Original Research Article

Pregnancy induced hypertension: lipid peroxidation and antioxidant status

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Received: 21 May 2019
Revised: 06 June 2019
Accepted: 26 June 2019

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ABSTRACT

Background: Pregnancy is a stressful condition accompanied by a high energy demand and increased oxygen requirement. Oxidative stress has been recognized as a significant factor linked to hypertension. Elucidation of antioxidant cascade in patients with pregnancy induced hypertension (PIH) can give insights about the oxidative stress and lead to better management of the condition. It was a prospective case control study to elucidate the parameters of oxidative stress in patients with PIH.

Methods: Levels of Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were elucidated using enzyme linked immunosorbent assay (ELISA) in hypertensive mothers and their age matched pregnant and non-pregnant controls to determine the lipid peroxidation and oxidative stress.

Results: A total of four hundred and twenty study subjects were enrolled in the study. Malondialdehyde levels from mothers with hypertension were significantly higher than their age matched pregnant controls. The results indicate that oxidative stress induced by pregnancy induced hypertension manifests as increased lipid peroxidation.

Conclusion: There is a decrement in anti-oxidant status reflecting the ineffective scavenging of reactive oxygen species resulting in oxidative damage and tissue injury.

Keywords: Catalase, Hypertension, Oxidative stress, Pregnancy induced hypertension, Superoxide dismutase

INTRODUCTION

Pregnancy is a demanding state and imposes nutritional, physiological and biochemical stress on maternal organism.² The adrenal respond to the stress of pregnancy by increased cortical activity, unusually kept within bound by body’s adaptation to stress. When however, the stress is prolonged a conflicting situation occurs and diseases manifest.² Pregnancy is confronted with aggressive and periodic changes in metabolic and physiological profile. Consequently, remarkable and dramatic events occur during this period for sustaining mother and fostering the growth and maintenance of fetus.² Pregnancy while not a disease often accompanied by a high energy demand of many bodily functions and an increased oxygen requirement. One of the adaptive changes in respiratory physiology from 8th week onwards where minute ventilation initially increased by 36% and ultimately reaches to the maximum of 50% or more to meet the requirement of fetus which could rise up to 30-
This triggered aerobic environment should primarily be responsible for raised oxidative stress in pregnancy. Further, over enthusiastic and uncontrolled iron supplementation and inclement environmental factors may further add to oxidative stress. Above all, the feeble anti-oxidant defense, as it is often visible in developing population, could lead to an undesirable level of oxidative stress which could become a discovering cause of many diseases due to oxidative damage of cellular or tissue components. There is increased energy demand and oxygen requirement during pregnancy. As a result, various compensatory adaptive changes occur with advancing pregnancy to meet the increasing demands for proper bodily functions of mother to fulfill the requirement of fetus, and thereafter such conditions may be responsible for raised oxidative stress in pregnancy. From early pregnancy the human placenta influences maternal homeostasis which is rich in mitochondria and when fully developed consumes about 1% of the basal metabolic rate of the pregnant women. It is also highly vascular and is exposed to high maternal oxygen partial pressure. These characteristics explain in part, the generation of superoxide because about 5% of all electrons in the mitochondrial respiratory chain leak out of mitochondria. The human placenta is hemomonochorial, meaning that only one chorioc layer exists between maternal and fetal bloods, favoring exchange of gases, nutrients and metabolic products. Initially, the placenta has a hypoxic environment. As it matures its vascularization develops, it changes to an oxygen rich environment and its abundant mitochondrial mass favors the production of reactive oxygen species (ROS), which increases free iron liberated from iron-sulphur clusters. Oxidative stress peaks in the second trimester of pregnancy, ending what appears to be a vulnerable period for fetal health and gestational progress. Conditions restricted to pregnancy such as gestational hypertension, insulin resistance and diabetes exhibit exaggerated indications of free radicals. The International Society for the Study of Hypertension in Pregnancy (ISSHP) defines pregnancy induced hypertension as de novo hypertension with blood pressure ≥ 140/90 after 20 week of gestation. Oxidative stress has been identified as an important factor associated with hypertension. An imbalance caused due to increased reactive oxygen species (ROS) production and/or reduced antioxidant systems results in oxidative stress. However, the mechanisms by which vascular oxidative stress contributes to hypertension are not completely understood. Also, whether oxidative stress is a cause or a consequence of hypertension remains to be established. Needless, to say that pregnant women are more prone to health problems especially in our population and these observations naturally call for determining the pattern of oxidative stress and anti-oxidant cascade in pregnancy. So the present study is designed to study lipid peroxidation product (MDA; indicator of oxidative insults) and enzymatic antioxidants (SOD and CAT) (free radical scavenging players) in hypertensive mothers and their age matched pregnant and non-pregnant controls.

