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ORIGINAL ARTICLE

Antioxidant Enzymes And Vitamins In Gestational Diabetes.

SURAPANENI K M, VISHNU PRIYA V

ABSTRACT

The exact pro-oxidant and antioxidant status in gestational diabetes is still not clear. To add a new insight to the question, levels of erythrocyte antioxidant vitamin Ascorbic acid and plasma vitamin E (non enzymatic antioxidant vitamin parameters) and activities of antioxidant enzymes Super Oxide Dismutase (SOD), Glutathione Peroxidase (GP_X) and Catalase in erythrocytes were studied in patients with gestational diabetes and compared to controls. Statistical analysis between controls and patients was performed by Mann Whitney U test using stat - view package. It was observed that there was a significant increase in activities of SOD, GP_X and a significant decrease in erythrocyte ascorbic acid, plasma vitamin E levels and Catalase activity in patients with gestational diabetes when compared to controls. Results of our study depict higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of GSH, ascorbic acid, vitamin E and Catalase activity, supporting the evidence of oxidative stress in gestational diabetes patients. Increased activities of antioxidant enzymes might be a compensatory regulation of body in response to increased oxidative stress. Decreased concentrations of antioxidant vitamins support the hypothesis that gestational diabetes is an important causative factor in pathogenesis of lipid peroxidation. These data reveal that antioxidant defense mechanisms might be impaired in patients with gestational diabetes. These findings also provide a theoretical basis for development of novel therapeutic strategies, such as antioxidant supplementation.

Key Words

Ascorbic acid, vitamin E, superoxide dismutase (SOD), glutathione peroxidase, catalase, gestational diabetes.

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Introduction

Gestational diabetes is occurrence of diabetes in previously normal women and is associated with an increased incidence of congenital abnormalities when compared with normal pregnancy. Frequency of congenital malformations 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 formation of anomalies in fetuses born to women with diabetes. Moreover, body's defense mechanism would play a role in formation of antioxidants and attempt to minimize 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 might contribute to formation of anomalies [4]. In diabetes, excess oxygen radicals may result from auto oxidation of glucose [5] and increased glycated

hemoglobin levels, because of increased glucose levels in body [4]. There is considerable evidence that antioxidant defense system is depleted and activity of antioxidant enzymes is reduced in diabetes [6].

In the present study, following parameters were assessed in erythrocytes and plasma to elucidate the oxidant – antioxidant status in patients with gestational diabetes. Erythrocyte ascorbic acid, plasma vitamin E which serve as non enzymatic antioxidant parameters, antioxidant enzymes Super Oxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GP_X) in erythrocytes. The present study is an attempt to examine oxidative stress and status of protective antioxidants under conditions of stress due to gestational diabetes. Alterations in antioxidant enzymes have been reported by various studies [3]. In our previous study we showed that lipid peroxidation was significantly increased along with decreased glutathione in patients with gestational diabetes when compared to control subjects [7]. However antioxidant vitamins' and antioxidant enzymes' status were not assessed. Therefore in present study, concentrations of erythrocyte ascorbic acid and plasma vitamin E status were estimated along with status of antioxidant enzyme activities in patients with gestational diabetes.

Materials And Methods

The study was conducted in Department of Biochemistry, Saveetha Medical College & Hospital, Saveetha University, Chennai, T.N, India. Twenty patients (mean age: 26.40 ± 0.82 years) with proven gestational diabetes were chosen from Obstetrics & Gynaecology department as study subjects (patients). They were diagnosed to have gestational diabetes after undergoing glucose tolerance test with 100gms of glucose as per criteria suggested by O'Sullivan and Mahan [8]. Twenty age- and gestational age- matched normal pregnant females with similar socio-economic status were taken as controls. All 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 study. Subjects with normal nutritional habits without any vitamins supplement since last 6 months were included. Due permission was obtained from ethical committee of the institution before the study. Written consents were also taken from patients prior to study and objectives of study fully explained. Eight of the participants were dropped out at the end of selection, as they did not like the idea of giving blood.

Two groups of participants were included in study. Cases were the ones who had gestational diabetes and controls were healthy pregnant age matched women.

[Table/Fig 1] Demographic Details Of Gestational Diabetics And Control Subjects.

