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Research Article

Association between serum paraoxonase and plasma nitric oxide in pre-eclampsia

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ABSTRACT

Background: It is well accepted that oxidative stress plays a crucial role in the development of endothelial dysfunction leading to Pregnancy Induced Hypertension (PIH). However, studies related to altered levels of paraoxonase and nitric oxide in PIH subjects are still in obscure. In this context, the present study was undertaken to assess serum Paraoxonase (PON), Nitric Oxide (NO) levels along with other markers of oxidative stress i.e. erythrocyte Malondialdehyde (MDA), plasma ascorbic acid levels in the blood samples of PIH subjects and to determine their relation in etio-pathogenesis of PIH complication.
Methods: Markers of oxidative stress (PON, MDA, and ascorbic acid) and endothelial dysfunction (serum NO) were estimated in 20 normotensive pregnant women (Group I) and 20 PIH women (Group II) by using standard methods. Data obtained from both the groups were statistically compared with age matched 20 healthy non pregnant women (Control group) by using student's t-test.
Results: Serum PON and erythrocyte MDA levels were increased significantly whereas marked depletion in plasma NO and vitamin C levels ($P < 0.05$, $P < 0.001$) were observed in PIH subjects. In addition, PON levels were inversely correlated with endothelial dysfunction. However, only plasma NO levels were decreased significantly in group I subjects whereas other parameters were altered insignificantly ($P < 0.1$) in group I subjects.
Conclusion: These findings reflects the importance of antioxidant rich diet in the prevention of PIH complication and emphasizes the early assessment of serum paraoxonase and NO levels as efficient diagnostic marker to predict future risk of PIH development.

Keywords: Ascorbic acid, Pre-eclampsia, Reactive oxygen species, Endothelial dysfunction

INTRODUCTION

Oxygen free radicals have been identified as mediators of cell injury in a wide range of pathological processes including Pregnancy Induced Hypertension (PIH). Increasing interest has been focused on the role of oxidative stress in the etiopathogenesis of PIH, which are convincingly linked to the altered antioxidant defense system and biomolecular deterioration including lipid peroxidation.[1,2]

Most common target of free radicals attack and incidence of biomolecular deterioration is lipid peroxidation of membrane lipids. It generates a variety of hydroperoxide and aldehyde products that are highly reactive with cellular components and extracellular matrix. Malondialdehyde (MDA) is a well-known toxic aldehy-

dic end product of lipid peroxidation.[3] Gupta et al. also documented that excess endogenous aldehyde production (lipid peroxides) plays a significantly role in blood pressure elevation by binding sulphydryl groups of membrane proteins, altering Ca^{2+} channels and increasing cytosolic free Ca^{2+} that cause further extensive membrane damage leading to peripheral vascular resistance and hypertension.[4]

Amongst various enzymes, Paraoxonase (PON), a calcium-dependent A-esterase synthesized primarily in the liver and secreted into the serum as HDL-associated enzyme, prevents oxidation of Low Density Lipoprotein (LDL) and responsible for anti-atherogenic property of High Density Lipoprotein (HDL), has received much attention in exploring hidden facts related to vascular complications.[5]

Moreover, cardioprotective and oxidant scavenging role of PON are well supported by cooperative action of non-enzymic antioxidants such as ascorbic acid (vitamin C) etc. These non-enzymic antioxidants have a significant role in regulating the oxidative stress mediated cascade responsible for biomolecular deterioration leading to the development of PIH.[6] In addition, their role in scavenging free radicals, in inhibiting lipid peroxidation and in the prevention of vascular disorders, are well documented.[5]

Nitric Oxide (NO) is a powerful endothelium derived vasodilator, produced from the precursor L-arginine in all human body cells. It plays a significant role in circulatory, digestive, neural and immunological systems function regulation. It takes part in blood pressure control, inhibits mast cells degranulation, possesses anti-aggregant properties, and regulates vascular tone. It also inhibits both proliferation of smooth muscle cells and adhesion of leukocytes and platelets.[7] However, the relationship between NO and PON along with non-enzymic antioxidants in PIH subjects is yet not clarified and need further attention. In addition, it is conceivable that alteration in the levels of these important biomolecules may induce series of events and adversely affect the pregnancy which in-turn leads to the development of PIH. Early recognition may help the women to receive treatment and prevent the development of PIH and its related complications. Therefore, the overall objective of present study was to estimate serum paraoxonase and plasma nitric oxide levels along with non-enzymic antioxidants i.e. plasma ascorbic acid levels in normotensive pregnant (NTP) and hypertensive pregnant women (PIH) and to determine their relation in the development of hypertension during pregnancy.