**METHODS**

**Study design**

The present study is a hospital-based study conducted in the Department of Clinical Biochemistry in collaboration with Department of Obstetrics and Gynecology at Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Soura. All the study subjects were born in the Kashmir division of J&K state India and were attending Department of Gynecology, Sheri-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar were included in the study. A pre-informed consent obtained from each patient were recorded in a questionnaire.

The study was approved by the Ethical Clearance Committee of Sher-I-Kashmir Institute of Medical Sciences (SKIMS) (SIMS 1 131/IEC-SKIMS/2018-228, dated: 28.03.2018).

**Sample size and collection**

A total of four hundred and twenty (n=420) study subjects ranging in the age from 20-40 years attending the clinic of Obstetrics and Gynecology were enrolled in the study. Out of total 420 subjects, 150 normal non-pregnant; 150 normal pregnant and 120 hypertensive pregnant women were studied.

**Inclusion and exclusion criteria**

Clinically diagnosed hypertensive mothers with gestational age of >20 weeks; normal pregnant women in the same gestational age and normal non pregnant women were included while as women with twin pregnancies, known hypertension, renal diseases, liver diseases, severe anemia and diabetes were excluded from the study.

**Sample collection**

There were 1 mL of peripheral blood was obtained from each subject in a sterile 02 mL microfuge clot activator tubes which were later centrifuged in a refrigerated centrifuge and serum was obtained. Serum obtained was stored at -80°C till the enzyme investigations were performed. Serum samples were used for analysis of various oxidative (MDA) and anti-oxidative enzymes (SOD and Catalase).

**Estimation of enzyme concentration**

Enzyme concentrations were estimated by commercially available kits using enzyme linked immunosorbent assay (ELISA) (Lisa Scan II, TransAsia Biomedicals, Pvt, Ltd)
**Estimation of MDA levels (TBARS Assay)**

MDA is a marker a lipid per-oxidation. TBARS assay was measured calorimetrically using TBARS assay kit (Cayman Chemical, USA) according to the manufacturer’s protocol. Thiobarbituric acid was reacted with MDA in the samples at 100°C and the product, MDA-TBA2 was then measured calorimetrically. MDA is a naturally occurring product of lipid peroxidation derived from polyunsaturated fatty acids, and the results therefore reflected the lipid peroxidation in the samples. Absorbance of each sample at 530nm was measured by micro plate reader and plotted on a standard curve generated by serially diluted standard provided by the manufacturer to obtain the amount of TBARS (nmol/mm). The MDA-TBA adduct formed by the reaction of MDA & TBA under high temperature and acidic conditions is measured calorimetrically at 530 nm.

**Estimation of SOD Levels (SOD Assay)**

SOD activity was measured calorimetrically using super oxide assay kit (Cayman Chemical, USA) according to the manufacturer’s protocol. This assay used tetrazolium to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD is defined as the amount of enzyme needed to produce 50% dismutation of superoxide radicals. Absorbance of each sample at 440nm was measured by micro-plate reader, and then plotted on a standard curve generated by serially diluted standard provided by the manufacturer to obtain SOD activity. The SOD assay measures all types of of SOD. The assay provides reproducible, simple and fast tool for assaying SOD activity in serum. The reaction is initiated by adding 20 microliters of diluted xanthine oxidase to all the wells being used. The absorbance is measured at 440-460 nanometers using plate reader.

**Estimation of CAT levels (Catalase Assay)**

The activity of catalase in the plasma using the CAT peroxidase ability for enzyme activity determination where aliphatic alcohols function as specific CAT substrates to form an aldehyde was analyzed using the Cayman’s Catalase Assay kit. The formaldehyde formed in the reaction between the enzyme and methanol in the presence of optimal hydrogen peroxide concentration was measured calorimetrically with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole (Purpald). The absorbance was measured at 540nm on the micro plate reader and the reaction rate was determined using the formaldehyde standard curve. Results were expressed as units per milliliter plasma. One unit was defined as the amount of enzyme causing the formation of 1.0 nmol formaldehyde per minute at 25°C.

