

ANTIOXIDANTS AND LIPID PEROXIDATION IN GESTATIONAL DIABETES – A PRELIMINARY STUDY

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Abstract : Increased free radical activity in gestational diabetes (GDM) can lead to a host of damaging and degenerative maternal and fetal complications. Hence antioxidant levels in blood of GDM mothers and cord blood were estimated. Erythrocyte glutathione (GSH), superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS), plasma vitamins C and E and serum total glutathione-S-transferase (GST), protein thiols and ceruloplasmin (Cp) were estimated spectrophotometrically in maternal blood of age matched controls and mothers with GDM and also in cord blood samples of the above. There was a significant increase in the erythrocytic GSH, serum total GST and protein thiols in GDM maternal blood when compared to controls whereas erythrocytic SOD exhibited a marked decrease in GDM cases. The changes in plasma vitamins C and E, Cp and erythrocytic TBARS in GDM were not significantly different from controls. Cord blood levels of protein thiols were also significantly increased in GDM. No significant changes were observed in the serum Cp and GST levels of the same. Hence, elevated glucose levels can induce oxidative stress in GDM mothers.

Key words : antioxidant status
gestational diabetes

cord blood
maternal blood

INTRODUCTION

Gestational diabetes is a condition exhibiting increased susceptibility to oxidative stress leading to potential damage (1, 2). The incidence of GDM world wide is approximately 4% of mothers every year. As

many as 16% of mothers exhibit GDM in India. 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 (3). Further, the oxidative stress status of the GDM mother will

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influence the outcome (4).

Therefore, in this study the erythrocytic lipid peroxidation in maternal blood and the antioxidant levels in maternal and cord blood were assessed in GDM to analyze the relation between the parameters and GDM and evaluate the response to the oxidative challenge in this condition.

MATERIAL AND METHODS

This study was approved by the Institutional Review committee. Informed consent was obtained from each patient before removal of blood sample. The study group consisted of 36 subjects. GDM patients were diagnosed at the out patient section of the department of Obstetrics and Gynecology of Kasturba Hospital, Manipal, a tertiary care hospital.

They were classified into 2 groups, namely

- **Controls :** Comprising of 18 healthy pregnant women.
- **Patients :** Consisting of 18 pregnant women who were diagnosed as having GDM after undergoing an oral glucose tolerance test with 100 g of glucose taken orally, as laid down by the American College of Obstetricians and Gynecologists (1994, 5) for diagnosis of GDM. On glucose challenge the normal pattern for glucose levels in blood was as follows : Fasting-60-110 mg%, at 1 hr.-120-180 mg%, at 2 hrs.-120-150 mg% and at 3 hrs.-70-110 mg%. In cases with GDM, the ranges observed were as follows : Fasting-90-120 mg%, at 1 hr.-110-220 mg%, at 2 hrs.

160-200 mg% and at 3 hrs. 110-140 mg%.

Every patient underwent routine history taking, physical examination and other blood investigations. None of the subjects were administered any kind of supplements/antioxidants. In this study glycosylated hemoglobin levels were not estimated as it was economically not feasible for the subjects to bear the cost of this investigation. Patients and controls belong to the same socioeconomic and nutritional status. Hence, difference due to dietary habits in our estimations will be very minimal, if any.

Exclusion criteria

Patients with diabetes mellitus before pregnancy and pre-eclampsia were excluded from the study.

Inclusion criteria :

Patients diagnosed to have GDM and on therapy irrespective of the type of treatment namely oral hypoglycemic agents/insulin therapy, were all included. Two patients were on diet regulation and the rest were on dietary as well as metformin (500 mg 8 hourly) therapy. None of the patients were obese and did not have any habit of smoking and alcohol consumption.

Clinical profile : Maternal age (y), maternal hemoglobin level (g%), and birth weight (kg) in normal pregnancies and pregnancies complicated with GDM are presented in Table I. No birth mortality was observed.