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

Heparinised venous blood samples obtained from these subjects were used for analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for estimation of vitamin E. Buffy coat was removed and packed cells were washed three times with physiological saline. Erythrocyte suspension was prepared by method of Dodge et al.,[9] modified by Quist [10]. Packed cells were used for analysis of ascorbic acid, SOD, Catalse and GP_X. Erythrocyte ascorbic acid levels were estimated in plasma by method of Tietz [11]. Plasma vitamin E levels were estimated by method of Baker H et al (12). SOD (EC 1.15.1.1)

activity was determined in hemolysate according to method described by Murklund and Murklund [13] with some modifications as described by Nandi and Chatterjea (14). Catalase (EC 1.11.1.6) activity was measured in hemolysate by method of Sinha [15] and activity of Glutathione Peroxidase (GPX, EC 1.11.1.9) was measured as described by Paglia and Valentine [16] in erythrocytes. All reagents used were of analytical reagent grade. All chemicals were obtained from sigma chemicals, St.Louis, MO. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Mann Whitney U test. Data were expressed as mean $\pm$ SD. P value < 0.05 was considered as significant.

Statistical Analysis

Statistical analysis between controls and Study Subjects (patients) was performed by Mann Whitney U test.

Results And Discussion

Mean $\pm$ SD of erythrocyte GSH & MDA and plasma GST are indicated in [Table/Fig 2].

[Table/Fig 2] Mean $\pm$ SD Values Of Ascorbic Acid, Vitamin E, Superoxide Dismutase (SOD), Catalase And Glutathione Peroxidase (GP_X) In Controls And Study Subjects (Patients) With Gestational Diabetes.

Parameter	Controls n=20	Study Subjects (Patients) n =20
Ascorbic Acid (mg/dl)	6.17 ± 0.18	4.75 ± 0.29 ***
Vitamin E(µmoles/L)	9.23 ± 0.23	7.87 ± 0.18 ***
SOD(U/gm of Hb)	392.66 ± 18.22	419.82 ± 18.11 ***
Catalase(U/gm of Hb)	11.58 ± 0.34	10.75 ± 0.20 ***
GP_X(U/gm of Hb)	68.66 ± 1.76	73.34 ± 2.66 ***

*** p <0.001 compared to controls

There was a significantly lower level of erythrocyte ascorbic acid, activity of catalase and plasma vitamin E levels in patients compared to controls. Levels of erythrocyte SOD and GP_X were significantly higher in gestational diabetic patients as compared to controls. In diabetes mellitus, increased blood glucose levels induce oxidative stress and decrease antioxidant defenses [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 [17]. 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 oxidative stress [18]. Abnormalities in regulation of peroxide and transition metal metabolites are postulated to result in establishment of disease as well as its long-term complications [19].

In this study we observed a significant decrease in levels of erythrocyte ascorbic acid and plasma vitamin E (non enzymatic antioxidant vitamins defense system) in gestational diabetes patients when compared to controls. GSH, vitamin E, and ascorbic acid are important chain breaking antisoxidants responsible for scavenging free radicals and suppression of peroxidation in aqueous and lipid region of cell [20],[21]. Decrease in levels of these non enzymatic antioxidant parameters may be due to increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in gestational diabetics. Similar reports of decreased GSH, ascorbic acid and vitamin E levels in gestational diabetic mothers compared to control mothers were reported by Griss O et al [22]. In contrast to our observations Santra D et al reported decreased lipid peroxidation levels and increased alpha tocopherol levels in gestational diabetes patients compared to controls [23].

In this study, erythrocyte antioxidant enzyme i.e. super oxide dismutase (SOD) & glutathione peroxidase (GP_x) activities were increased significantly in patients with gestational diabetes compared to controls. Increased activity of SOD may be indicative of increased superoxide generation by whichever mechanism, like increased catecholamine metabolism. SOD is an important antioxidant enzyme having an antitoxic effect against super

oxide anion. Over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme plays a significant role in peroxyl scavenging mechanism and in maintaining functional integration of cell membranes. Rise in activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. GP_X provides an effective protective mechanism against cytosolic injury because it eliminates H_2O_2 and lipid peroxides by reduction, utilizing GSH. Decrease in antioxidant enzyme status was reported in various studies [24].

In the present study, we observed a significant decrease in activity of catalase in gestational diabetics compared to controls. Catalase is an enzyme which protects cells from accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant, a process in which it works as a peroxidase [25].

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 [26],[27]. But in diabetic pregnancy there is increased oxidative stress leading to increased free radical generation and decreased antioxidant defenses.

Conclusion

So, gestational diabetes induces oxidative stress leading to an easiest membrane lipid peroxidation and consequently results in membrane damage during diabetic gestation. Decreased concentrations of antioxidant vitamins supports the hypothesis that gestational diabetes is an important causative factor in pathogenesis of lipid peroxidation. These data reveal that antioxidant defense mechanisms might be impaired in patients with gestational diabetes. These findings also provide a theoretical basis for development of novel therapeutic strategies, such as antioxidant supplementation. However due to the limited number of cases included in this study, more studies may be required to substantiate these results.