**Statistical analysis**

Values were reported as the mean ± standard error of the mean. P values were calculated using Student’s t test (2 tailed) with a significance level of P<0.05 using Graph Pad Prism 5 (Graph Pad Software, Inc., San Diego, Calif).

**RESULTS**

The parameters of oxidative stress that were estimated in the serum of non-pregnant, normal healthy pregnancy and pregnancy with hypertension are MDA, SOD and catalase. Table 1 shows the maternal age and gestational age of the respective groups. Mean systolic blood pressure (BP) of normotensive pregnant women was found to be 118.2± 6.1 while as for hypertensive mothers had a mean systolic BP of 148.2±5.5 A significant difference in the mean diastolic BP was also observed, the mean diastolic BP for the normotensive women was 77.3±6.2 while as for the hypertensive mothers was the 96.4±5.8 (Table 2).

**Table 1: Age (in years), gestational age (in weeks) in normotensive pregnant and hypertensive pregnant.**

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Non-Pregnant group</th>
<th>Normotensive pregnant</th>
<th>Hypertensive pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age in weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive pregnant</td>
<td>38.29±2.32</td>
<td>38.32±3.23</td>
<td></td>
</tr>
<tr>
<td>Hypertensive pregnant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All values are mean±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Systolic and Diastolic Blood Pressure (BP) of normotensive pregnant women and hypertensive pregnant.**

<table>
<thead>
<tr>
<th>Blood Pressure (BP) (mmHg)</th>
<th>Normotensive Pregnant (n=150)</th>
<th>Hypertensive Pregnant (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Systolic</td>
<td>118.1±6.1</td>
<td>148.2±5.5</td>
</tr>
<tr>
<td>Mean Diastolic</td>
<td>77.3±6.2</td>
<td>96.2±5.8</td>
</tr>
</tbody>
</table>

The levels of MDA (lipid peroxidation marker) in the pregnant group, hypertensive pregnant and normal subjects is compared in Table 3. The MDA level is significantly higher in the hypertensive pregnant coupled with gestation as compared with the normotensive pregnant group. The levels of MDA with normal pregnancy are in turn higher than the age matched non-pregnant group.

The levels of anti-oxidant enzymes super oxide dismutase and catalase which are used as markers of oxidative stress are summarized in (Table 4).

A decline in the levels of SOD is observed in pregnant groups (with hypertensive pregnant and normotensive pregnant) as compared with the non-pregnant group. The levels of catalase were also found to be considerably lower in the pregnancy induced hypertension group as
compared with the pregnant and normal healthy subjects (Table 5).

Table 3: MDA levels obtained in study groups.

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>MDA (^*) Levels in (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Pregnant group</td>
<td>2.98±0.82</td>
</tr>
<tr>
<td>Normotensive Pregnant group</td>
<td>3.65±1.52</td>
</tr>
<tr>
<td>Hypertensive pregnant group</td>
<td>9.28±0.99**</td>
</tr>
</tbody>
</table>

\(^*\) Lipid Peroxidation levels as indicators of free radical damage
\(^*\) As compared to non-pregnant group
\(^*\) As compared to pregnant group (P<0.05)

Table 4: SOD enzyme levels obtained in study groups.

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>SOD (^*) (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant group</td>
<td>3.99±0.07</td>
</tr>
<tr>
<td>Normotensive Pregnant group</td>
<td>1.38±0.28</td>
</tr>
<tr>
<td>Hypertensive pregnant group</td>
<td>0.96±0.08**</td>
</tr>
</tbody>
</table>

\(^*\) As compared to non-pregnant group
\(^*\) As compared to pregnant group (P<0.05)

Table 5: Catalase enzyme levels obtained in study groups.

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Catalase levels (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant group</td>
<td>97.33±2.53</td>
</tr>
<tr>
<td>Normotensive Pregnant group</td>
<td>85.38±3.20</td>
</tr>
<tr>
<td>Hypertensive group</td>
<td>70.56±4.98**</td>
</tr>
</tbody>
</table>

\(^*\) As compared to non-pregnant group
\(^*\) As compared to pregnant group (P<0.05)

The MDA level is significantly higher in hypertensive mothers as compared with the normotensive pregnant group. The levels of MDA with normal pregnancy are in turn higher than the age matched non-pregnant. A decline in the levels of SOD is observed in pregnant groups (with hypertension and without hypertension) as compared with the non-pregnant group. The levels of catalase were also found to be considerably lower in the gestational hypertension group as compared with the pregnant and normal healthy subject.

