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LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN GESTATIONAL DIABETICS

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Abstract : The exact pro-oxidant and antioxidant status in gestational diabetes is still unclear. To add new insight to the question, changes in the lipid peroxidation products (MDA) and activities of antioxidant enzymes: superoxide dismutase (SOD) and catalase in red blood cell hemolysates were evaluated in 20 women with gestational diabetes. 20 healthy pregnant women served as the control group. Statistical analysis was done using unpaired Student' t – test. Pregnant women with gestational diabetes showed an increase in lipoperoxidation products ($P<0.001$) and a decrease in SOD activity ($P<0.01$) as compared to normal pregnant women while no significant change was observed in catalase activity. These findings suggest increased oxidative stress and decreased detoxification or free radical scavenging capacity in pregnancy complicated by diabetes.

Key words: lipid peroxidation, gestational diabetes, antioxidant enzymes, free radicals

INTRODUCTION

Gestational diabetes is associated with a greater incidence of congenital anomalies in comparison with non diabetic pregnancy. Frequency of congenital malformation among infants of diabetic mothers is estimated to be 6– 10% (1, 2). Factors responsible for these abnormalities are not fully understood but there are reports suggesting that increased free radical production and antioxidant depletion in diabetic pregnant female may contribute to this risk (3). In diabetes excess oxygen radicals may result from auto oxidation of glucose (4), increased generation of non enzymatically glycated proteins and elevated activity of mitochondrial electron transport chain because of increased glucose load (3). Also there are considerable evidences that antioxidant defence system is depleted and activity of antioxidant enzymes is reduced in diabetes (5). The imbalance between free radical production and antioxidant defenses in diabetes is reflected by greater levels of biological markers of free radical activity including Malondialdehyde (MDA), Conjugated dienes etc.

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Glucose can oxidize when catalyzed by trace amount of transition metals, leading to generation of free radicals, hydrogen peroxide and reactive ketoaldehyde. The process of glycation and free radical production are intimately linked by the propensity of glucose to undergo auto oxidation. Proteins exposed to glucose is cleaved, undergo conformational change and develops fluorescent adducts. The fragmentation and conformational changes observed are dependent upon hydroxyl radicals produced by glucose auto–oxidation. Antioxidants dissociate structural damage caused by exposure of glucose to protein from incorporation of monosaccharide into protein (6).

Normal pregnancy is also associated with increased oxidative stress causing increase in lipoperoxidation products but this lipid peroxidation is balanced by adequate antioxidative responses (7). So far data available regarding pro–oxidant and antioxidant status in gestational diabetes is insufficient and controversial. In an earlier study significant increase in lipid peroxidation levels and decrease in SOD activity in diabetic pregnancy as compared to normal pregnancy was reported (8). In contrast to this another study in early diabetic pregnancy found no evidence of greater lipid peroxidation in pregnant diabetics as compared to normal pregnant women and total antioxidant capacity was also reported to be similar in both the groups (9). Therefore present study was undertaken to assess lipid peroxidation and anti oxidant enzymes – superoxide dismutase (SOD) and catalase in females with gestational diabetes.

METHODS

Subjects

Study was conducted on 40 pregnant females reporting to Gynecology and Obstetrics department of Guru Teg Bahadur Hospital, Delhi. Study included 20 pregnant women with single pregnancy that were diagnosed as having gestational diabetes after undergoing glucose tolerance test with 100 grams of glucose as per criteria suggested by O' Sullivan and Mahan (10). Twenty age and gestational age matched normal pregnant females were taken as control. Age (Mean ± S.D.) of the subjects in control group was 25.42 ± 7 years (range 19 – 32) and in diabetic group was 26.06 ± 7.5 years (range 20 – 35). Gestational age in control group was 31.58 ± 6 weeks (range 26 – 38) and in diabetic pregnant group was 32.85 ± 4 weeks (range 29 – 36). Mean Hemoglobin concentration in control group was 11.1 ± 2.3 g/dL (range 9.2 – 13) and in study group was 10.9 ± 1.9 g/dL (range 8.6 –12.4). All the subjects in the study were normotensive and had a negative family history of diabetes, hypertension and obesity. Clearance was obtained from college ethical committee. An informed consent was taken from all the subjects.

1. *Serum TBARS level*: Assay was done by method described by Satoh (11). The thiobarbituric acid (TBA) reacting substances (TBARS) assay has been used as an indicator of lipid peroxidation and levels of free radicals in serum samples. The assay was based upon the reaction of TBA with Malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation. The serum

sample was heated with TBA under acidic conditions and the amount of MDA – TBA adduct produced was measured. For increased sensitivity, the complex was extracted into an organic solvent such as butanol and measured spectrophotometrically at 530 nm and the values were expressed in terms of Malondialdehyde (nMol/ ml).

