Estimation of malondialdehyde as oxidative factor & glutathione as early detectors of hypertensive pregnant women

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Abstract

Gestational hypertension is characterized by the onset of hypertension after 20 weeks of gestation without any maternal or fetal features of preeclampsia & occurs at about 6% to 8% of pregnancies. The exact cause is not known. Oxidative stress describes the damage that occurs when reactive oxygen species (ROS) overwhelm the antioxidant defenses of the host. Oxidant stress may play an important role in the pathogenesis of hypertension in pregnancy & may be a final common pathway leading to tissue damage. A cross sectional study was conducted in Tikrit teaching hospital during the period from the beginning of September 2007 to the end of may 2008. There were 81 pregnant women participated in this study & were classified into 4 groups:-

1-Group 1: consists of 34 primigravida hypertensive pregnant.
2-Group 2: consists of 12 primigravida normotensive pregnant.
3-Group 3: consists of 27 multiparaus hypertensive pregnant women.
4-Group 4: consists of 8 multiparaus normotensive pregnant women.

The serum MDA level & serum glutathione (GSH) level for hypertensive pregnant women & normotensive pregnant women were measured. There are no significant differences between hypertensive pregnant women & normotensive pregnant regarding age, body weight, height & BMI. There was highly significant increase in serum level of MDA in hypertensive pregnant women. This is becaus MDA consider to be the most sensitive & final stage of peroxidation. In the present study, it was observed significant decreases in the activity of catalase represent by glutathione in hypertensive patients as compared with controls.

Key words: pregnancy, hypertension, MDA, serum glutathione

Introduction

Normal pregnancy is characterized by a fall in blood pressure, detectable in the first trimester & usually reaching a nadir in the second trimester, blood pressure rises towards pre-conception levels towards the end of the third trimester (1). Normal pregnancy associated with physiological changes such as, increased in cardiac output of about 30 to 40%, increase in blood volume about 50%, decrease in peripheral vascular resistance, increase in renal blood flow about 70% & decrease in blood pressure (2,3).

Gestational hypertension is characterized by the onset of hypertension after 20 weeks of gestation without any maternal or fetal features of preeclampsia & occurs at about 6% to 8% of pregnancies. The exact cause is not known. The primary characteristic of this condition, is a high blood pressure reading higher than 140 / 90 mmHg significant increase in one or both pressures (1, 2,3,4).

There is no satisfactory treatment of this disease, except steps to avoid or reduce complications such as hypertension and eclamptic seizures and early delivery.

It is hypothesized that preeclamptic condition could be explained by alterations in the function of vascular endothelium and placenta, likely in response to reduced perfusion, produces circulating factor(s) that alters endothelial function. All these factor(s) are products of oxidative stress (5,6). However, oxidative stress describes
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the damage that occurs when reactive oxygen species (ROS) overwhelm the antioxidant defenses of the host. Oxidant stress may play an important role in the pathogenesis of hypertension in pregnancy & may be a final common pathway leading to tissue damage (5).

Malondialdehyde is an aldehyde considered to be the terminal compound & the most important marker for monitoring lipid peroxidation & oxidative damage induced by ROS which is strongly associated with development of serious disease, it is also considered as a thiobarbituric reactive substance (6, 7).

In view of the contradictory reports and the seriousness of preeclampsia (3,4,5 ), the present study included the determination of the activities of MDA & GSH in the blood of normal pregnant women and hypertensive non-preeclamptic pregnant women. Therefore, the aim of the study is to determine the concentration of MDA as marker for oxidative stress & glutathione as a marker of antioxidant incase of gestational pregnancy in primigravida & multigravida pregnant women.

**Patients & Methods**

A cross sectional study was conducted in Tikrit teaching hospital during the period from the beginning of September 2007 to the end of may 2008.

All pregnant women were asked by special form of questionnaire about the age, present of any disease, gestational period & family history.