DISCUSSION

Pregnancy is confronted with aggressive episodes of progressive and periodic changes in metabolic and physiological profile. Pregnancy though not a disease is often accompanied by a high energy demand and increased oxygen requirement. Hypertension during pregnancy continues to be one of the leading causes of maternal and perinatal mortality and morbidity globally. During pregnancy, apoptosis may be a normal part of a tissue to embryonic development and placental functioning, or the response of tissue to exogenous stimuli such as cytotoxic agents, oxidative stress, or hypoxia. The role of oxidative stress in this progress is suggested by finding that ROS appear in many forms of apoptosis, and the exogenous slow dose administration of oxidants can initiate the apoptotic process. Several reports have indicated increased apoptosis and shedding of placental fragments into the maternal circulation during pathological pregnancy. A longer-term burden of gestational hypertension exists as women who develop it are two and a half times more likely to display ischemic heart disease later in life and infants born to hypertensive mothers are at a increased risk of developing respiratory diseases and experiencing long-term neurological morbidity. Persistent hypertension causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose oxidation and protein glycosylation. Free radicals are generated as by-products of normal cellular metabolism, however several conditions are known to disturb the balance between ROS production and cellular defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. ROS attack the phospholipids of cell membranes and react with polyunsaturated fatty acids to form lipid peroxides, resulting in cell injury and have been proposed to be a promotor of lipid peroxidation and the endothelial cell dysfunction that is commonly associated with disorders of pregnancy. Authors found that the parameters of oxidative stress thus vary with the gravidity status. Serum MDA, SOD and catalase were analysed. It was found that pregnant women have increased oxidative stress as shown by remarkable increase in lipid peroxidation (MDA). However, the malonaldehyde levels were significantly increased in mothers with pregnancy induced hypertension when compared to controls. These results show that oxidative stress as coupled by alterations in the levels of MDA may be contributing factor in the pathogenesis of pregnancy induced hypertension. A significant increase in this circulating indicator of oxidative stress in pre-eclamptic mothers has been reported by Khatri et al. Increase in oxidative stress markers in turn have been found to damage the maternal vascular endothelium leading to the elevation in diastolic pressure which further worsened the state of hypertensive mothers. A delicate balance of anti-oxidant enzymes is necessary for reducing oxidative stresses. Anti-oxidant molecules such as SOD and catalase work in unison within the cell to metabolize these harmful forms of oxygen into non-injurious substances. SOD is an intracellular anti-oxidant enzyme helping in removing superoxide anion. We noticed a significantly lower activity of SOD and catalase in blood of mothers with hypertension as compared to their age matched pregnant controls and non-pregnant controls. A significant decrease in SOD activity in women with hypertensive disorders as compared to normal women has been reported by Pandey et al. The concentration of...
catalase was decreased significantly in women with pre-eclampsia as compared to normotensive pregnant women which is in accordance with the previous reports.\textsuperscript{26-28} However, some studies have depicted increased activity of catalase enzyme in hypertensive disorders.\textsuperscript{29,30} Taken together the results show that lipid peroxidation plays a major role in the pathogenesis of pregnancy induced hypertension and to prevent further injury more of anti-oxidants are utilized and hence the levels of anti-oxidants decrease. Consequently, the enzymes of the first line of defense i.e SOD and Catalase decrease. A simultaneous decline in the activities of both SOD and Catalase was observed thereby damaging the important avenue of enzymatic anti-oxidant defense. These observations further confirm that pregnancy coupled with hypertension may lead to ineffective scavenging of reactive oxygen species resulting in oxidative damage and tissue injury.

CONCLUSION

Our results show substantial decline in endogenous antioxidant status and rise in oxidative stress markers by pregnancy induced hypertension pointing to the key role of hypertension in initiating stress induced oxidative damage.

ACKNOWLEDGEMENTS

The authors acknowledge the staff of the Department of Obstetrics and Gynaecology, SKIMS, Soura who facilitated the blood sampling.

\textit{Funding: The Study was Funded by Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India (Grant no# SIMS/Acad/156 )

Conflict of interest: None declared}

Ethical approval: The study was approved by the Institutional Ethics Committee of Sher-I-Kashmir Institute of Medical Sciences (SKIMS) (SIMS I 131/IEC-SKIMS/2018-228, dated: 28.03.2018)

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