2. *Estimation of catalase activity :* Catalase activity in red cell hemolysate was determined according to method of Sinha (12). It is based on the principle that dichromate in acetic acid as an unstable intermediate. The chromic acetate thus produced, was measured colorimetrically at 570 nm. Since dichromate has no absorbance in this region the presence of compound in the assay mixture does not interfere at all with colorimetric determination of chromic acetate. The reaction was stopped at a particular time by addition of dichromate/ acetic acid mixture and the remaining H_2O_2 was determined by measuring absorbance at 570 nm in spectrophotometer. Activity was expressed as U/ g Hb.

3. *Assay of erythrocyte Superoxide dismutase activity (SOD):* The activity of SOD in erythrocytes was determined by method described by Marklund and Marklund (13) with some modifications as described by Nandi and Chatterjee (14). The auto oxidation reaction was started by addition of freshly prepared pyrogallol solution to Tris–Hcl buffer at pH 8.5. The 50% inhibition of pyrogallol by SOD was measured by spectrophotometer at 420 nm. The activity was expressed as U/g Hemoglobin. Hemoglobin estimation was done by Cynomethhemoglobin method.

Statistical significance was assessed by unpaired Student's *t* – test.

RESULTS

As shown in Fig. 1 levels of TBARS (expressed in terms of Malondialdehyde n Mol/ ml of serum) were significantly higher in gestational diabetics as compared to normal pregnant females (P<0.001). One of

Table I: Levels of lipid peroxides† and antioxidant enzymes: SOD and catalase in normal pregnant women and gestational diabetics.

	Normal pregnancy (n = 20)	*gestational diabetes (n = 20)*
MDA (nMol/ml)	2.71±0.36	3.33±0.64**
SOD (U/g Hb)	407.83±38.21	372.77±38.11*
Catalase (U/g Hb)	6.27±0.74	6.12±0.63

†Expressed in terms of Malondialdehyde (MDA)
*P<0.001; **P<0.001

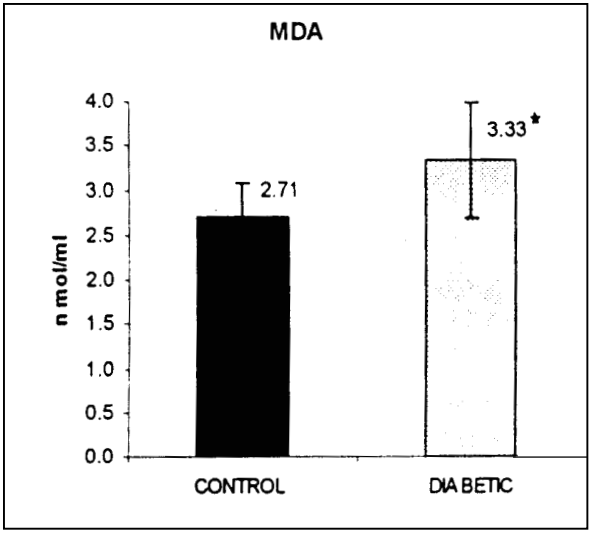

Fig. 1: Levels of lipid peroxides† in normal pregnant controls and gestational diabetics.
†Expressed in terms of Malondialdehyde (MDA)
*P<0.001

the indirect methods to correlate the changes of TBARS levels with oxygen radical mediated process is the evaluation of antioxidant enzyme activities. Statistical analysis of the data showed a significant decrease in the superoxide dismutase (SOD) activity in gestational diabetes ($P<0.01$) as compared to controls (Fig. 2) whereas catalase (CAT) activity in women with gestational diabetes did not show any significant difference as compared to normal pregnant women (Fig. 3).

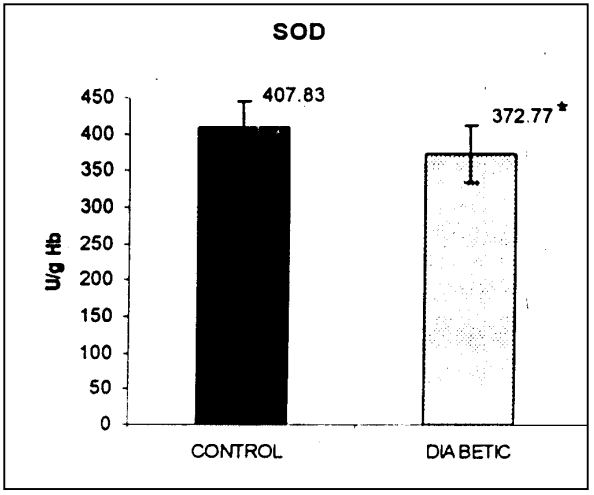

Fig. 2: Levels of sod in normal pregnant controls and gestational diabetics.
*P<0.001