Gestational age ranged from 34 to 39 weeks (36.85 ± 1.90). Of the 18 mothers with

TABLE I: Clinical profile of normal and GDM mothers and respective newborns (Mean±SD).

| <i>Parameter</i> | <i>Control (n=18)</i> | <i>Gestational diabetes (n=18)</i> |
|--------------------------|---------------------------|--|
| Maternal age (y) | 26.89±2.76 | 29.50±4.22 |
| Maternal hemoglobin (g%) | 11.30±1.32 | 10.81±0.79 |
| Birth weight (kg) | 2.89±0.43 | 3.00±0.49 |
| Apgar score <5 at 5 min | 0(0)* | 0(0)* |

*Apgar scores are presented as the number of newborns in either group having Apgar score <5 at 5 min and percentage of such cases in each group has been given in parentheses.

GDM, 8 were spontaneous deliveries, 5 by elective Caesarean section, 4 by vacuum extraction and 1 by forceps. Of the 18 mothers in control, 13 were spontaneous deliveries, 3 by Caesarean section and 2 by vacuum extraction. The duration of labour varied from 8–10 hours. All deliveries were prima gravida except 3 which were multipara (2 in control, 1 in GDM).

Collection of sample: Blood (fasting) was collected in the later half of the third trimester in EDTA and serum vacutainers respectively and immediately after delivery, samples of cord blood were also collected in serum vacutainers. Erythrocyte suspension was prepared according to the method of Beutler et al (6). It was immediately centrifuged under refrigeration at 3000 g for ten minutes. Plasma and buffy coat were carefully removed and separated cells were washed thrice with cold saline; pH 7.4 (sodium phosphate buffer containing 0.15 mol/L sodium chloride). The erythrocytes were then suspended in an equal volume of physiological saline. All analyses were completed within 4–8 hours of collection. Appropriately diluted hemolysates were then

prepared from erythrocyte suspension by the addition of distilled water for the estimation of GSH, SOD and TBARS. The plasma was used for the estimation of vitamins C and E.

Serum separation

Two milliliters of venous blood was collected from patients and control groups under aseptic precautions, in a clean dry centrifuge tube and was allowed to clot for 1 h and then centrifuged at 1600 g under refrigeration for 30 min. at 4°C. The cord blood was also similarly treated for serum separation.

The erythrocyte GSH was estimated colorimetrically (7). Serum total GST activity was analyzed spectrophotometrically (8). Serum total protein thiols were estimated by a spectrophotometric method using 5', 5' dithio-bis (2-nitrobenzoic acid) (9). Serum Cp was determined colorimetrically (10). SOD activity was analyzed by the method of Beauchamp and Fridovich (11). Plasma vitamin C was determined colorimetrically using dinitrophenyl hydrazine as a color compound (12). Plasma vitamin E was measured colorimetrically (13). Lipid peroxidation products were quantified by the thiobarbituric acid method (14). The hemoglobin content of the erythrocyte was estimated by the cyanmethemoglobin method (15). A limitation of our study was the volume of cord blood collected wherein we could estimate only 3 parameters compared to 8 in the maternal blood samples.

Statistical analysis was carried out using Independent samples test and Mann-Whitney test. Correlation was done by Spearman Rho and Pearson correlation (SPSS package).

RESULTS

Maternal blood

The plasma vitamin C and E levels were found to be increased in GDM patients when compared to controls. But the values were not significant. The plasma Cp levels were found to be insignificantly increased in GDM patients when compared to controls. The erythrocytic GSH was significantly increased in GDM patients when compared to controls. On the other hand, erythrocytic SOD activity was markedly decreased in GDM patients when compared to normal subjects. The serum total GST and protein thiol levels exhibited a highly significant increase in GDM patients when compared to controls. The erythrocytic TBARS levels were found to be increased insignificantly in GDM patients when compared to normal pregnant women (Table II).

Cord blood

Cerum Cp and GST levels were found to be increased in cord blood of GDM mothers when compared to control cord blood. But the change was not significant. However, the serum protein thiol levels were significantly increased in cord blood of GDM patients when compared to controls (Table III).

