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Study of gestational diabetes mellitus with special reference to oxygen free radicals

Shivaji Mane[*1] and Anisa B. Bebal[2]

[1]*Additional Professor, Department of Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, Maharashtra-400022*
[2]*Lab Technician, A/p Miraj Tal-Miraj, Dist. Sangli, Maharashtra-416410*

Corresponding author*
Dr. Shivaji Mane,
Additional Professor,
Department of Biochemistry,
Lokmanya Tilak Municipal Medical College and General Hospital, Sion,
Mumbai, Maharashtra, India-400022,
E-mail: maneshivajid64@gmail.com

Abstract
Background: The exact pro-oxidant and antioxidant status in gestational diabetes is still not clear. Increased free radical activity in gestational diabetes can lead to a host of damaging and degenerative maternal and fetal complications. Hence the present study was undertaken to study effect of oxidative stress (lipid peroxidation) and antioxidants status in patients with gestational diabetes mellitus (GDM) and compare them with normal healthy pregnant controls of age and gestational age matched group.
Method: A total 68 samples as healthy pregnant controls (n=35) and GDM patients (n=33) were enrolled in the study. The biochemical parameters estimated in both the groups were fasting and post prandial blood glucose, glycosylated hemoglobin, malondialdehyde, superoxide dismutase, vitamin C and E, Uric acid, bilirubin, ceruloplasmin, copper and zinc.
Results: There was a significant increase in malondialdehyde, ceruloplasmin and uric acid in GDM maternal blood when compared to controls whereas superoxide dismutase exhibited a significant decrease in GDM cases. Also, we found highly significant decreased in plasma vitamins C, vitamin E and zinc while there was significantly increased in copper level in GDM cases as compared to controls.
Conclusion: Our findings showed that antioxidant capacity is lower in women with GDM, possibly related to lower intakes of vitamin E & C and zinc. Elevated glucose levels can induce oxidative stress in GDM mothers.
Keywords: Antioxidant, Gestational diabetes, Oxidative stress, Hemoglobin, Malondialdehyde, Superoxide dismutase, Ceruloplasmin.

1. Introduction

Gestational diabetes mellitus (GDM), defined as hyperglycaemia with onset or first recognition during pregnancy, is one of the most common metabolic disorders occurring during pregnancy that affects from 3% to 34% of all pregnancies, depending on the screening criteria and ethnic group[1,2]. However, the incidence of GDM worldwide is approximately 4% of mothers every year, as many as 16% of mothers exhibit GDM in India. This prevalence of GDM differs depending on the regions and the country[3]. No specific cause has been identified for developing diabetes but it is believed that the hormonal changes during pregnancy increase a woman's resistance to insulin, resulting in impaired glucose tolerance[4].

Moreover, the hyperglycemia of GDM causes increased generation of free radicals. These free radicals damage the lipid membrane called as lipid peroxidation. Further rise in free radicals can damage proteins and DNA results into cell death. Free radicals are difficult to measure directly because of their highly unstable nature so level of MDA, end product of lipid peroxidation has been used as an indicator of free radical activity[5]. Antioxidants defense system against free radical counter balances generation and lipid peroxidation e.g., vitamin C, a first line defense against free radical, vitamin E as a membrane protector, enzymatic SOD, ceruloplasmin, endogenous generated uric acid and bilirubin. The elements required in enzymatic defense system are copper and zinc is estimated in the blood. Along with glycosylated hemoglobin is an indicator of GDM patients.

Although gestational and pre-existing diabetes can cause damage for gestational age babies, a sudden drop in a neonate's blood sugar after birth, and has a high risk for preterm delivery and other complications. Hence, it is of paramount importance to identify women at risk of GDM and to keep a tight metabolic control in order to avoid immediate and long-term consequences for their offspring[6]. Further, the oxidative stress status of the GDM mother will influence the outcome[7]. Therefore, it is necessary to study the effect of oxidative stress and antioxidant status in GDM.