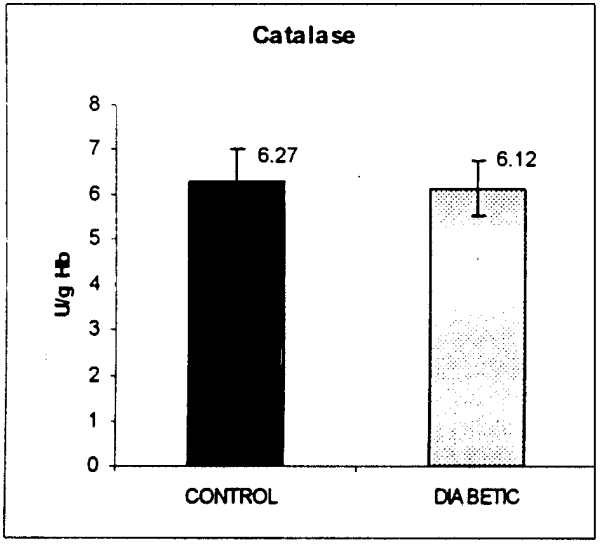

Fig. 3: Catalase levels in normal pregnant controls and gestational diabetics.

DISCUSSION

Diabetes mellitus is a syndrome characterized by a perturbation of glucose homeostasis. It has been seen that increased blood glucose levels induce oxidative stress and decrease antioxidant defenses apparent in diabetes [5]. 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. 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 exacerbates the oxidative stress (15). Abnormality in the regulation of peroxide and transition metal metabolites are postulated to result in establishment of disease as well as its long term complications (6).

Free radicals are difficult to measure directly because of their highly unstable nature so levels of various lipid peroxidation products has been used as an indicator of free radical activity.

In the present study there is a significant increase in levels of MDA, a marker of lipid peroxidation in gestational diabetes as compared to normal pregnancy. SOD activity was found to be significantly lower in

pregnant females with gestational diabetes as compared to normal pregnant females. Similar results were also reported by Carone et al (8). They observed a significant rise in levels of TBARS values and a lower SOD activity in diabetic pregnancy as compared to normal pregnancy. However no change in catalase activity was reported in diabetic pregnancy. Increase in levels of MDA in diabetic pregnancy was also reported by kamath et al (16). Bates et al (9) in their study of early diabetic pregnancy of less than 12 weeks gestation found no evidence of greater lipid peroxidation as compared to normal pregnancy and total antioxidant capacity was similar in both the groups. Data available regarding pro–oxidant and antioxidant status in diabetic pregnancy is scanty and controversial.

In present study the activity of antioxidant enzyme SOD, which detoxifies the superoxide anion radical, were found to be decreased in gestational diabetes as compared to normal pregnancy. Decreased SOD activity in gestational diabetics might indicate decreased detoxification or free radical scavenging capacity in pregnancy complicated by diabetes. This decrease in SOD activity may result from decrease enzyme production or enzyme inactivation. It has been found that human erythrocyte contain glucosylated and non–glucosylated SOD. The percentage of glucosylated form, which has a lower enzymatic activity, is significantly increased in erythrocytes of diabetic patients as compared to normal erythrocytes. Thus a mechanism for in–vivo inactivation of SOD during diabetes was suggested (17).

It has been reported that in normal pregnancy there is an increase of lipoperoxidation products in serum with advancing gestation which is balanced by an adequate antioxidative response (7). But in diabetic pregnancy there is increased oxidative stress leading to increased free radical generation and decreased antioxidant defences. The proposed mechanism for oxygen free radical generation at higher glucose concentration in pregnancy include–

i. Non–enzymatic protein glycation, which may induce production of oxygen free radicals (18).

ii. increased mitochondrial electron transport chain flow and

iii. Oxidative activities of the fetus (19).

Primary effect of increased oxygen free radical in gestational diabetes is believed to be on enhanced lipid peroxidation. Hydro–peroxides which are major products of lipid peroxidation have been shown to alter prostaglandin biosynthesis which may be responsible for development of diabetes related embryopathy (19).

In conclusion gestational diabetes induces a condition of oxidative stress leading to an easier membrane lipoperoxidability and consequently easier membrane damage during diabetic gestation.

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