References

[1]. Hagay ZJ, weiss Y. Zusman I, Kumar MP, ReeceEA, Errikson UJ, Groner Y. Prevention of diabetes-associated embryopathy by over expression of the free radical scavenger copper Zinc superoxide dismutase in transgenic mouse embryos. Am j obstet gynecol 1995; 173:1036-41.

[2]. Reece EA, Wu YK, Wiznitzer. A role of free radical in membrane lipid induced congenital malformation. J Soc Gynaecol Invest.1998; 5(9): 178-87.

[3]. Carone D. Lipid Peroxidation Products and antioxidant enzymes in red blood cells during normal and diabetic pregnancy. Eur J Obstet Gynaecol Reprod Biol. 1993; 51: 103-9.

[4]. Eriksson UJ, Borg LAH. Protection by free oxygen radical scavenging enzymes against glucose induced embryonic malformation invitro. Diabetologia. 1991; 34: 325-31.

[5]. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radical Biology and Medicine. 1991; 10: 339-52.

[6]. Tho LL, Candlish JK, Thai AC. Correlation of diabetes markers with erythrocyte enzyme decomposing reactive oxygen Species. Annals of clinical biochemistry. 1988; 25: 426-31.

[7]. Surapaneni KM. Oxidant – Antioxidant Status in gestational diabetes patients. Journal of Clinical and Diagnostic Research. 2007; 1 (4): 235 – 38.

[8]. O'Sullivan JB. Mahan CM.Criterion for oral glucose tolerance test in pregnancy Diabetes. 1964; 13: 278-85.

[9]. Dodge JF, Mitchell G, and Hanahan DJ. The preparation and chemical characterization of hemoglobin free ghosts of human red blood cells. Arch. Biochem. Biophys. 1968; 110: 119-30.

[10]. Quist EH. Regulation of erythrocyte membrane shape by calcium ion. Biochem Biophys Res Commun. 1980; 92:631-37.

[11]. Tietz NW. In; Text book of clinical chemistry, Edited by N W Tietz, W B Saunders company, Philadelphia, London, Toronto. 1986; 960-62.

[12]. Baker H, Frank D, and Winley N C. Clinical Vitaminology. 1968; 772.
[13]. Marklund S, Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur. J. Biochem 1974; 47: 469.
[14]. Nandi A, Chatterjea IB. Assay of SOD activity in animal tissues. J. Biosc 1988; 13(3): 305-15.
[15]. Sinha AK. Colorimetric assay of catalase. Annal. Biochem. 1972; 47: 389-94.
[16]. Paglia DE. and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J.Lab.Clin.Med. 1967; 70: 158-59.
[17]. Haliwell B and J.M.C Gutteridge. The chemistry of oxygen radicals and other oxygen derived species. Free radicals in Biology and Medicine. New York: Oxford University Press, 1985; P 20-64.
[18]. Baynes JW. Role of oxidative stress in development of complication of diabetes. Diabetes. 1991; 40: 405-12.
[19]. Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and auto oxidative glycosylation. Biochem J. 1988; 256: P 205-12.
[20]. Niki, ER. Antioxidants in relation to lipid peroxidation. Chem. Of Physiol. Lipids. 1987; 44: 227-253.
[21]. Niki E. Interaction of ascorbate and alpha tocopherol. Ann. N. Y. acad. Sci. 1987; 498: 186-99.
[22]. Grissa O, Ategbo JM, Yessoufou A, Tabaka Z, Miled A, Jerbi M, Dramane KL, Moutairou K, Prost J, Hichami A and Khan NA. Antioxidant status and circulating lipids are altered in human gestational diabetes and macrosomia. Transl Res. 2007 Sep; 150 (3): 164 – 71.
[23]. Santra D, Sawhney H, Aggarwal N, Majumdar S, Vasishta K. Lipid peroxidation and vitamin E status in gestational diabetes mellitus. J Obstet Gynaecol Res. 2003 Oct; 29 (5): 300 – 4.
[24]. Coughlan MT, Vervaart PP, Pemezel M, Georgiou HM, Rice GE. Altered placental oxidative stress status in gestational diabetes mellitus. Placenta. 2004 Jan; 25 (1): 78 – 84.
[25]. Lenzi A, Cualosso F, Gandini L, Lombardo F and Dondero F. Placebo controlled double-blind cross over trial glutathione therapy, in male infertility. Hum. Reprod. 1993; 9: 2044.
[26]. Uotila J, Tuimala R, Aarnio T, Pyykko K, Ahotupa M. Lipid peroxidation products, selenium dependent Glutathione Peroxidase and vitamin E in pregnancy. Eur J Obstet Gynaecol Reprod Biol. 1991; 42: 95-100.
[27]. Oxidative stress: Changes in pregnancy and with gestational diabetes mellitus. Curr Diab Rep. 2005; 5(4): 282 – 8. Review.