pm 14.6^{\beta,\gamma,\lambda,\partial}$	122 ± 21.3
Albumin (g/dL)	3.19 ± 0.26^{O}	$3.13 \pm 0.29^{\delta}$	$2.95 \pm 0.32^{\beta,\zeta,\lambda}$	$2.72 \pm 0.27^{\beta,\gamma,\ \varepsilon,\alpha}$	3.37 ± 0.31
AOA (mmol/L)	$1.207 \pm 0.349^{\alpha}$	$1.196 \pm 0.489^{\alpha,\mu}$	$1.02 \pm 0.117^{\beta,\varepsilon,\vartheta}$	$0.085 \pm 0.0.207^{\beta,\psi,\partial,\alpha}$	1.42 ± 0.29

$^{\alpha}$ $p < 0.001$ versus control; $^{\beta}$ $p < 0.0001$ versus control; $^{\delta}$ $p < 0.01$ versus control; ^{O} $p < 0.05$ versus control; $^{\zeta}$ $p < 0.05$ versus gestational hypertension; $^{\varepsilon}$ $p < 0.01$ versus gestational hypertension; $^{\gamma}$ $p < 0.05$ versus pre eclampsia; $^{\lambda}$ $p < 0.01$ versus chronic hypertension; $^{\partial}$ $p < 0.001$ versus gestational hypertension; $^{\alpha}$ $p < 0.001$ versus chronic hypertension; $^{\psi}$ $p < 0.01$ versus pre eclampsia; $^{\vartheta}$ $p < 0.01$ versus chronic hypertension; $^{\mu}$ $p < 0.05$ versus chronic hypertension

Table 4 Correlation analysis between protein oxidation and antioxidants parameters in different HDP groups

		AOA		Albumin		Total thiols		AOPP	
		r	*p*	r	*p*	r	*p*	r	*p*
Chronic	AOPP	−0.412	<0.01	−0.457	<0.005	−0.259	>0.05	–	
Hypertension	IMA	−0.389	<0.05	−0.383	<0.05	−0.211	>0.05	0.461	<0.005
Gestational	AOPP	−0.395	<0.05	−0.424	<0.01	−0.243	>0.05	–	
Hypertension	IMA	−0.379	<0.05	−0.401	<0.05	−0.234	>0.05	0.454	<0.005
Preeclampsia	AOPP	−0.454	<0.005	−0.511	<0.001	−0.312	<0.05	–	
	IMA	−0.326	<0.05	−0.504	<0.001	−0.254	>0.05	0.511	<0.001
Eclampsia	AOPP	−0.596	<0.0001	−0.513	<0.001	−0.496	<0.01	–	
	IMA	−0.479	<0.005	−0.507	<0.001	−0.467	<0.01	0.584	<0.0001
Pregnant	AOPP	−0.289	>0.05	−0.293	>0.05	−0.213	0.186	–	
controls	IMA	−0.262	>0.05	−0.307	>0.05	−0.197	0.223	0.341	<0.05

r Spearman correlation coefficient

($p < 0.05$ vs. gestational hypertension). The decrease in AOA was more significant in eclampsia group compared to other groups ($p < 0.01$ vs. preeclampsia, $p < 0.001$ vs. gestational hypertension and chronic hypertension). Preeclampsia group showed a significant decrease compared to gestational hypertension and chronic hypertension ($p < 0.01$ vs. gestational hypertension and chronic hypertension), followed by gestational hypertension ($p < 0.05$ vs. chronic hypertension).

Albumin showed a significant decrease in all the groups compared to controls ($p < 0.05$ vs. chronic hypertension, $p < 0.01$ vs. gestational hypertension, $p < 0.0001$ vs. preeclampsia and eclampsia). The decrease in albumin was highest in eclampsia group ($p < 0.05$ vs. preeclampsia, $p < 0.01$ vs. gestational hypertension, $p < 0.001$ vs. chronic hypertension) followed by preeclampsia group ($p < 0.05$ vs. gestational hypertension, $p < 0.01$ vs. chronic hypertension). Albumin was decreased in gestational hypertension compared to chronic hypertension but was not statistically significant.

Table 4 shows the correlation analysis between protein oxidation and antioxidants parameters in different hypertensive groups. There was a significant positive correlation between AOPP and IMA in all the hypertensive groups. The association was stronger for eclampsia (<0.0001) compared to preeclampsia ($p < 0.001$), gestational hypertension and chronic hypertension ($p < 0.005$). Significant negative correlation between IMA and albumin was stronger for preeclampsia and eclampsia ($p < 0.001$) compared to gestational hypertension and chronic hypertension ($p < 0.05$). Significant negative correlation of IMA and AOPP with AOA was seen in all the hypertensive groups and was more stronger in eclampsia group (AOA with IMA, $p < 0.005$, AOA with AOPP $p < 0.0001$) than the other hypertensive groups. AOPP showed a significant negative correlation with total thiols only in preeclampsia ($p < 0.05$) and eclampsia ($p < 0.01$). IMA showed an significant negative correlation with total thiols only in eclampsia ($p < 0.01$).

Discussion

Oxidative stress is known to play a central role in the pathogenesis of hypertension in pregnancy. Considerable attention has been focused on the role of protein oxidation/impaired redox homeostasis in preeclampsia [14–16]. Evidence for imbalance of prooxidants and antioxidants as an underlying factor in the biochemical derangement of other different types of hypertensive disorders that

complicate pregnancy are limited [23, 24]. The present study is the first study to document a comparative exploration of protein oxidation parameters in diverse types of HDP.

