Histological and Physiological Effects of Diabetes on Placenta of Pregnant Females

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Abstract

Gestational diabetes mellitus is a state of glucose intolerance occurring with the onset of pregnancy causing much hazard for the mother and baby. It may be associated with macrosomia and disturbed Redox status which may be an index for great threat for the fetus in this condition.

The present study aimed to find out the effect of the oxidative stress on the redox status (GSH, SOD, CAT & GPx) and to investigate the histological status of placenta in GDM compared with healthy pregnant women.

Twenty women with GDM and ten control healthy pregnant cases were investigated. All women underwent caesarean sections. Antioxidant parameters were studied in blood and placenta of GDM compared with control ones. The results of this study showed that maternal oxidative stress has overwhelmed its defense system, where GSH, SOD and CAT levels, except that of GPx, were decreased, effects that were accompanied by increased lipid peroxidation. On the placental side, the redox system picture was better, where contents/activity of GSH, CAT and GPx were elevated, however, this was associated by an increased MDA content and a decreased SOD activity. The study also revealed significant correlations between blood and placental oxidative stress parameters. It is believed that administration of antioxidants is required as complementary therapy, once the mother's blood sample has shown change in the redox parameters. So, the clinician can use the oxidative stress parameters as a good new method to follow-up pregnant women, to avoid any dramatic effects that may influence the outcome of pregnancy.

Key Words: Gestational diabetes mellitus (GDM) — Placenta Pregnant females.

Introduction

GESTATIONAL diabetes mellitus (GDM), a "pre-diabetic" state, defined as a glucose intolerance of varying severity occurring with the onset of pregnancy. It is associated with significant maternal and fetal morbidity.

There are findings which indicate the association of pregnancy complicated with diabetes and/or macrosomia with disturbed redox status that may represent sensitive indices of fetal/neonatal threat in GDM mothers [2].

Gestational diabetes mellitus (GDM), a "pre-diabetic" state, is defined as a glucose intolerance of varying severity with onset or first recognition during pregnancy [3] and is characterized by hyperglycemia, insulin resistance and hyperlipidaemia [4]. It complicates about 7% of pregnancy [5] and is a significant cause of fetal macrosomia, perinatal mortality and adverse maternal outcomes of pregnancy, such as preeclampsia, cesarean delivery and birth trauma [6]. Most neonatal clinical problems attributed to GDM include hypoglycemia, polycythemia, hyperbilirubinaemia, hypocalcaemia, and respiratory distress syndrome (RDS) [7]. It also participates in cardiovascular diseases after pregnancy as compared with their counter parts who remained normo-glycemic during pregnancy. Furthermore, women with GDM and their offspring are at high risk for the development of diabetes later in life [8].

Macrosomia is one of neonatal complications and defined as birth weight above 4kg [5,9]. It is associated with visceral organ hypertrophy, accompanied by increased muscle mass and fat deposition with the exception of the kidney and brain [7].

GDM can be halted or at least slowed by medical nutrition therapy (MNT) and exercise, insulin therapy or oral medication [5]. Education for a woman with GDM can include dietary counseling, technique and demonstration of insulin utilization. Additional education pertains to lifestyle measures that patients can employ for optimal management of GDM and for reducing the postpartum long-term complications that may affect both mother and child [10].

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Oxidative stress refers to the overwhelming of endogenous defense systems by the overproduction of reactive oxygen species (ROS) that jeopardize cell survival. Ample evidence exists that links diabetes, particularly type 2, with oxidative stress, as identified by increased oxygen radical damage biomarkers along with abnormalities in the antioxidant defenses. Nevertheless, there is limited data available regarding the presence of oxidative stress in women with GDM.

Apart from the diabetic maternal status, normal pregnancy per se, increases oxidative stress, compared to normal non-pregnant women. This stress state arises from increasing placental mitochondrial activity and excessive release of ROS, which occur at certain windows in placental development and may contribute to pathological processes in GDM.

Placenta being an interface between maternal and fetal circulations may play a crucial role in protecting the fetus from adverse effects of the maternal dysfunctions. The protective effect of placenta involves several biochemical safety mechanisms, including defense enzymes and antioxidants. Of the endogenous defense tactics against superoxide and hydrogen peroxide mediated injury are the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Thus, variation of placental development may increase risk of complicated pregnancy associated with diabetes.