2. Method

A total 68 samples as pregnant controls and GDM patients were collected from outpatient Department of Obstetrics and Gynecology of Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai. Patients with diagnosed GDM and on therapy irrespective of the type of treatment, oral hypoglycemic agents/insulin therapy were all included. The Ethical Committee approved the study design and informed consent was obtained from all patients. Patients were classified into two groups i.e., Controls: Healthy pregnant women (n=35) and study group: consisting of 33 pregnant women who were diagnosed as having GDM after undergoing an oral glucose tolerance test with 75g glucose taken orally, as laid down by National diabetes data. The normal pattern of glucose levels in blood after oral glucose tolerance test (OGTT): Fasting- 60-110 mg%, at 1 hr- 120-180mg%, at 2 hrs – 120-150 mg% and at 3 hrs- 70-110 mg%. Whereas pattern for glucose levels in subjects with GDM after OGTT: Fasting 90-120 mg%, at 1 hr- 110-220 mg%, at 2 hrs 160-200mg%, and 3 hrs 110-140 mg%. Patients with previous diabetes mellitus, hypertension and other related disorders in pregnancy were excluded from the study.

Age and gestational age of the study group and control were noted. Routine history was obtained from all the subjects. None of the subjects were obese and did not have any habit of smoking and alcohol consumption. They were not on any kind of supplements or antioxidants. Patients and controls belonged to the same socioeconomic and nutritional status. Hence differences due to dietary habits were minimal, if any. For biochemical investigation blood samples were collected from patients and controls. Total 10 ml of blood collected in fasting condition and transferred 3 ml in EDTA, 2ml in sodium fluoride and 5 ml bulb for serum respectively. EDTA whole blood was used for glycosylated hemoglobin. Remaining EDTA and fluoride blood was immediately centrifuged at 3000 rpm for 15 mins. Plasma and buffy coat were carefully separated. The plasma was used for the estimation of vit C and E and fluoride plasma is used for blood sugar estimation. Glucose, vitamin C and E were analyzed within 1-2 hours of collection. Similarly post prandial 2 ml blood was collected in fluoride bulb and immediately centrifuged at 3000 rpm for 15 minutes. The plasma was separated and used for blood sugar.

Remaining 5 ml of blood was allowed to clot for 1 h and then centrifuged at 1600 rpm for 30 min. the serum was stored in plastic vials in the refrigerator at 4^0 C. the serum sample was used for the estimation of malondialdehyde (MAD), superoxide dismutase (SOD), ceruloplasmin (CP), uric acid, bilirubin, copper and zinc. Standardization was done for each of parameters studied viz glucose, glycosylated hemoglobin serum MDA, vitamin C, vitamin E, bilirubin, uric acid, copper and zinc.

2.1 Statistical analysis

Mean and standard deviations were analyzed for all parameters of study group and controls. Z test was carried out for statistical analysis and the 'P' value of significance was calculated as <0.05.

3. Observations and Results

The demographic characteristics of GDM and control group are presented in Table 1. There were no significant differences about age, gestational age, parity and weight gain between the groups. All women were taking multivitamin and minerals supplements routinely used during pregnancy.

Table 1: Demographic details of gestational diabetics and control subjects

Parameters	Controls (n=35)	GDM patients (n=33)	P value
Age (years)	24.31±0.5	26.41±0.83	>0.05
Gestational age (weeks)	32.18±0.42	34.14±0.30	>0.05
Parity	2.50±1.21	2.71±1.12	>0.05
Weight (kgs)	71.13±0.54	73.45±1.23	>0.05

Results of biochemical parameters such as serum malondialdehyde (MDA) level, enzymatic antioxidant superoxide dismutase, ceruloplasmin, uric acid and various fractions of bilirubin in patients with gestational diabetes and normal controls are shown in table 2.

Table 2: Biochemical parameters

Parameters	Controls	GDM patients	P value
Malondialdehyde (µmol/L)	3.79±1.01	6.86±1.30	<0.01
Superoxide Dismutase (Units/ml)	1.78±0.17	0.96±0.30	<0.01
Ceruloplasmin (mg/dl)	69.16±11.58	77.09±18.64	<0.05
Uric acid (mg/dl)	4.59±0.56	6.0±0.34	<0.01
Total bilirubin (mg/dl)	0.54±0.14	0.65±0.15	NS
Direct bilirubin (mg/dl)	0.23±0.08	0.25±0.10	NS
Indirect bilirubin (mg/dl)	0.35±0.08	0.39±0.08	NS

<0.01= Highly significant, p<0.05= significant

Table 3 showed the levels of dietary constituents those acting as antioxidants, vitamin C, Vitamin E, Copper and zinc in the blood of patients with GDM and normal pregnant controls. The groups had highly statistically significant differences in vitamin C and E, copper and zinc.