81 pregnant women were participating in this study (46 primigravida & 35 multiparaus pregnant women); & were classified into 4 groups:-

1-Group 1: consists of 34 primigravida hypertensive pregnant.
2-Group 2: consists of 12 primigravida normotensive pregnant.
3-Group 3: consists of 27 multiparaus hypertensive pregnant women.
4-Group 4: consists of 8 multiparaus normotensive pregnant women.

Patients with chronic hypertension, diabetes mellitus, hyperthyroidism, edema, protein urea, & kidney diseases were excluded from the study.

Physical measurements include:-

Body weight was measured to the nearest 100 gm, height was measured to nearest cm. Body mass index was calculated from body weight in Kg divided by height in meter square (BMI). Blood pressure was measured after 5 minute rest.

The present study was carried out with the prior approval of the local ethical committee. All the patients mentioned above gave their consent in writing, and the objectives of the study were fully explained to them in detail prior to taking consent. They were always instructed to fast between 9 p.m. till time of blood collection.

Blood sample was done by taking five ml of venous blood & was obtained from the basalic vein in the anticubital fossa of each pregnant woman & drawn by using plastic syringe.

Venous blood transferred to free tubes to allow clotting, centrifuged at 3000 rpm for 5 minutes, & the serum was then removed to another plain tube & store at -2C until the time of analysis, sample showing hemolysis was discarded.

Clinical examination and history taking excluded patients with diabetes, ischemic heart disease, a history of stroke, kidney disorders or other conditions of known free radical etiology & chronic hypertension. The criteria for dividing women into normotensive and hypertensive groups have been set at a blood pressure of 140/90 mmHg or higher, without proteinuria and edema.

Determination of MDA Principle

Malondialdehyde in serum was separated & determined as conjugate with TBA. Serum proteins were precipitated by TCA & then removed by
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Centrifugation. The MDA – TBA complex was measured at 534 nm (8).

Reagents
Reagent 1:
(Trichloroacetic acid 17.5%): 17.5 grams of TCA were dissolved in 100ml of distilled water; the reagent was kept into a sealed glass bottle to prevent evaporation.
Reagent -2 :(Trichloroacetic acid 70%).
Reagent – 3 (Thiobarbituric acid 0.6%): 600 mg of TBA were dissolved in 100ml of distilled water; the reagent was kept into a sealed glass bottle to prevent evaporation.

Procedure
The reaction was performed in 18 x 150 mm Pyrex test tube labeled as: test & blank, into which the following reagents were pipette as follow:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Dist. Water</td>
<td>……</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

The tubes were mixed well & incubated in boiling bath for 15 minutes, allowed to cool, then the tubes let to stand at room temperature for 20 minutes. Then the tubes centrifuged at 2000 rpm for 15 minutes & then the supernatant layer was read at 534 nm.

D- Calculation;
The concentration of MDA (nmol/ml) was calculated by using the following formula:
Concentration of the test= Abs (test) – Abs (blank) / 1.56 x 1000000

Determination of reduced glutathione (GSH).
Serum (0.2 ml) were used in the assay. The GSH was made to react with 5’5’-dithiobis (2-nitrobenzoic acid) [DTNB], which reacts with sulfhydryl groups, to develop a stable color. The absorbance was measured at 412 nm and GSH content was expressed as µmol/gHb.

Statistical analysis was done by using student T test. The statistical t-test was considered significant with a p-value of 0.05 or less. All data were presented as a mean & standard deviation (SD).

Results
Table 1 Show the characteristics of pregnant women (mean & standard deviation). While, table 2 show the distribution of pregnant women according to the presence or absence of hypertension

The value of systolic blood pressure in hypertensive pregnant women is highly significant than that of normotensive pregnant women (table 1). There are no significant differences between hypertensive pregnant women & normotensive pregnant regarding age, body weight, height, & BMI. The mean value of hemoglobin for hypertensive pregnant was 11.6 ± 0.11 gm/dl. While the heamoglobin value for normotensive pregnant women was 10.77 ± 0.49. There is no significant difference between hypertensive pregnant women & normotensive pregnant regarding blood hemoglobin.