Material and Methods

In this study a total of thirty pregnant women, twenty with GDM and ten healthy non-diabetic, were recruited from the antenatal outpatient clinic at Om El Masreen Maternity Hospital. The women were aged ranging between 20-35 years, carrying singleton alive fetus and were undergoing caesarian section for obstetric cause. Patients were in good general health, and those with cardiac, hepatic, renal, or other chronic diseases, as well as pre-eclampsia, or antepartum complications were excluded. In addition, women with smoking habit, alcohol addiction, twin pregnancy or any other conditions, which had been documented to affect oxidative stress measurement, other than gestational diabetes mellitus, were considered ineligible.

The twenty GDM patients were diagnosed following the criteria of the American Diabetes Association (2010). Oral glucose tolerance test (OGTT) was performed in all pregnant woman at 24-28 weeks of gestation, where 100gm glucose was administered orally and the blood level was traced 1,2 and 3 hours thereafter. The other ten healthy pregnant women participated in the study as normal control group. Patients with GDM were controlled by both dietary instructions and insulin.

Gestational age in both GDM and healthy pregnant women was confirmed by menstrual history and clinical examination during the first trimester and by ultrasound examination after 20 weeks. At delivery placental tissues and blood samples were collected from all pregnant women.

1- Placental tissue collection:

Tissue processing commenced within 5 min of delivery and a fragment of the placenta was obtained from the central region of the fetal surface including placental villi, but excluding the amniotic layer.

Collected tissues were rinsed with a phosphate buffered saline solution (PBS, pH 7.4) containing 0.16mg/m1 heparin to remove any red blood cells and clots. Afterwards, tissue was homogenized using glass homogenizer (Universal Lab. Aid MPW-309, mechanikaprecyzyjna, Poland), with cold buffer (50mM potassium phosphate, pH 7.5) to prepare 20% w/v homogenate. The homogenate was centrifuged at 4000 rpm for 15min. and then the supernatant was separated and stored in deep freeze till assay.

The tissue homogenate was used to assess the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), along with the tissue contents of reduced glutathione (GSH) and lipid peroxide measured as malondialdehyde (MDA).

2- Maternal blood sampling:

Just before delivery, venous blood was obtained immediately into heparinized evacuated tubes.

a- Plasma separation:

Plasma was separated by centrifugation at 4000rpm for 10min. The top yellow plasma layer was pipetted off, and then put in Eppendorf tubes and freezed in deep freeze till assay. The plasma separated was used to estimate GSH and MDA contents, as well as, CAT activity.

b- Erythrocyte lysate preparation:

The buffy coat layer above the RBCs was removed and the packed red blood cells (RBCs) were washed with physiological saline. The red cell pellets were lysed by adding 4 volumes of cold deionized water to the estimated pellet volume and the red cell stroma was removed by centrifuging (4000 rpm, 10min, 4°C). The resulting clarified supernatant was collected and freezed till assay.
The oxidative stress profiles including lipid peroxidation, measured as MDA content, the non-enzymic antioxidant GSH, as well as the antioxidant enzymes, SOD, and GPx, were assessed.

**Histopathological examination:**

Placental tissues from fetal side were available for histopathological examination, washed with heparinized phosphate buffered saline (pH 7.4). To prevent tissue degeneration, samples were fixed in 10% buffered formalin then washed in saline to remove formalin.

Dehydration was performed in alcohol descending grading manner (90%, 70%, 50%, 30%), after which, samples were cleared by xylol, impregnated in soft paraffin and embedded in hard paraffin.

Five micron thick sections were cut out from the blocks, mounted on slide using canda balsam, followed by deparaffinization by xylol, while hydration was done using ascending grades of alcohol. The sections were stained in haematoxylin for 10min. then counter stained in eosin for 1mM. followed by rapid rinsing in distilled water.

**Statistical analysis:**

All data are expressed as mean±standard deviation (S.D.M.). Parametric statistical analysis was performed using unpaired Student’s t-test to compare the mean values of quantitative variables among the groups. The minimal level of significance was identified at p<0.05.

Histopathological examination of placental tissue obtained from normal healthy pregnant women and gestational diabetic (GDM) ones.

The high incidence of histological abnormalities was in particularly observed when gestational diabetic placenta was compared with that obtained from normal control one. These alterations include thickening of the blood vessels' wall of the chorionic villi with hyperplasia of the trophoblast, and thickening of the trophoblastic cell membranes, with potential angiogenesis of new blood vessels. In addition, hypertrophy of chorionic villi with enlarged syncytiotrophoblast may be detected. Besides, hyaline and vacicular degeneration, as well as necrosis of syncytiotrophoblast can be seen, which indicate loss of normal chorionic villi architecture.

