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RESEARCH ARTICLE

A comparative study of oxidative stress among gestational diabetics and normal pregnancy

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ABSTRACT

Background: Pregnancy causes progressive changes in maternal carbohydrate metabolism which can induce stress. In subjects with gestational diabetes mellitus (GDM), there can be alteration in oxidant-antioxidant profile producing oxidative stress. Malondialdehyde (MDA) produces damage by acting as free radicals. Ceruloplasmin and uric acid possess antioxidant properties. **Aims and Objectives:** The present study was done (1) to determine the levels of MDA and the antioxidants namely uric acid and ceruloplasmin among GDM and controls and (2) to correlate the level of oxidant and antioxidant in the GDM subjects. **Materials and Methods:** A total of 130 pregnant women attending the antenatal outpatient department were recruited for the study after obtaining permission from the Institutional Ethical Committee. 30 had GDM and were designated as cases. The remaining 100 with normal glucose tolerance served as controls. Serum levels of MDA, ceruloplasmin, and uric acid were measured. Statistical analysis was done. **Results:** Compared to the controls, the GDM subjects showed a significant increase in the level of MDA ($P < 0.001$) and significant elevation of uric acid levels ($P < 0.001$) in the second trimester. **Conclusion:** The results suggest that there is increase in the oxidant levels in GDM subjects. The uric acid levels were increased and ceruloplasmin levels slightly decreased in the GDM subjects, suggesting that excessive free radical production evokes a response to combat oxidative stress.

KEY WORDS: Gestational Diabetes; Malondialdehyde; Uric Acid; Ceruloplasmin; Oxidative Stress

INTRODUCTION

Pregnancy induces progressive changes in maternal carbohydrate metabolism to accommodate the rapidly increasing metabolic demand of the fetus. The fuel metabolism in uncomplicated pregnancy is characterized by facilitated insulin action during the first half of pregnancy and diabetogenic stress during the second half of pregnancy.[1] Gestational diabetes mellitus (GDM) results from sluggish first-phase insulin release, and in addition, there is excessive resistance to the action of insulin on glucose utilization due to the placental hormones such as placental lactogen, progestin, prolactin, and cortisol.

GDM occurs usually around 24–28 weeks. However, diagnosis by oral glucose tolerance test before this period is done to prevent serious complications to the offspring[1]. The studies by Kamana *et al*. have suggested that maternal hyperglycemia can result in complications in fetus such as macrosomia, congenital defects, and neonatal hypoglycemia.[2]

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Prooxidant reactive oxygen species (ROS), which are normal products of aerobic metabolism, can increase under pathological conditions, exceeding the body's detoxification capacity. Antioxidants present in tissues have the capacity to balance or neutralize these free radicals. Oxidative stress occurs when the homeostatic processes fail and free radical generation is much beyond the capacity of the body's defenses, thus promoting cellular injury and tissue damage.[3-5] Adenji *et al*. in their case–control study showed that in subjects with GDM, there is alteration in oxidant-antioxidant profile.[6] Excess oxygen radicals may result from autoxidation of glucose.[7]

ROS react with polyunsaturated lipids forming malondialdehyde (MDA). This reactive aldehyde causes toxic stress in cells and forms covalent protein adducts referred to as advanced lipoxidation end products.[8] MDA produces DNA and protein damage by acting as free radicals.[9] Ceruloplasmin is an important intravascular antioxidant and protects tunica intima against free radical injury.[10] Uric acid possesses antioxidant properties and free radical scavenging activity in serum.[11] Studies have shown that the uric acid levels in the women with GDM were higher than those of the controls.[12]

The present study was done to evaluate oxidative stress in GDM by estimating serum levels of MDA (oxidant), serum ceruloplasmin (antioxidant), and uric acid (antioxidant). The study was also done to correlate the levels of oxidant and antioxidant in the GDM subjects.

