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ORIGINAL ARTICLE / RESEARCH

Oxidant–Antioxidant Status in Gestational Diabetes Patients

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ABSTRACT

The exact pro-oxidant and antioxidant status in gestational diabetes is still not clear. To add a new insight to the question, erythrocyte antioxidant glutathione (GSH) and malondialdehyde (MDA) levels in erythrocytes and plasma glutathione-S-transferase (GST) activity were estimated in patients with gestational diabetes and compared to controls. Statistical analysis between controls and patients was performed by the unpaired *t*-test using the stat-view package. It was observed that there was a significantly lower erythrocyte GSH levels (9.6 ± 6.1 vs. 16.8 ± 5.9 mg/g of Hb, $p = 0.0005$) and plasma GST activity (3.1 ± 1.3 vs. 10.3 ± 2.3 micromoles/dl of plasma, $p < 0.0001$) in patients with gestational diabetes when compared to controls. There was a significantly higher erythrocyte MDA levels in patients with gestational diabetes when compared to controls (21.0 ± 4.7 vs. 10.3 ± 2.7, $p < 0.001$). The results of our study suggests that there was higher oxygen free radical production, as evidenced by higher MDA and lower GSH, supporting the hypothesis that there is increased oxidative stress in patients with gestational diabetes and the decreased GST activity supports the decreased detoxification capacity in pregnancy-complicated diabetes.

Key words: Glutathione (GSH), malondialdehyde (MDA), glutathione-S-transferase (GST), gestational diabetes

Introduction

Gestational diabetes is the occurrence of diabetes in previous normal women and is associated with an increase incidence of congenital abnormalities when compared with normal pregnancy. Frequency of congenital malformation in infants of diabetic mothers is estimated to be 6–10% [1],[2]. Alteration in oxidant–antioxidant profile is known to occur in diabetic pregnancy [3]. Oxidative stress due to the damage brought about by free radicals is also known to influence the formation of anomalies in foetuses born to women with diabetes. Moreover, body's defence mechanisms would play a role in the formation of antioxidants and try to minimise the damage. Factors responsible for these anomalies are not fully understood, but there are several reports showing that increased free radical production and antioxidant depletion in diabetic pregnant women may contribute to the formation of anomalies [4]. In diabetes excess oxygen radicals may result from the auto oxidation of glucose [5] and increased level of glycated haemoglobin levels, because of increased glucose levels in the body [4]. There is considerable evidence that antioxidant defence system is depleted and activity of antioxidant enzymes is reduced in diabetes [6].

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In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant–antioxidant status in patients with gestational diabetes. Erythrocyte glutathione (GSH) levels were estimated as an index of antioxidant status. Malondialdehyde (MDA) levels were measured as thiobarbituric acid-reacting substances (TBARS), which serve as an index of extent of lipid peroxidation. These parameters were estimated in RBCs to assess the disturbances in oxidant–antioxidant status and their effect on erythrocytes. Glutathione–S–transferase (GST) levels were estimated in plasma. GST is an enzyme involved in antioxidant defence and also involved in detoxication. Alterations in antioxidant enzymes have been reported in various studies [3].

Materials and Methods

The study was conducted in 20 pregnant women, reporting to Gynaecology & Obstetrics Department of Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation, Chinoutpally, Gannavaram (Mandal), A.P, India. They were diagnosed to have gestational diabetes after undergoing glucose tolerance test with 100 g of glucose, as per criteria suggested by O'Sullivan and Mahan [7]. Twenty age- and gestational age-matched normal pregnant females with similar socioeconomic status were taken as controls. All the subjects in the study were normotensive and had no family history of diabetes, hypertension and obesity. Patients suffering from disease of any origin other than gestational diabetes were excluded from the study. Subjects with normal nutritional habits without any vitamins supplement during last 6 months were included. Due permission was obtained from the ethical committee of the Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation, Chinoutpally, Gannavaram (Mandal), A.P, India, before the start of the study. The written consents were also taken from the patients prior to the study and the objectives of the study were fully explained. Eight of the participants were dropped out at the end of the selection, as they did not like the idea of giving blood.