**Results**

The results of the present study showed that there was significant difference in gestational age mean between GDM pregnancies and control ones, where the GDM females gave birth about one week earlier than the control ones. It was revealed in Table (1) that the fasting and postprandial blood glucose levels in the GDM participants exceeded the normal values by about 25% and 15% respectively.

Table (2) illustrated the change of enzymatic and non-enzymatic antioxidant parameters in the placenta of GDM compared with control ones. Regarding the non-enzymatic parameters, the endogenous defense molecule, placental glutathione (GSH), was increased significantly compared to control group. Moreover, the GDM placental malondialdehyde (MDA), an indicator of lipid peroxide formation, was elevated markedly by about 60%. On the other hand, the antioxidant enzymatic parameters were also altered significantly by the GDM state, as the, superoxide dismutase (SOD) decreased nearly by about 50% while the, catalase (CAT) and glutathione peroxidase (GPx) were increased by 28% and 100%, respectively.

Table (3) illustrated the effect of GDM on oxidative stress parameters in maternal blood. The non-enzymatic parameters were evaluated in the plasma. The plasma glutathione (GSH) decreased significantly in the GDM patients by 43% compared to those in the control group. The plasma CAT activity was inhibited significantly by 17% as compared to control group. Concerning the other two enzymes, SOD and GPx the gestational diabetic condition raised up the GPx activity by about 26% compared to the control level. On the other hand, the SOD activity was decreased in the GDM group by about 48% compared to the control level.

With normal control pregnant women (***, **** using unpaired t-test, p<0.05, 0.01 or 0.001, respectively. Was used for the statistical comparisons between the two groups.

Table (2) effect of gestational diabetes mellitus (GDM) on non-enzymatic [glutathione (GSH) and malondialdehyde (MDA)] and enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] oxidative stress parameters in placental tissues homogenates.

Values are represented as mean±S.D.M., as compared with normal control group (***, ***, ***). Statistical significance was carried out using unpaired t-test, p<0.001.

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Histological & Physiological Effects of Diabetes

Histological picture of placenta x 100 showing capillary obliteration and peri vascular hyaline degeneration.

Histological picture of placenta x 400 showing congestion and dilatation of blood vessels.

Histological picture of placenta x 400 showing dilated placental blood vessels.

Histological picture of placenta x 100 showing hyaline degeneration and fibrosis in placental capillaries.

Histological picture of placenta x 100 showing thickening, obliteration, fibrosis of blood vessels.

Histological picture of placenta x 100 showing perivascular fibrosis in placental capillaries.
Histological picture of placenta x 100 showing pericapillary fibrosis.

Histological picture of placenta x 100 showing pericapillary degeneration.

Histological picture of placenta x 100 showing massive fibrosis in chorionic villi.

Histological picture of placenta x 400 showing congestion and dilatation of blood vessels.

Histological picture of placenta x 100 showing massive fibrosis in the placenta.

Histological picture of placenta x 100 showing obliterated congested placental capillaries.
**Table (1):** Show the maternal age, gestational age, fasting blood glucose, and post-prandial blood glucose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>GDM (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>25.18±4.13</td>
<td>28.6±6.8</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>37.56±1.07</td>
<td>36.8±0.7**</td>
</tr>
<tr>
<td>Fasting blood glucose level</td>
<td>74.8±5.22</td>
<td>92.8±21.3***</td>
</tr>
<tr>
<td>Post-prandial blood glucose</td>
<td>135.13±8.27</td>
<td>156±31.64*</td>
</tr>
</tbody>
</table>

**Table (2):** Effect of gestational diabetes mellitus (GDM) on non-enzymatic [glutathione (GSH) and malondialdehyde (MDA)] and enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] oxidative stress parameters in placental tissues homogenates.