Table 3: Levels of dietary constituents

Constituents	Controls (n=35)	GDM patients (n=33)	P value
Vitamin C (mg/dl)	1.08±0.54	0.45±0.12	<0.01
Vitamin E (mg/l)	11.02±0.99	8.65±0.86	<0.01
Copper (µg/dl)	112.31±26.96	206.53±6.04	<0.01
Zinc (µg/dl)	67.11±15.10	26.8±9.58	<0.01

4. Discussion

Oxidative stress (OS) simply means imbalance in free radicals (oxidants) and antioxidants in excess of oxidants[8]. This study measured plasma malondialdehyde (MDA) which is one of the intermediate products of free radical injury. This comes as a result of free radical attack on membrane lipid in the process of lipid peroxidation. Though, previous studies[9, 10] found not statistically significant, the increased plasma value of MDA observed in patient with GDM may signify increase free radical production and subsequently injury to the membrane lipid. Some in their findings reported the oxidative stress to be due to reduced antioxidant contents in the system[10]. In the present study, the plasma MDA level was significantly elevated in the subjects with GDM than the controlssimilar to the study done by Surapaneni[11] and Kamath *et al*[12]. MDA concentration increase, in GDM cases because the oxygen free radicals and lipid peroxidation products increase in GDM (excessive production of free radicals observed in group of study), its insufficient removal results in damage to cellular proteins, membrane lipids, and nucleic acids, the presence of high levels of MDA in the present study may relate to the glycemic control, that's lined with other studies[12,13].

Among various antioxidants that could be considered but not given routinely are catalases, glutathione peroxidase, superoxide dismutase (SOD) and so on.The reduced plasma SOD observed in subjects with GDM in our study may be as a result of overwhelming use of this antioxidant in counteracting free radical formation and its injury. Superoxide dismutase is an important antioxidant defence in nearly all living cells exposed to oxygen. It helps in degrading hydrogen peroxide which is one of the oxygens related free radicals. The finding of present study concerning the SOD is in total agreement with the work of Lobo *et al*[8] and Kharb[14]. This may be due to consumption of this antioxidant in clearing the free radicals from the system. The present study as described above observed lower antioxidant in the phase of increasing product of free radical injury. This defines oxidative stress in the GDM patients recruited in the study. The available antioxidants (SOD) were not sufficient to degrade the free radical production. The antioxidant component from the diet of the majority of the subjects may not also be adequate in counteracting the effect of free radical injury[8].

Loven *et al* found that no significant difference in serum concentrations of ceruloplasminbetween GDM case and control groups, while the activity of ceruloplasmin in women with GDM was slightly higher[15]. While in current study there was a significant increase in ceruloplasmin in GDM maternal blood when compared to controls. Also, we found no significant difference in serum concentrations of totalbilirubin, direct and indirect bilirubin between two groups. In the other hand, our results of UA levels improved fact, the antioxidant system was strongerthan peroxidation during pregnancy, that's confirm the antioxidant role for uric acid, that's agreement with the study of Uotila *et al*[16]. UA levels increase, because the placenta leads to overproduction of UA which serves as a marker of the disease, hyperuricemia may predate proteinuria by several weeks[17]. This elevation predicates a GDM as our results show, furthermore it is considered a risk factor for development GDM because the correlation between UA and IR, probably because hyperisulinemia would cause lower renal UA excretion, and different role for the UA it is an antioxidant role in the GDM group. Besides that, these increases suggesting excessive free radical production evokes a response to combat OS because different role for UA an antioxidant property, our result in oxidative factor confirms that and that lined with other study.

