Estimation of Primary Enzymatic Antioxidants in Pregnancy Induced Hypertension

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Abstract

Introduction: Pregnancy Induced Hypertension (PIH) is a leading cause of fetal growth retardation, infant morbidity, mortality and maternal death. Despite extensive research, there is a limited knowledge of pathophysiology and etiology of PIH. Recent studies have suggested the role of oxidative stress and altered endothelial function in PIH. However, there are very limited studies concerning the antioxidant status throughout normal gestation and in PIH.

Aim: To determine the levels of antioxidants Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in normal pregnancy and in pregnancy induced hypertension. Also, to compare the levels of these antioxidants with controls.

Materials and Methods: 30 non pregnant healthy controls, 30 normal pregnant females and 30 pregnant females with PIH of age group 18-35 years were evaluated for antioxidant levels in blood. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) levels were estimated in erythrocytes and compared with controls. Mean arterial pressure (MAP) was also correlated with antioxidant levels.

Results: The values of SOD were 140.7 ± 37.7 (U/ml), 213.8 ± 68.4 (U/ml) and 195.6 ± 82.7 (U/ml) respectively in PIH, normal pregnancy (NP) and normal control (NC) groups. The values of glutathione peroxidase were 18,602 ± 6,823 (U/L), 20,378 ± 5,871 (U/L) and 20,765 ± 2,986 (U/L) respectively in the above groups. Mean arterial pressures in the above groups were 119.49 ± 11 mm Hg (PIH), 90.46 ± 4.57 mm Hg (NP) and 90.59 ± 6.8 mmHg (NC).

Discussion: There was a significant decrease in SOD levels in PIH compared to normal pregnant and normal controls. There was no statistically significant change in GPx levels among the groups. There was a significant increase in MAP in PIH group compared to the other groups. A negative correlation between MAP and antioxidant levels was observed, which was not significant statistically.

Conclusion: This study showed an altered antioxidant status in PIH, which may be an indirect proof for the existence of oxidative stress in PIH.

Introduction

Pregnancy induced Hypertension (PIH) or preeclampsia is a serious complication of the second half of pregnancy that occurs with a frequency of 5-15%. It is characterised by high blood pressure, urinary loss of proteins, edema and activation of hemostatic mechanisms. According to WHO, this disease is a leading cause of fetal growth retardation, infant morbidity, mortality and maternal death. The etiology of PIH has always remained elusive. Increasing evidence supports the role of oxygen free radicals and lipid peroxidation in the pathophysiologic mechanisms of preeclampsia. Uncontrolled lipid peroxidation is known to play an important role in the pathophysiology of preeclampsia by causing vascular endothelial cell dysfunction. Recent studies suggest that oxidative stress and altered endothelial cell function may occur in PIH. Many studies have shown increase in free radical generation as indicated by an increase in the level of lipid peroxides.

But little is known concerning the enzymatic antioxidant status in normal and preeclamptic pregnancies and there is only scattered information regarding baseline levels of antioxidants in healthy pregnant women. Therefore in the present study, the erythrocyte levels of primary enzymatic antioxidants Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) were studied in non pregnant, healthy pregnant and PIH females.

Methods

The present study was carried out on diagnosed patients of pregnancy induced hypertension (PIH) attending the outpatient and inpatient departments of J.S.S. Medical College hospital, Mysore, Karnataka. Institutional ethical committee clearance was obtained. The study was undertaken on a total of 90 cases of which, 30 pregnancy induced hypertension (PIH) cases, 30 normotensive pregnant females and 30 healthy controls. The diagnosis of PIH was based on the norms of American College of Obstetrics & Gynecologists, i.e, Systolic blood pressure greater.
than 140 mm of Hg or a rise of atleast 30mm of Hg or diastolic blood pressure greater than 90 mm of Hg or a rise of atleast 15 mm of Hg (manifested on two occasions atleast 6 hrs apart) ,with or without proteinuria of 300 mg or greater in a 24 hrs urine collection or 100 mg/dl or > in at least 2 random urine specimens collected 6 or more hours apart. The patients were compared with 30 normal pregnancy cases of similar age group. 30 normal, age matched, healthy non-pregnant females without any h/o hypertension, diabetes mellitus, major illness or factors which may affect the antioxidant status formed the control group.

Samples were collected between 24-36 weeks of gestation from pregnant women and randomly from control. Personal history, which included diet, history of parity, use of vitamin supplements and other drugs, smoking, use of alcohol, previous history of hypertension or diabetes mellitus were noted in all the three groups. Blood pressure, urinary protein estimation to check for associated proteinuria, BMI (kg/m²) was measured at the time of sample collection. Mean arterial pressure (MAP) was calculated.

Patients with conditions that may affect the antioxidant concentrations, such as hypertension, diabetes mellitus and other chronic illnesses were excluded from the study. Patients with family history of hypertension and diabetes, patients taking vitamin A, E and C supplements and other drugs, which are known to affect antioxidant concentrations were also excluded from the study.