MATERIALS AND METHODS

This case–control study was conducted at Sree Balaji Medical College and Hospital, Chromepet, Chennai, from July 2010 to June 2011. The study was approved by the Institutional Ethical Committee. A total of 130 pregnant women (second trimester) attending the antenatal outpatient were recruited for the study after obtaining written informed consent from each person. Each subject underwent routine history, examination, and blood investigations. They were aged between 19 and 35 years with similar nutritional habits and belonged to the same socioeconomic status. None of them had the habit of alcohol consumption or smoking. The pregnant women who developed GDM in the second trimester were designated as cases. The antenatal subjects who had normal glucose tolerance were designated as controls.

Exclusion Criteria

Patients with a history of hypertension, diabetes, asthma, recent surgeries, preeclampsia, or any other chronic ailments were excluded from the study. Major diseases which would alter oxidant and antioxidant levels were eliminated by doing hemoglobin, erythrocyte sedimentation rate (ESR), liver function tests, and serum creatinine.

Methodology

After obtaining written informed consent, venous blood samples were collected from each of the subjects, in the morning in ethylenediaminetetraacetic acid and plain tubes, in the second trimester of pregnancy. Oral glucose tolerance test was done based on the WHO recommendations, by taking blood samples, 2 h after intake of 70 g of oral glucose.[13,14]

The blood samples were subjected to the estimation of hemoglobin, ESR, blood sugar, serum creatinine, and liver function tests. The plain samples were subjected to centrifugation and serum was obtained. Serum MDA was estimated using thiobarbituric acid reactive substances method.[15] Ceruloplasmin was estimated by paraphenylene diamine oxidase method.[16] Serum uric acid was measured using uricase-peroxidase method.[17]

Statistical Analysis

Data were expressed as mean ± standard deviation. The significance of the results was assessed by the Student's *t*-test. $P < 0.05$ was considered statistically significant. The correlation between the parameters was performed using Pearson correlation coefficient (r).

RESULTS

The 130 pregnant women were divided into two groups based on oral glucose tolerance test in the second trimester. The antenatal subjects who developed GDM in the second trimester were designated as cases. The antenatal subjects who had normal glucose tolerance throughout pregnancy were designated as controls. They were age matched and there was no significant difference in blood pressure between the groups. The body mass index and the routine biochemical parameters are shown in Table 1.

In the second trimester, the mean serum level of MDA in the GDM subjects was 4.74 ± 1.02 nmol/ml and was significantly increased compared to 2.87 ± 0.86 nmol/ml among the controls with $P < 0.001$ [Table 2]. The mean serum uric acid level in the GDM subjects was 4.62 ± 0.37 mg/ml, which was significantly increased ($P < 0.001$) compared to 3.3 ± 0.33 mg/ml among the controls [Figure 1]. There was an insignificant decrease in mean ceruloplasmin levels in GDM subjects (44.8 ± 4.09 mg/dl) when compared to the controls (46.1 ± 3.8 mg/dl) in the second trimester.

There was negative correlation between the MDA levels (oxidant) and uric acid levels (antioxidant) with $r = -0.27$, but this correlation was not statistically significant ($P > 0.05$).

DISCUSSION

In the present study, the serum levels of oxidant MDA in the GDM subjects were significantly increased compared to the controls in the second trimester of pregnancy. The levels of the antioxidant uric acid levels were significantly raised in GDM subjects than the controls. The present study showed an insignificant decrease in mean ceruloplasmin levels in GDM subjects.

The prevalence of GDM is high in India.[18] The increased MDA levels in the GDM subjects in the present study suggest the presence of oxidative stress. The studies by Pandey *et al*. on neurological subjects have shown increase in the MDA levels as a measure of oxidative stress.[19] A study conducted by Adenji *et al*.[6] had found significant increase in MDA levels in GDM subjects compared to uncomplicated pregnancies. Bates *et al*.[20] in their study found no evidence of greater lipid peroxidation in GDM as compared to uncomplicated pregnancy, and total antioxidant capacity was similar in both the groups. The present study showed that in GDM, there is increased oxidative stress, leading to increased free radical generation. The proposed mechanism for oxygen free radical generation at higher glucose concentration in pregnancy includes non-enzymatic protein glycation, which may induce production of oxygen free radicals,[7] increased mitochondrial electron transport chain flow, and oxidative activities of the fetus. Hydroperoxides which are major products of lipid peroxidation have been shown to alter prostaglandin biosynthesis which may be responsible for the development of diabetes-related embryopathy.[21,22]