The oxidation of the protein side chains by ROS results in the chemically stable dityrosine-containing protein products, AOPP often carried by albumin in vivo. Its accumulation represents the global index of protein damage involving side chains of amino acids [25].

In the present study increased AOPP was seen in all the hypertensive groups compared to normal pregnant women that underlies the oxidation of proteins in the pathogenesis of HDP. The significant negative correlation between albumin and AOPP which was associated with other hypertensive disorders such as chronic hypertension and gestational hypertension is suggestive of AOPP being a product of the free radical mediated oxidative damage to albumin, the most abundant plasma protein and a powerful extracellular antioxidant in these entities of hypertension as well. However the elevation of AOPP and its association with albumin was found to be more stronger in eclampsia, that explains the further increase of oxidative stress in this group compared to the other hypertensive disorders. This finding is in contrast to an earlier study which showed that the levels of AOPP did not change significantly among the control, pre-eclampsia and eclampsia groups [26]. AOPP's are important mediators in the regulation and signaling of angiogenic pathways of trophoblasts, enhancing soluble Fms-like tyrosine kinase 1 expression in trophoblasts, promoting the development of preeclampsia [27]. Whether AOPP mediates similar signaling pathways as an attributable mechanism for development of other different hypertensive disorders is yet to be explored.

Albumin the most abundant plasma protein and a powerful extra cellular antioxidant accounts to more than 70 % of the free radical-trapping activity of serum. In the present study albumin were decreased in all the hypertensive groups compared to the pregnant controls. The urinary loss due to nephrosis is one of the most important causes of this hypoalbuminemia, but there is no selectivity in hypertensive disorders and hence this cause may have very little influence in producing low albumin levels in pregnancy induced hypertensive disorders such as gestational hypertension. Its utilization could be attributed to the increased free radical generation from the hypoxic placental environment in the hypertensive groups. The decrease in albumin in the present study was associated with an increase in IMA, formed due to the damage of N-terminal region of albumin by ROS. Negative correlation between albumin and IMA seen in the hypertensive groups is a clear indication of oxidative modification of albumin during oxidative stress. We have earlier reported an increase in serum IMA, its negative correlation with albumin and clinical characteristics in preeclampsia [13]. The significant increase in AOPP and IMA and a stronger positive correlation between IMA and AOPP in the eclamptic group compared to the other hypertensive groups, reflects further augmentation of protein oxidation in this hypertensive group than the others. This finding was enhanced with the severity of pregnancy induced hypertensive disorders. Though there was a significant elevation of AOPP in chronic hypertension on comparison to gestational hypertension, such a consistency was lost with IMA. This incompatibility probably could be due to differences in albumin levels in these conditions, as IMA is formed by the damage of N-terminal region of albumin by ROS.

The total thiol status, especially thiol (-SH) groups in the body are considered as major plasma antioxidants in vivo. The major part of thiols in plasma is contributed by albumin, that contains an exposed –SH group over cysteine-34 residue and provide the bulk of total plasma thiol. The oxidation of plasma –SH group, indicated as thiol stress, reflects protein oxidation, as the conformation of albumin is altered, allowing –SH groups to be oxidized [28, 29]. A significant decrease in total thiols in the present study may indicate increased consumption of these thiol groups to neutralize ROS in the hypertensive disorders. Earlier studies have reported decrease in plasma thiol status in preeclampsia [30, 31]. The inverse relation of AOPP's with plasma thiols seen in eclampsia is an evidence of increased protein oxidation under conditions of elevated oxidative stress, thereby emphasizing the crucial role of antioxidants in protecting against ROS mediated protein damage.

AOA, a measure of the sum total plasma antioxidants, was decreased in the other different types of hypertensive disorders in addition to preeclampsia, which implies that there is an imbalance of antioxidants, owing to their usage to counteract the excess free radicals generated in these conditions. The decrease in AOA was in parallel to the decrease in albumin in HDP, indicating that albumin may be a major oxidation target since most plasma-free thiol groups are found in albumin. Moreover the negative correlation between AOA and protein damage markers IMA and AOPP in the hypertensive groups suggests that the antioxidants could be used for the free radicals generated due to protein damage in these conditions. The stronger relationship of this correlation in eclampsia that indicates further imbalance of protein oxidants and antioxidants in this group compared to the others. Though earlier studies have shown an impaired antioxidant activity in preeclampsia [32], this finding has not been uniformly reproducible [33]. Our study is the first of its kind to measure antioxidant parameters AOA and total thiols in various types of hypertensive disorders.

Though the findings of this study strengthens the hypothesis that increased protein oxidation and decreased

capacity of antioxidant systems is seen in different types of hypertensive disorders, simultaneous assay of these parameters in placental tissues and cord blood could probably give more conclusive indication on the origin of oxidative stress and help us understand whether similar biochemical changes are seen in the newborns of these hypertensive groups.

To conclude, the data from the present study strengthens the hypothesis that oxidative stress is involved not only in preeclampsia but in every HDP, as evidenced by increased protein oxidation, decreased antioxidant status and significant negative association between protein oxidation and AOA in every HDP group. The imbalance of prooxidants and antioxidants was further augmented in eclampsia group.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of the paper.

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