Correlation

In the present study there was a significant positive correlation between the levels of serum protein thiol in normal and GDM maternal and cord blood samples. A significant positive correlation was also obtained for serum total GST levels in maternal and cord blood of GDM patients (Table IV)

TABLE II: Antioxidant levels and lipid peroxidation in maternal blood of GDM patients, Mean±SD.

| Parameter | Control | GDM | Significance |
|-------------------|---|---|-----------------|
| Vitamin C (µM/L) | 1.62±0.42 (0.41–3.96) n=8 | 1.97±0.26 (0.15–4.1) n=18 | NS ^a |
| Vitamin E (mg/dl) | 1.09±0.22 (0.25–3.45) n=13 | 2.19±0.39 (0.5–6.1) n=14 | NS ^a |
| Cp (mg/dl) | 61.83±8.94 (25–88) n=6 | 49.63±11.25 (4.26–134.37) n=13 | NS ^a |
| GSH (mg/g Hb) | 17.27±1.12 (14.00–29.74) n=14 | 23.57±2.60 (15.85–44.77) n=11 | P<0.01 |
| SOD (U/g Hb) | 2267.84±363.64 (706.73–4162.42) n=8 | 1005.21±115.95 (275–2051.43) n=15 | P<0.01 |
| GST (IU/L) | 1.18±0.18 (0.2–2.9) n=14 | 3.19±0.37 (0.6–5.6) n=15 | P<0.01 |
| Thiol (µM/L) | 86.02±19.95 (29–273) n=13 | 206.62±29.32 (19.52–449.3) n=18 | P<0.01 |
| TBARS (nM/g Hb) | 5.24±0.67 (1.94–9.16) n=11 | 6.68±1.02 (2.02–16.57) n=16 | NS ^a |

NS^a=Not significant, Independent Samples Test. P<0.01, Mann-Whitney Test. n=Sample size (various due to unavoidable practical difficulties), Range in parentheses.

TABLE III: Antioxidant levels in cord blood of GDM patients, Mean±SD.

| Parameter | Control | GDM | Significance |
|--------------|---------------------------------------|--|-----------------|
| Cp (mg/dl) | 15.08±2.96 (6.87–26.5) n=6 | 22.47±3.09 (7.58–36.26) n=10 | NS ^a |
| GST (IU/L) | 2.42±0.41 (1.03–5.2) n=9 | 4.52±0.56 (3.5–7.1) n=6 | NS ^a |
| Thiol (µM/L) | 70.29±11.46 (19.42–195.23) n=14 | 262.59±54.04 (17.62–536.89) n=12 | P<0.05 |

NS^a=Not significant, Independent Samples Test. P<0.05, Mann-Whitney Test. n=Sample size. Range in parentheses.

TABLE IV: Correlation between maternal and cord blood Cp, GST and Thiol levels in GDM patients.

| Parameter | Control | GDM | Significance |
|--------------------|-----------------------|-------------------------|--|
| Cp (mg/dl) | $\rho=0.5$ n = 3 | $\rho = 0.262$ n = 8 | NS ^c NS ^g |
| GST (IU/L) | $\rho=0.238$ n = 8 | $\rho = 1.0$ n = 5 | NS ^c P<0.01 ^g |
| Thiol (μ M/L) | $r=0.795$ n = 10 | $r=0.737$ n = 10 | P<0.01 ^c P<0.05 ^g |

ρ = Spearman Rho.

r = Pearson correlation.

NS^c = Not Significant (Control).

NS^g = Not Significant (GDM).

n = Sample size.

DISCUSSIONS

In the present study, a significant increase in maternal erythrocytic GSH, serum total GST and protein thiol was observed in patients with GDM when compared to controls. This may be due to the increased oxidative stress prevalent in GDM (4, 16–18). Further, free radical mediated oxidative stress has been implicated in the pathogenesis of diabetes mellitus (DM) and its complications (19–21). It has been reported earlier that oxidative stress is associated with a prooxidative shift of the GSH redox state in the blood (22). It may lead to an increase in the turnover of GSH, hence the elevation in the levels in our study. Besides, lower concentrations of erythrocyte GSH have been observed in diabetic mothers before and after delivery and also in their infants (23). Protein thiols which act as antioxidant may have effects at the genomic and epigenomic levels eg. post translational modification of proteins and cellular signaling through thiol redox and thiolation (24). Distinct from the genotype, epigenomics encompasses the modulation of