METHODS

In the present study, 20 NTP women & 20 subjects of PIH were taken in study group i.e. group I and group II respectively and 20 age matched NP women were taken in control group.

Inclusion criteria

The pregnant women having blood pressure greater than 140/90 mm Hg diagnosed after 20 weeks of gestation with evidence of proteinuria (~200 mg/l) and fulfilled National Institute for Health and Clinical Excellence (NICE) guidelines 107, were grouped as PIH subjects (Group II). A general information or pre-experimental questionnaire regarding anthropometric and clinical data was completed from all the patients after taking their informed consent and approval of protocol by ethics committee of college.

Exclusion criteria

Patients with diabetes mellitus, family history of hypertension, smoking habit, renal insufficiency, hepatic disease, taking lipid lowering drugs, diuretics or vitamin and minerals supplements were excluded.

Fasting blood samples were collected in a plane vial from each subjects followed by serum separation after measurement of blood pressure and recognition of other symptoms of PIH i.e. proteinuria, edema and sudden weight gain etc. Serum paraoxonase activity was estimated by Gan et al. method using p-nitrophenyl acetate (5.5 mM/L) as a substrate.[8]

The increase in the absorbance of p-nitrophenol formed at 412 nm was measured spectrophtometrically. The activity of PON was measured in Tris buffer (20 mM/L; pH 8.0) containing 1mM $CaCl_2$. The generated product p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole/cm at pH 8. Results are expressed as Units/ml (1 nmol p-nitrophenol formed per minute).

Plasma ascorbic acid levels were estimated by Mc Cormick and Greene method.[9] Ascorbic acid in plasma is oxidized by Cu^{2+} to form dehydroascorbic acid which reacts with acidic 2,4–dinitrophenyl hydrazine to form a red bishydrazone, which is measured at 520 nm.

The measurement of plasma NO is difficult because this radical is poorly soluble in water and has a short half-life in tissue (10-60s), but its half-life may be as long as 4 minutes in the presence of oxygen. For these reasons, the end products of the phenomenon, nitrate and nitrite, are preferentially used in clinical biochemistry. Plasma total nitrate and nitrite levels were measured with the use of Griess reagent as described earlier.[10]

Erythrocyte Malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate.[11] The heat induced reaction of Malondialdehyde (MDA) with Thio Barbituric Acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532 nm.

Statistical analysis

Values were expressed as Mean ± SD. The significance of mean difference between groups was compared by using Student's 't'-test. Linear regression analysis and Pearson correlation test were performed to determine the association between above said indexes or parameters.

RESULTS

In the present study, demographic profile of the study group subjects is depicted in Table 1. Blood pressure measurement revealed significant elevation in group II subjects with respect to group I and control which indicates their direct relation with disease process. Marked alteration in the levels of erythrocyte MDA, serum PON, plasma NO and ascorbic acid were observed in the study group subjects, as represented in Table 2.

Table 1: Demographic profile of study group subjects (Mean ± SD).

Particulars	Control group (n=20)	Group I (n=20)	Group II (n=20)
Age (years)	26.5 ± 2.4	29.0 ± 3.0	30.4 ± 3.3
Systolic blood pressure (mmHg)	110 ± 4.0	122 ± 5.0	146 ± 6.2
Diastolic blood pressure (mmHg)	74.0 ± 2.4	80 ± 3.5	96 ± 4.2

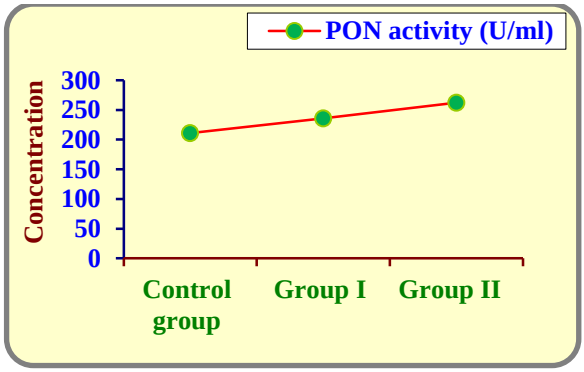

Figure 1: Serum paraoxonase activity in study group subjects.