The mean serum MDA level for primigravida hypertensive pregnant women was found to be 81.9 ± 11 micromole/L. While, the mean serum MDA level for normotensive pregnant women was found to be 36.3 ± 4.5 micromol / L.

There is significant difference at p value of less than 0.05 between hypertensive pregnant women & normotensive pregnant regarding serum MDA level in of primigravida pregnant women (Table 3).

In regard to multigravida pregnant women, the mean serum MDA level for multigravida hypertensive pregnant women was found to be 72.8 ± 12 micromole/L. While, the mean serum MDA level for normotensive pregnant women was found to be 31.8 ± 8.0 micromol / L.
There is significant difference at p value of less than 0.05 between hypertensive multigravida pregnant women & normotensive pregnant regarding serum MDA level in of multigravida pregnant women (Table 4).

The mean serum GSH level for primigravida hypertensive pregnant women was found to be $408 \pm 58$ micromole/L. While, the mean serum GSH level for normotensive pregnant women was found to be higher than that of hypertensive pregnant women ($933 \pm 115$ micromol / L). There is significant difference at p value of less than 0.05 between hypertensive pregnant women & normotensive pregnant regarding serum GSH level in of primigravida pregnant women (table 3).

In regard to multigravida pregnant women, the mean serum GSH level for multigravida hypertensive pregnant women was found to be $299.2 \pm 69$ micromole/L. While, the mean serum level for normotensive pregnant women was found to be higher than that of hypertensive pregnant women $371.48 \pm 98$ micromol / L. There is significant difference at p value of less than 0.05 between hypertensive multigravida pregnant women & normotensive pregnant regarding serum GSH level in of multigravida pregnant women (table 4).

There was highly significant increase in serum level of MDA in hypertensive pregnant women. This because MDA consider to be the most sensitive & final stage of peroxidation & it considered as a marker of pro-oxidant level & indicator of oxidative stress, & it is the end product of lipid peroxidation.

**Discussion**

In the present study, the lipid peroxidation product like malondialdehyde (MDA) levels has been measured in plasma of hypertensive pregnant women. It was found that higher O2 free radical production, evidenced by increase levels of MDA in hypertensive pregnant women. The present study show that, there is significant difference between hypertensive & normotensive pregnant regarding serum MDA level in of primigravida pregnant women.

So, as in table 3, the mean serum MDA level for primigravida hypertensive pregnant women was found to be $81.9 \pm 11$ micromole/L. While, the mean serum MDA level for normotensive pregnant women was found to be $36.3 \pm 4.5$ micromol / L.

In regard to multigravida pregnant women, the mean serum MDA level for multigravida hypertensive pregnant women was found to be $72.8 \pm 12$ micromole/L. While, the mean serum MDA level for normotensive pregnant women was found to be $31.8 \pm 8.0$ micromol / L.

There is significant difference between hypertensive multigravida pregnant women & normotensive pregnant regarding serum MDA level in of multigravida pregnant women.

Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in the hypertensive patients (9, 10). These O2 species in turn can oxidize many other important biomolecules including membrane lipids. The lipid peroxides & free radicals may be important in pathogenesis of PIH (11,12,13, 14).

In similar previous study was done on pregnant women with pregnancy induced hypertension, it was found that there was a significant increase in erythrocytes MDA levels, activates of SOD, & GP level (9).

In contrast to the present study, some studies have reported that there are no evidences of increased lipid peroxidation in PIH (15).

However, in regarded to glutathione (GSH), the mean serum GSH
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level for primigravida hypertensive pregnant women was found to be lower than that the mean serum GSH level for normotensive pregnant women. Also, in regard to multigravida pregnant women, the mean serum GSH level for multigravida hypertensive pregnant women were found to be (299.2 ± 69 micromole/L) lower than that of normotensive pregnant women (371.48 ± 98 micromol / L). In both primigravida & multigravida, there is a significant reduction in the levels of serum GSH of hypertensive pregnant women as compare with normotensive pregnant women. The decrease in this non-enzymatic antioxidant parameter may be due to the increased turn over, for preventing oxidative damage in these hypertensive patients suggesting an increased defense against oxidant damage in PIH (16). In the present study, we have observed significant decreases in the activity of glutathione (GSH) in hypertensive patients as compared with controls.