Antioxidants are known to play protective roles against oxidative stress. Several reports have reviewed the interaction of vitamin C and E, both as prooxidant and antioxidant[18,19]. The plasma antioxidant vitamins C and E exhibit a significant decrease in cases of GDM when compared to healthy pregnant women in this study. Previous studies have reported decrease of vitamin E in GDM[20,21], however, other study demonstrated rise in vitamin E level during GDM[22]. However, conflicting findings have shown that women who consumed < 70 mg of vitamin C daily were at a 1.8-fold increasing risk of GDM as compared with women who consumed higher amounts[23] and in another study, concentrations of vitamin C significantly increases in women with GDM[24].

The current study demonstrated decreased maternal serum zinc in GDM compared to control group and the changes were significant in case of normal pregnancy. Significantly lower serum zinc levels in pregnancy have been reported in different studies[25]. Other researches[26], reported decreased plasma zinc level as pregnancy progresses, which was further supported by Ejezie and Nwagha[25]. The intakes of zinc were negatively associated with gestational hyperglycemia and daily intake of zinc, 1 mg per day, caused 11% reduction in the risk of gestational hyperglycemia. It is conceivable that zinc could limit oxidant-induced damage with protection against vitamin E depletion, thereby increase the stabilization of membrane structure, restriction of endogenous free radical production, contribution to the structure of the antioxidant enzyme extracellular superoxide dismutase, maintenance of tissue concentrations of metallothionein, as a possible scavenger of free radicals. It was reported that mild zinc deficiency in rats can exhibit high serum levels of ceruloplasmin, the acute phase protein. High concentration of this protein may be a sign of inflammation, which is associated with above-normal phagocyte secretion rates of free radicals[27].

We found that plasma copper (Cu) concentrations were significantly higher in patients with GDM as compared to

in control. In the case-control study conducted by Wang *et al*., in 2002,[28] Cu concentrations in GDM group (n=46) were significantly higher than those in healthy pregnant group (n=90), which was consistent with our results. Gambling *et al*[29] indicated that the increase in serum copper was parallel with the increased serum ceruloplasmin. In addition, ceruloplasmin acts as a ferrioxidase which is able to convert Fe^{+2} to Fe^{+3} and stimulate iron efflux from the liver. During pregnancy, the metabolism of copper and iron are correlated and the deficiency of one has marked effects on the metabolism of the other metal. In the mother, iron deficiency resulted in increased liver copper levels, which is associated with increased both serum copper and ceruloplasmin activity in maternal serum. Therefore, the significant increase in serum copper in this study might be due to the imbalance in iron status during pregnancy that alters copper levels; as a result of enhanced gene expression; leading to elevated serum copper in pregnant women[30].

There are some limitations in the current study- The sample size of study was small and our study did not provide information about serum micronutrient concentrations. However, this study has strengthened by the close matching of control subjects against GDM subjects. Subjects were matched based on gestational and maternal ages thus negating these parameters as confounders.

5. Conclusion

The findings of present study showed that antioxidant capacity is lower in women with GDM, possibly related to lower intakes of vitamin E & C and zinc. Elevated glucose levels can induce oxidative stress in GDM mothers. It has been reported that in normal pregnancythere is an increase of lipid peroxidationproducts in serum with advancing gestation, which is balanced by an adequate antioxidativeresponse. But in diabetic pregnancy, it can be seen that the hyperglycemia of diabetes can enhance free radical generation through at least ten differing pathways. Some of these include the polyol pathway, formation of age, activation of protein kinase C (PKC), hexosamine pathway, and increased oxidative stress generation by enhanced ROS production in the mitochondria. In addition, other molecular mechanisms such as the Fenton reaction, metabolic changes, and the accumulation of ketone bodies may also be involved. The differing pathways interact in a complex fashion that may lead to their induction or inhibition of each other in both a direct and indirect fashion. Unrestrained free radical formation leads to tissue damage through increased oxidative stress and addressing these molecular mechanisms may lead to new therapeutic strategies.

The present study suggested that antioxidant status could be enhanced by consumption of food rich in antioxidant and high dietary intake of fruit and vegetables and other rich antioxidant sources in GDM, which may be ultimately beneficial in the prevention and management of GDM.Also, low intake of simple sugar to control hyperglycemia which further may reduce oxidative stress and complications in infants also suggested waking exercise.

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