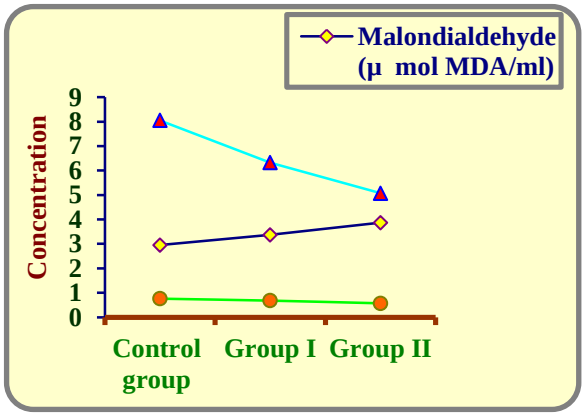

Figure 2: Plasma NO, ascorbic acid and erythrocyte MDA levels in study group subjects.

Table 2: Serum paraoxonase, plasma NO and ascorbic acid levels in study group subjects (Mean ± SD).

Particulars	Control group (n=20)	Group I (n=20)	Group II (n=20)
PON (U/ml)	210.8 ± 15.2	235.7 ± 16..4*	262.04 ± 16.8**
NO level (µmol/L)	8.05 ± 2.5	6.32± 2.1**	5.07± 1.8**
Ascorbic acid (mg%)	0.76 ± 0.16	0.68 ± 0.12*	0.57 ± 0.08**
Malondialdehyde (µmol MDA/ml)	2.95 ± 0.15	3.37 ± 0.17*	3.87 ± 0.20**

*P <0.1: Non-significant; **P <0.05: Significant

Serum PON activity and erythrocyte MDA levels were increased significantly (P <0.05; 24.30% and 31.24% high) in group II patients while these levels were increased insignificantly (P <0.1) in group I subjects (11.82% and 14.43% high) as compared to controls. On the other hand, plasma ascorbic acid levels were found to be decreased significantly (P <0.05) in group II (PIH women) subjects i.e. 25.80% low respectively, as compared to controls but these values do not differ significantly in group I subjects. However, plasma NO levels were significantly low (P <0.05) in both group I (NTP women) and group II (PIH women) subjects i.e. 21.52% and 37.05 % low respectively, as compared to controls. Correlation coefficient (r) between Blood Pressure (BP) and various markers (oxidative stress and endothelial dysfunction) in PIH subjects (Table 3) reflect the association of hypertension with alteration in PON, NO and ascorbic acid; and exert their cumulative effect in the disease pathology. Similarly, elevated PON activity was inversely related with endothelial dysfunction (NO

levels) whereas positively correlated with lipid peroxidation in PIH subjects (Table 4).

Table 3: Correlation coefficient (r) between blood pressure (BP) and various markers (endothelial dysfunction and oxidative stress) in PIH subjects.

Particulars	NO group II	PON group II	MDA group II	Ascorbic acid group II
Blood pressure	- 0.530	+ 0.621	+ 0.724	- 0.653

Table 4: Correlation coefficient (r) between serum PON activity and other parameters (plasma NO, ascorbic acid and erythrocyte MDA) in PIH subjects.