Simple random sampling technique was used to select the cases. After obtaining the informed consent, 5 ml of venous blood was drawn from the patients and controls under aseptic precautions in fasting state. Sample was collected in labelled heparinised tubes. It was immediately centrifuged, and RBC’s were washed. The antioxidants superoxide dismutase (SOD) (EC 1.15.1.1) and glutathione peroxidases (GPx) EC 1.11.1.9) were estimated in erythrocytes and plasma. (Kit purchased from Randox laboratories. U.K., RANSOD Cat No. SD 125 , RANSEL Cat No. RS505).

Estimation of Superoxide Dismutase (SOD) :

0.5 ml of whole blood was centrifuged for 10 minutes at 3000 rpm and plasma was aspirated. Then Erythrocytes were washed four times with 3 ml of 0.9% NaCl solution centrifuging for 10 minutes at 3000 rpm after each wash. The washed centrifuged erythrocytes were made up to 2 ml with cold re-distilled water, mixed and left to stand at +4°C for 15 minutes. Superoxide dismutase (SOD) was estimated in erythrocyte lysate by McCord and Fridovich method 6 in a kinetic assay at 37°C. The absorbance was measured at 505nm and the results were expressed as U/mL.

Estimation of Glutathione Peroxidase (GSH-Px):

0.05 ml of heparinised whole blood was diluted with 1 ml of diluting agent and incubated for 5 minutes. Later 1 ml of double strength Drabkin’s reagent was added, mix well and assayed within 20 minutes of adding Drabkins reagent. GSH-Px activity was estimated in the plasma according to Paglia and Valentine7 by a kinetic assay at 37°C by using cumene hydroperoxide as the substrate. The absorbance was measured at 340nm and the results were expressed as U/L. Appropriate Statistical tools like t-test and Karl-Pearson correlation coefficient were applied to test the significant difference among the average SOD, GPx, and MAP parameters and relationship among them.

Results

Illustration-1 shows demographic details of the subjects. There was no statistically significant difference in the mean age group, gestational age and BMI (Body mass index) of PIH and normal pregnancy groups. In the present study, there was a significant increase in the systolic, diastolic and mean arterial pressures (MAP) in PIH compared to normal pregnancy and controls (p<0.001). In the present study erythrocyte SOD levels were 140.7±37.70, 213.8±68.40, 195.6±82.70 U/ml in PIH, normal pregnancy and control groups respectively. Erythrocyte SOD levels were found to be decreased in PIH patients compared to normal pregnancy (p<0.001) and controls (p<0.001). (Illustration-2 & 3). But an increase in SOD levels were seen in normal pregnancy compared to controls, however it was not statistically significant (p>0.05).

Levels of GPx were 18,602 ± 6422.74, 20,378 ± 5870.8 and 20,765 ± 4986.5 U/L respectively in PIH, normal pregnancy and control groups(Illustration -4 & 5).

Discussion

In this study, there is decrease in erythrocyte SOD levels in PIH which is comparable with the previous studies by Sharma et.al 8, Kumar et al 9, Chen et.al 10, Jendryczko11, who have demonstrated a significant decrease in SOD levels in PIH compared to normal pregnancy and non-pregnant controls. Whereas Llurba
et al\textsuperscript{12}, have demonstrated a significant increase in superoxide dismutase levels in PIH compared to normal pregnancy and controls. A significantly reduced SOD activity in PIH may be due to increased attack of free radicals and thus resulted in low production of the enzyme. Lalthenglian et al.\textsuperscript{13} have speculated that the enzyme SOD plays a major antioxidant role in erythrocytes where super oxide anions are continuously generated by the auto oxidation of Hemoglobin. It has been implied that in PIH there could be an increase in the production of super oxide anion, which may inactivate NO (Nitric acid) leading to decreased relaxation and increased vasoconstriction.\textsuperscript{14} Hence erythrocyte SOD activity found low in PIH as compared to the control groups in conformity with most findings.

Ilhan et al\textsuperscript{15} and Kumar\textsuperscript{9} have shown a decrease in SOD levels in normal pregnancy compared to controls whereas Sharma et al\textsuperscript{8} and Hubel et al\textsuperscript{8}, have shown an increase in SOD levels in normal pregnancy.

Data on changes of GPx activity during pregnancy and in PIH are conflicting. In the present study GPx levels indicate no statistically significant change in Glutathione peroxidase levels in normal controls, normal pregnancies and in PIH (P>0.05).(Table -3,Fig-2). The results are in conformity with that of Funei et al,\textsuperscript{16} Carone et al,\textsuperscript{17} and Pyska et al,\textsuperscript{18} studies that showed no change in Glutathione peroxidase levels throughout pregnancy and in PIH. However Kumar et al\textsuperscript{8}, Wang et al,\textsuperscript{19} Hubel et al,\textsuperscript{8}, and Orzan et al,\textsuperscript{20} have demonstrated an increase in Glutathione peroxidase in PIH, whereas Alexa et al,\textsuperscript{21}, Jendryzco et al,\textsuperscript{21}, have demonstrated decreased GPx in PIH similar to SOD.