The results of the present study conclude that in hypertensive pregnant women have a high oxygen free radical productions & decreased catalase activity supports the higher oxidative stress hypothesis in pregnancy induced hypertension. In some previous study, the increased activities of antioxidanant enzymes may be a compensatory regulation in response to increased oxidative stress (9,17,18).

Lipid peroxides could be a part of the cytotoxic mechanisms leading to the endothelial injury. The decreased concentrations of the glutathione & antioxidant vitamin status supports the hypothesis that lipid per oxidation is an important causative factor in the pathogenesis of PIH (2, 4, 6, 9,18,19).

Glutathione and glutathione related enzymes are one of the major antioxidant systems within body. GSH is the most abundant thiol-based antioxidant, and is found primarily in the reduced form, with intracellular concentration up to 11 mM and provides sulfhydryl-buffering capacity. It also conveys an antioxidant power through the direct inactivation of ROS or by acting as an electron donor for GPx that reduces H2O2 to water (20). It has been suggested that the high levels of GSH may act as a compensatory mechanism in preeclampsia to prevent excessive lipid peroxidation via membrane bound GPx (21).

In present study the serum GSH concentration was very significantly lowered in hypertensive non-preeclamptics pregnant women. The present study clearly indicate that GSH in serum decreased profoundly in PIH pathophysiological conditions with a parallel increase in MDA. The present findings regarding serum MDA & GSH in agreement with previous work (22).

Therefore, the present study recommended that PIH should be treated with antioxidant substances such as vitamin C & E in the initial stage of the disease may be useful as secondary treatment to prevent the adverse effect of oxidative stress in hypertensive pregnant women. Groups revealed a significant difference (22–29%), which shows that vitamins C and E consumption prevented the fall in antioxidant enzymes activities (6,13,17,19).

References
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**Table(1)** Show the characteristics of pregnant women (mean & standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive pregnant women</th>
<th>Normotensive pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.8 ± 6.8</td>
<td>27.2 ± 5.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>80.53 ± 8.5</td>
<td>75.54 ± 8.85</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.8 ± 2.2</td>
<td>159.3 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/M2)</td>
<td>30.11 ± 2.6</td>
<td>29.6 ± 2.8</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>11.6 ± 0.11</td>
<td>10.77 ± 0.49</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>149.3 ± 7.7</td>
<td>114.5 ± 10.4</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>98 ± 9.6</td>
<td>70.5 ± 10.4</td>
</tr>
</tbody>
</table>

**Table(2)** Distribution of pregnant women according to the presence or absence of hypertension

<table>
<thead>
<tr>
<th></th>
<th>Primigravida</th>
<th>Multipars pregnant women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive women</td>
<td>34</td>
<td>27</td>
<td>61</td>
</tr>
<tr>
<td>Normotensive</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>35</td>
<td>81</td>
</tr>
</tbody>
</table>

**Table(3)** Show the values of serum MDA & GSH of primigravida pregnant women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypertensive women n=34</th>
<th>Normotensive n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (micromole/L)</td>
<td>81.9 ± 11</td>
<td>36.5 ± 4.5</td>
</tr>
<tr>
<td>GSH (micromole/L)</td>
<td>408 ± 58</td>
<td>933 ± 115</td>
</tr>
</tbody>
</table>

**Table(4)** Show the values of serum MDA & GSH of multigravida pregnant women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypertensive women</th>
<th>Normotensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (micromole/L)</td>
<td>72.8 ± 12</td>
<td>31.8 ± 8.0</td>
</tr>
<tr>
<td>GSH (micromole/L)</td>
<td>299.2 ± 69</td>
<td>371.48 ± 98</td>
</tr>
</tbody>
</table>