Particulars	Plasma NO (Group II)	Plasma Ascorbic acid (Group II)	Erythrocyte MDA (Group II)
Serum PON	- 0.472	-0.348	+ 0.535

DISCUSSION

Vascular dysfunction related to pre-eclampsia may occur because of the loss of endothelial cell homeostasis due to perturb cell mechanisms which include oxidative stress mediated biomolecular destruction (proteins, lipids, and nucleic acids). In particular, Malondialdehyde (MDA), a toxic aldehydic end product of lipid peroxidation, initiates a complex cascade that promotes prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance leading to hypertension.[4] In the present study, erythrocyte MDA levels were significantly high ($p<0.05$) in study group subjects which suggest the crucial role of lipid peroxidation in hypertension progression and development of pre-eclampsia. Similarly, Adiga et al. documented a significant role of enhanced lipid peroxidation in the development of preeclampsia.[12]

Oxidative stress mediated lipid peroxidation is well controlled by antioxidant enzymes including plasma paraoxonase, an enzyme found in association with HDL contributing it to anti-atherogenic and antioxidant capability. Alteration in the PON activity may have significant effect in cardiovascular complications possibly due to increased production of reactive aldehydes.[5] In the present study, plasma PON activity was found to be increased significantly in PIH subjects with increase in blood pressure. It could be explained on the basis of increased expression of genes responsible for enzyme production in order to overcome the burden of lipid peroxidation and thereby prevent the culprit effect of oxidative stress. Consistent findings have been documented by Yaghmaei et al. who observed a significant increase in serum PON activity due to high 192QR genotype of PON1 in preeclampsia patients.[13] In contrast, Saxena et al. observed depleted levels of PON in patients with hypertension as well as cardiovascular complications.[14,15]

Moreover, association of oxidative stress and development of pre-eclampsia is well supported by marked alteration in non-enzymic antioxidant and marker of endothelial dysfunction such as vitamin C and NO. It has been observed that vitamin C alone can afford protection against the oxidant mediated damage to LDL even though it is not lipid soluble.[16] Gupta et al. in their study also documented that free radicals, mediators of vascular injury, under physiological conditions inactivate and destroy endothelial NO (well-known vasodilator) and thereby leads to increased blood pressure.[3] Plasma ascorbate scavenges oxygen free radicals and increases the availability of NO, thereby reducing the risk of vascular disorders.[17]

In the present study, plasma ascorbate levels were found to be significantly low ($P <0.05$) in PIH patients as compared to healthy controls which direct towards its protective and radical scavenging action in PIH patients. Similarly, marked reduction in vitamin C levels in pre-eclampsia were reported by Gupta et al. and suggested that vitamin C supplementation may reduce the progression of PIH and its complications.[19] Despite its massive role in reduction of PIH development, its synergistic role in the regeneration of α-tocopherol by reducing the α-tocopheroxyl radical and in the prevention of cardiovascular complications is well documented.[18]

In particular, NO is known to be implicated in a number of crucial physiological functions such as gestational vasodilation during pregnancy, penile erection, cerebral blood flow, microbicidal and tumoricidal activities of macrophages and neutrophils.[7,20] Alterations in NO levels have a detrimental role on maternal health leading disease precipitation. In the present study, marked depletion in plasma NO levels were observed in PIH subjects. It could be explained on the basis of its interaction with free radicals. Superoxide anion, produced in excess amount during oxidative stress reacts with NO to form toxic product peroxynitrite anion ($ONOO^-$) and thereby reduces NO bioavailability i.e. an important event in progression of pregnancy induced hypertension.

Furthermore, depleted levels of plasma NO in NTP women, as observed in our study may be due to increased transfer of L-arginine to fetus leading to deficiency of precursor molecule followed by insufficient synthesis of NO during pregnancy. Our findings were also in agreement with the findings of Sahu et al. who also reported the role of decreased NO production in etiopathogenesis of preeclampsia and emphasizes the assessment of NO level as a diagnostic marker of preeclampsia.[21] However, Ranta et al in their study observed increased production of NO in preeclamptic women.[22]

In conclusion, it is obvious that oxidative stress in association with endothelial dysfunction plays a crucial role in the development of preeclampsia. Furthermore, increased plasma paraoxonase activity in order to regulate the free radicals mediated lipid peroxidation and depleted levels of NO are excellent marker of future prediction of PIH development during pregnancy. In addition, our findings also suggest that supplementation of diet rich in antioxidant vitamins may be an effective effort to impede the development of preeclampsia.

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