An explanation for unchanged GPx in present study could be, because normal gestation induces an increase of lipid peroxidation products, whereas antioxidant activity appears stable or increased. In pregnancy complicated by hypertension, a deficiency of the oxidative system balance is detectable, which consistently progresses in the second and third trimester, strongly suggesting a failure of the protective mechanisms.

The protective antioxidant mechanisms are complex and multifactorial, which may also be the reason for conflicting reports regarding antioxidant activities in normal gestation and in PIH. The nature of these multifactorial mechanisms are not yet understood.\textsuperscript{22}

A study by Covas et al,\textsuperscript{23} regarding biological variation of super oxide dismutase in erythrocytes and GPx in whole blood mentions, higher biological variability and lesser diagnostic or screening potential for GPx whereas SOD showed a lower biological variation and lesser sensitivity to environmental and physiological changes, showing SOD as the scavenger enzyme of choice for diagnosis of an alteration in antioxidant status in a pathological situation as well as for screening in population studies.

A negative correlation between antioxidants especially SOD and diastolic and mean arterial pressure (MAP) were observed but was not significant in our study, which is in conformity with that of a study by Sharma et.al,\textsuperscript{8} where as Madazli et.al,\textsuperscript{15}, have shown significant negative correlation between antioxidants and mean arterial pressure. The reason for this may be that it indicates a direct relation of severity of hypertension in PIH with lipid peroxidation, which signifies that lipid peroxidation may be the primary event in the pathogenesis of PIH. Physiological antioxidant defence may be expected to remarkably vary from one individual to another. Therefore, measuring antioxidant levels may be important in predicting one’s susceptibility to oxidative stress.\textsuperscript{24}

**Conclusion**

Based on the results of the present study and data available from literature, it is clear that in PIH there is altered antioxidant status, the decreased enzymes suggesting the indirect evidence for the presence of oxidative stress in PIH. But it remains to be known whether these changes are a cause or consequences of the disease. It is also not clear which is the primary event that triggers the onset of increase in BP in PIH. The decreased antioxidant status, as seen in the present study show a scope for therapeutic augmentation of antioxidant levels in PIH.

**References**

1. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet 2001; 357:53-56
Illustrations

Illustration 1

Shows demographic characteristics of subjects

Illustration -1: Shows demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PIH (N=30)</th>
<th>Normal pregnancy (N=30)</th>
<th>Normal controls (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (Yrs) +SD</td>
<td>22.8±4.14</td>
<td>21.6±2.86</td>
<td>23.8±3.98</td>
</tr>
<tr>
<td>Mean Gestational Age(weeks) +SD</td>
<td>33.4± 1.3</td>
<td>32.6±1.5</td>
<td>-</td>
</tr>
<tr>
<td>Mean Systolic BP</td>
<td>152.4±13.9</td>
<td>118.7±7.4</td>
<td>118.4±10.0</td>
</tr>
<tr>
<td>Mean Diastolic BP</td>
<td>103.7±10.7</td>
<td>76.5±5.2</td>
<td>76.3±6.4</td>
</tr>
<tr>
<td>MAP</td>
<td>119.49±11</td>
<td>90.46±4.57</td>
<td>90.59±6.8</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.2±3.3</td>
<td>23.8±3.23</td>
<td>-</td>
</tr>
</tbody>
</table>
Illustration 2

Showing the mean and standard deviation of SOD (U/ml) in groups PIH normal pregnancy and normal controls

<table>
<thead>
<tr>
<th>SOD (U/ml)</th>
<th>PIH</th>
<th>Normal pregnancy</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D</td>
<td>140.7 ± 37.7</td>
<td>213.8 ± 68.4</td>
<td>195.6 ± 82.7</td>
</tr>
</tbody>
</table>

PIH vs Normal Pregnancy - p<0.001  t-5.126
PIH VS Control - p<0.001  t-3.31
Normal Pregnancy VS Control - P>0.05  t-0.929
Illustration 3

Bar diagram showing mean and standard deviation of SOD between study groups

Illustration -3: Bar diagram showing mean and standard deviation of SOD between study groups
**Illustration 4**

Showing the mean and standard deviation of GPx (U/L) in groups PIH normal pregnancy and normal controls

<table>
<thead>
<tr>
<th>GPx (U/L)</th>
<th>PIH</th>
<th>Normal pregnancy</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±S.D</td>
<td>18,602± 6,822</td>
<td>20,378± 5,870</td>
<td>20,765± 4,986</td>
</tr>
</tbody>
</table>

PIH vs Normal Pregnancy - p>0.05 NS  
PIH VS Control - p> 0.05 NS  
Normal Pregnancy VS Control - P>0.05 NS
Illustration 5

Bar diagram showing mean and standard deviation of GPx between study groups

Illustration - 5: Bar diagram showing mean and standard deviation of GPx between study groups
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