The controls and patients were divided into two groups [Table/Fig 1]:

- *Controls*: 20 healthy pregnant women of matching age are treated as controls.
- *Study subjects*: 20 patients with proven gestational diabetes.

Table/Fig 1

Parameters	Controls (mean ± SD) (*n* = 20)	Range	Study subjects (patients) (mean ± SD) (*n* = 20)	Range
Age (years)	23.31 ± 0.5	20–30	26.40 ± 0.82	22–32
Gestational age (weeks)	32.18 ± 0.42	28–36	34.04 ± 0.28	29–38

Demographic details of gestational diabetics and control subjects

The venous blood samples obtained from these subjects were used for the estimation of GSH and MDA in erythrocytes and GST in plasma. GSH was estimated by the method of Beutler et al., using dithio-bis-nitrobenzoic acid (DTNB) [8]. MDA was determined as a measure of TBARS [9] and GST was measured by using 1-chloro-2,4-dinitrobenzene (CDNB) [10]. All reagents used were of analytical reagent grade. DTNB, CDNB and thiobarbituric acid were obtained form Sigma Chemicals, St. Louis, MO.

Statistical analysis

Statistical analysis between controls and study subjects (patients) was performed by the unpaired *t*-test using the stat-view package.

Results and Discussion

The mean ± SD of erythrocyte GSH and MDA and plasma GST are indicated in [Table/Fig 2].

Table/Fig 2

Parameter	Controls (*n* = 20)	Study subjects (patients) (n = 20)
GSH (mg/g of Hb)	16.8 ± 5.9	9.6 ± 6.1*
MDA (*n* mol/g of Hb)	10.29 ± 2.71	21.02 ± 4.7**
GST (μmol/dl of plasma)	10.3 ± 2.3	3.1 ± 1.3***

The mean ± SD values of GSH, MDA and GST in controls and study subjects (patients) with gestational diabetes. (*p = 0.0005 compared to controls, **p < 0.001 compared to controls, ***p < 0.0001 compared to controls.)

There was a significantly lower level of erythrocyte GSH and plasma GST levels in patients compared to controls. The levels of erythrocyte MDA were significantly higher in gestational diabetic patients as compared to controls. In diabetes mellitus, the increased blood glucose levels induce oxidative stress and decrease antioxidant defences [6]. Possible source of oxidative stress and damage to protein in diabetes include free radicals generated by auto-oxidation of unsaturated lipids in plasma and membrane proteins [11]. Oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage and cell death, leading to increased free radical production and compromised free radical scavenger system, which further exaggerates the oxidative stress [12]. Abnormalities in the regulation of peroxide and transition metal metabolites are postulated to result in establishment of disease as well as its long-term complications [13].

In the present study, GSH, an antioxidant, was significantly lower in patients with gestational diabetes when compared to controls. The lower GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in these patients. Similar reports of lowered GSH levels in diabetes have been reported earlier by Rajdl et al. [14].

In the present study there is a significantly higher the level of erythrocyte MDA, a marker of lipid peroxidation in gestational diabetes as compared to normal pregnancy. Increase in levels of MDA in diabetic pregnancy was also reported by Kamath et al. [3],[15]. Bates et al. [16] in their study found no evidence of greater lipid peroxidation as compared to normal pregnancy, and total antioxidant capacity was similar in both the groups.

The GST is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood [17]. In this study, a significant decrease is observed in the levels of plasma GST levels in pregnant females with gestational diabetes, as compared to normal pregnant females. Similar results in the status of antioxidant enzymes were observed by Carone et al. [3],[18]. Decrease in GST activity in patients with gestational diabetes might indicate decreased detoxification or free radical scavenging capacity in pregnancy complicated by diabetes. This decrease in GST activity may result from decreased enzyme production or enzyme inactivation.

It has been reported that in normal pregnancy there is an increase of lipid peroxidation products in serum with advancing gestation, which is balanced by an adequate antioxidative response [19],[20]. But in diabetic pregnancy there is increased oxidative stress, leading to increased free radical generation and decreased antioxidant defences.

So, gestational diabetes induces oxidative stress leading to an easiest membrane lipid peroxidation and consequently results in membrane damage during diabetic gestation. However, due to the limited number of cases included in this study, more studies may be required to substantiate the results.

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