Table 1: Demographic, hematological, and biochemical profile of the study subjects

Variables	Cases *n*=30	Controls *n*=100
Age	27.36±3.34	24.02±3.24
BMI kg/m^2	24.57±2.15	24.08±2.26
OGCT value mg/dl	157.8±10.47	107.97±9.33
Haemoglobin gm/dl	11.04±0.74	11.47±0.93
ESR (in one hour)	19.3±6.52	18.42±5.65
Creatinine mg/dl	0.86±0.23	0.72±0.17
Bilirubin mg/dl	0.64±0.92	0.59±0.11

Table 2: Mean serum MDA, Ceruloplasmin, and Uric acid levels of the study subjects in the second trimester

Variable	Cases *n*=30	Controls *n*=100	*p* value
MDA (nmol/ml)	4.74±1.02	2.87±0.86	<0.001*
Ceruloplasmin (mg.dl)	44.8±4.09	46.1±3.8	0.12
Uric acid (mg/dl)	4.62±0.37	3.3±0.33	<0.001*

*statistically significant

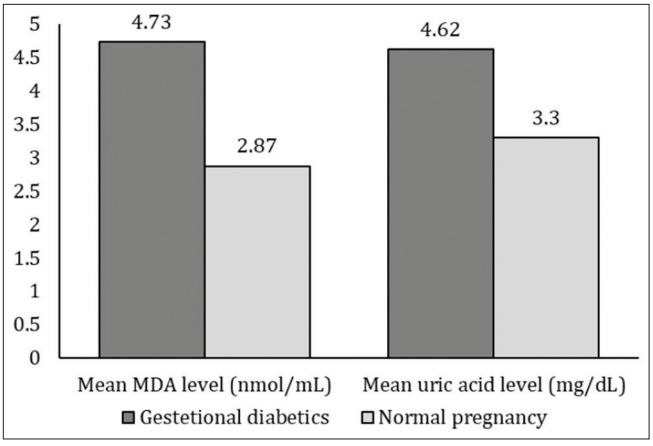

Figure 1: Mean malondialdehyde level (nmol/mL) and uric acid level (mg/dL) in the study population in the second trimester

The increase in uric acid levels in GDM subjects suggests a compensatory response to the increase in free radical levels. The study of Fabbrini *et al.* on oxidative stress and insulin sensitivity has emphasized that uric acid is a major circulating antioxidant in obese people and might provide a protective mechanism to prevent systemic by free radicals.[23] The studies by Coughlan *et al.* and Zhou *et al*. have shown the association of hyperuricemia with GDM.[24,25] The decrease in mean ceruloplasmin levels in GDM subjects could be due to its role as a free radical scavenger. The studies by Ganini *et al.* have concluded that ceruloplasmin influences the availability of iron in free radical scavenging reactions.[26] The studies by Huang *et al*. had shown that serum ceruloplasmin concentrations increase in stress conditions.[27] There was negative correlation between the MDA levels (oxidant) and uric acid levels (antioxidant), but this correlation was not statistically significant. This shows that increase in the MDA levels evokes antioxidant response to combat oxidative stress.

Strengths and Limitations of the study

Major factors which would alter oxidant and antioxidant levels were eliminated in the subjects by doing hemoglobin, ESR, liver function tests, and serum creatinine. Measurement of other oxidants and antioxidants could have been included in the study to do a better correlation.

CONCLUSION

In the second trimester, the levels of the lipid peroxidation parameter, MDA, were increased significantly in GDM subjects compared to uncomplicated antenatal subjects which confirms that hyperglycemia favors lipid peroxidation and causes oxidative stress. The present study showed higher uric acid levels in the second trimester in GDM subjects compared to controls, suggesting an adaptive response to combat oxidative stress. It is important to identify the women at risk of GDM and to keep a tight metabolic control to avoid

long-term consequences to the fetus. The role of exogenous antioxidants may be considered to minimize the adverse effects of oxidative stress in GDM subjects.

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