gene activity through particular global chromatin methylation patterns or histone modifications; these may be known as epigenetic marks. The chromatin pattern of epigenetic marks is modifiable over a lifespan and may influence disease progression. DNA methylation patterns or histone modifications are potentially reversible, but, in certain circumstances, such marks become imprinted and give rise to trans-generational effects. It is now recognized that an aberrant pattern of epigenetic marks may link the initiating mutation to the gene expression profile of a disease phenotype (24). It has been reported that RBC GST levels, both in maternal and cord blood are sensitive indicators of oxidative stress in insulin dependent diabetes patients before delivery. The same enzyme would act as a biomarker of oxidative stress upon sudden increase in oxygenation during delivery (20). Further, GSH acts as a substrate for GST. Moreover, GST is involved in detoxification of ROS, modulation and formation of ion channels (25) as well as in signal transduction pathways (26). Therefore, in view of the excessive generation of free radicals, erythrocytic GSH, serum total GST and protein thiols have markedly increased in the GDM cases, in our study. Erythrocytic SOD in maternal blood of GDM patients has significantly decreased when compared to controls. This would again be due to its role as an antioxidant enzyme for scavenging free radicals produced in this condition, as has also been observed earlier (27–30). Plasma-Cp levels exhibits a insignificant decrease in maternal blood of GDM cases when compared to controls. This could be probably due to its role as a free radical scavenger. Further, its role in iron and copper metabolism is well documented. Thus, it

influences the availability of the iron in free radical generating reactions (31). Moreover, hyperglycemia can favour nonenzymatic glycation which can induce ROS formation in presence of reactive transitional metals. It also induces reductions in serum transferrin and in activities of CuZnSOD and of Cp ferroxidase (32). The plasma antioxidant vitamins C and E also exhibit an insignificant increase in cases of GDM when compared to healthy pregnant women in this study. This effect seems to represent an adaptive response to increased oxidative stress. Such an increase in the diabetic state has also been reported earlier (33). Contrary to our findings, a slight decrease in vitamin E levels has been reported in GDM relative to controls (29, 34). No change in vitamin E levels was also observed in GDM elsewhere (35). Several reports have reviewed the interaction of vitamin C and E, both as prooxidant and antioxidant (36, 37). The lipid peroxidation parameter, erythrocytic TBARS also shows an insignificant increase in GDM patients when compared to controls. This may be due to the efficient antioxidant response brought about by GSH, GST, protein thiols, SOD etc. A similar observation was also reported earlier (34). Lipid peroxidation is known to increase in GDM (18). Hence, it would contribute to complications associated with it.

Elevated glucose levels are associated with increased production of ROS by several different mechanisms (38–42). Besides, diabetes in pregnant women is a known teratogen. Glycation products from excess glucose can chemically modify DNA causing mutations and complex DNA rearrangements. Therefore, DNA damage in fetal tissues as a result of maternal diabetes

may reflect a level of genomic injury sufficient to affect embryonic development (43). Therefore, the whole pathogenesis and spectrum of fetal and neonatal mortality and morbidity could primarily be attributed to the excessive transferal of glucose from mother to fetus. There is enough evidence to suggest that poor maternal homeostasis is at the core of the problem (3). The mechanism for induction of dysmorphogenesis in experimental diabetic pregnancy has been shown to include deficiency states of membrane lipids (myo-inositol, arachidonic acid, etc), alteration in the prostaglandin cascade and the generation of excess free oxygen radicals (43). Moreover, in the present study there was a significant increase in the serum protein thiol and a tendency of total GST and Cp to rise in cord blood of GDM patients when compared to control cord blood. This may be in response to the milieu of increased oxidative stress in case of GDM cord blood. It is already reported earlier (18) that GDM induces oxidative stress in the fetus. Furthermore, in this study there was a significant positive correlation between the levels of serum protein thiol in normal and GDM maternal and cord blood samples suggesting that excessive free radical production as seen in GDM state could lead to an adaptive response in terms of protein thiol as a sacrificial antioxidant. Moreover, a significant positive correlation that was observed in serum total GST level in GDM (maternal and cord blood) may be to combat the oxidative stress in such cases.

Thus, results of the present study suggest presence of oxidative stress in GDM probably due to increased free radical production.

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