<table>
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<th>Parameters</th>
<th>Control (n=10)</th>
<th>GDM (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/gm tissue)</td>
<td>45.69±5.68</td>
<td>61.24±4.89***</td>
</tr>
<tr>
<td>MDA (nmol/gm tissue)</td>
<td>163.88±9.24</td>
<td>263.14±11***</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>12.05±2.23</td>
<td>15.42±3.96***</td>
</tr>
<tr>
<td>GPx (mU/mg protein)</td>
<td>13±3.79</td>
<td>26.21±4.16***</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.1±0.11</td>
<td>0.053±0.11***</td>
</tr>
</tbody>
</table>

**Table (3):** Effect of gestational diabetes mellitus (GDM) on non-enzymatic [glutathione (GSH) and malondialdehyde (MDA)], as well as enzymatic [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)] oxidative stress parameters in maternal blood plasma/lysate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>GDM (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma GSH (mg/dl)</td>
<td>1.46±0.178</td>
<td>0.83±0.097***</td>
</tr>
<tr>
<td>Plasma MDA (nmol/ml)</td>
<td>4.2±0.71</td>
<td>6.7±0.76***</td>
</tr>
<tr>
<td>Plasma CAT (U/L)</td>
<td>391.98±13.84</td>
<td>324.34±20.99***</td>
</tr>
<tr>
<td>GPx lysate (mU/ml)</td>
<td>59.36±8.79</td>
<td>74.92±7.81***</td>
</tr>
<tr>
<td>SOD lysate (U/ml)</td>
<td>191±17.39</td>
<td>99.46±12.07***</td>
</tr>
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</table>

**Table (4):** Comparison between the placental and blood oxidative stress parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GDM in placental tissues homogenates</th>
<th>GDM in maternal plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/dl)</td>
<td>61.24±4.98</td>
<td>0.93±0.097</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>263.14±13</td>
<td>6.57±0.76</td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td>15.53±3.86</td>
<td>326.34±21.85</td>
</tr>
<tr>
<td>GPx (mU/ml)</td>
<td>27.23±4.16</td>
<td>73.82±6.81</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>0.063±0.11</td>
<td>98.45±11.16</td>
</tr>
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</table>

**Discussion**

Oxidative stress refers to the overwhelming of endogenous defense systems by the overproduction of reactive oxygen species (ROS) that jeopardize cell survival. Ample of evidence exists that links diabetes with oxidative stress, as identified by increased biomarkers of free radicals damage along with abnormalities in the antioxidant defenses. Gestational diabetes (GDM), is another type of diabetes in which oxidative stress may contribute to the GDM-accompanied histopathological processes; however, the exact pro-oxidant and antioxidant status is still not clear. Pregnancy per se, is a state of oxidative stress, which is normally higher during gestation than in the non-pregnant state, a fact that was documented by several studies, indicating that during pregnancy the antioxidative defense system is modified [16]. It is supposed that the GDM is of clinical importance, since it is associated with significant maternal and fetal morbidity. These include fetal macrosomia, perinatal mortality and maternal long-term risk of developing type 2 diabetes mellitus, as well as secondary fetal hyperinsulinemia.

The present study helped in tracing the histological and biochemical indices of oxidative damage and antioxidant status in maternal blood and intrauterine environment of gestational diabetic women at delivery, and plotted a correlation between the histological placental tissue changes and maternal blood redox parameters.

The present study agree with the work of [17], who stated that the human placenta is capable of mounting a well-built defence system, evidenced by the high antioxidant parameters which is reflected by histological changes of placenta around the central area of the cotyledon, where oxygen tension is known to be the greatest. This piece of information could be attributed to the placenta-producing excessive pro-oxidant agents, antioxidant enzyme/non-enzyme systems that could compensate for the adverse influence from maternal oxidative stress; such assumption is further consolidated by the histopathological findings of the placenta. All these findings uncover the placental adaptive response towards high maternal oxidative stress.

The increase in oxidative activity in GDM, in this study, appears to be secondary to an altered glycaemic control with a subsequent change in the antioxidant defence systems. There is an evidence between better birth outcome and maternal oxidative stress reduction. These results suggest that strategies of reducing oxidative stress in pregnant women are needed.
The results of the present study agree with the results of [18] where they stated that the oxidative stress-mediated alteration in antioxidant defense mechanisms of GDM mothers, can assure that complementary therapies with antioxidants, including vitamins and trace-elements, might help to shift the imbalance between production of free radicals and the decrease in antioxidant capacity. Such treatment may also be of value in women at risk of GDM to maintain their antioxidant capacity.

It was documented in this work that placenta showed altered redox homeostasis that may potentially lead to fetal compromise, hence, redox parameters may represent sensitive indices of fetal/neonatal threat in GDM mothers. Moreover, it is believed that these data can provide adequate measurements at the time of birth, which helps evaluating the proper therapy in newborn infants.

References