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ORIGINAL RESEARCH ARTICLE

Antioxidant Status in Type ll Diabetes Mellitus

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ABSTRACT

Diabetes, a lifelong progressive disease, is the result of body's inability to produce insulin or use insulin to its full potential, and is characterized by high circulating glucose. This disease has reached epidemic proportion and has become one of the most challenging health problems of the 21st century. In the present research work on type II diabetes mellitus, various biochemical parameters have been analyzed in a systematic manner. Blood and urine samples were collected from Sun and Apollo laboratory in Coimbatore. About thirty diabetic (type II) patients (15 males and 15 females) and thirty normal individuals (17 males and 13 females). Blood and urine samples were collected from the normal and diabetic individuals. The blood was obtained by vein puncture and collected in the centrifuge tubes. To analyse the various Enzymic and Non-enzymic antioxidants such as Superoxide dismutase, catalase, glutathione peroxidise, total reduced glutathione and lipid peroxidation. The study is significantly decreased level of GPX was noted in diabetic, when compared to normal control, whereas all the other antioxidants showed only little reduction in their levels but not significant, in diabetic than the normal control. Increased level of lipid peroxide was observed in diabetes as compared to respective control. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of functions of erythrocyte membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes.

Key words: Diabetes mellitus, Lipid peroxide, insulin, Superoxide dismutase, catalase, glutathione peroxidise, and total reduced glutathione.

INTRODUCTION

Diabetes, a lifelong progressive disease, is the result of body's inability to produce insulin or use insulin to its full potential, and is characterized by high circulating glucose. This disease has reached epidemic proportion and has become one of the most challenging health problems of the 21st century. It is the fourth leading cause of death by disease globally; every 10 seconds a person dies from diabetes related causes (Kowluru and Chan, 2007). Type 1 diabetes, prevuously known as insulin – dependent diabetes mellitus orjuvenile-onset diabetes, is the most severe form of the disorder. It is an autoimmune disease that develops when the body begins to destroy its insulinproducing pancreatic beta cells. The resulting shortage of insulin causes glucose to remain in the bloodstream instead of entering the cells to be converted into energy. Type 1 diabetes typically affects children and young adults and is the number one cause of diabetes in children; however, its onset can occur at any age. In order to survive, the patient must receive daily injections of insulin (U.S. Department of Health and Human Services, National Institutes of Health (NIH). 2008a).

Type 2 diabetes, previously referred as non-insulin dependent diabetes mellitus or adult-onset diabetes, is the most common form of diabetes. It accounts for 90 to 95 percent of the documented cases of diabetes (3). While the exact etiology of type 2 diabetes is unknown, studies indicate that the disorder begins in the form of insulin resistance. The pancreas produces sufficient insulin but the body cannot properly use the insulin to transport glucose into the cells. The pancreas responds to the resultant high blood

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sugar level by producing more insulin. Over time, the pancreas loses the ability to produce the amount of insulin needed to transport glucose into cells. Ultimately, the pancreas becomes permanently damaged due to the continual elevation of insulin production.

Gestational diabetes is diagnosed when any degree of glucose intolerance or high blood sugar is first discovered during pregnancy. This type of diabetes occurs inapproximately 3-8 percent of all pregnancies and usually disappears during the postpartum period (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC). 2009).

Maturity Onset Diabetes of the Young (MODY) occurs in individuals less than 20 years old and is considered a form of type 2 diabetes. MODY is known to be the result of genetic mutation and can be passed from parent to child. Several different gene mutations have been identified but each mutation results in lack of insulin production by the pancreas. Approximately 1-5 percent of all cases of diabetes in the U.S. are MODY (U.S. Department of Health and Human Services, National Institutes of Health (NIH). 2007).

Pre-diabetes is the state where a patient's blood glucose level is higher than normal but not high enough to be considered diabetes. This condition may also be referred to as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) depending on which test was used to measure the blood sugar level. U.S. Department of Health and Human Services, National Institutes of Health (NIH). 2008b).

MATERIALS AND METHODS

In the present research work on type II diabetes mellitus, various biochemical parameters have been analysed in a systematic manner. The methodology followed was given below. Blood and urine samples were collected from Sun and Apollo laboratory in Coimbatore. About thirty diabetic (type II) patients (15 males and 15 females) and thirty normal individuals (17 males and 13 females). Blood and urine samples were collected from the normal and diabetic individuals. The blood was obtained by vein puncture and collected in the centrifuge tubes.

Antioxidants

Antioxidants work to protect our body from free radicals. Antioxidant means "against oxidation ". Antioxidants are effective because they are willing to give up their own electrons to free radicals. To analyse the various Enzymic and Non-enzymic antioxidants such as Superoxide dismutase, catalase, glutathione peroxidise, and total reduced glutathione.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Statistical analysis

Results are Mean ± SD for 60 samples. Values are expressed as mg / dl. Values that have a different superscript (a,b,c) differ significantly with each other ($P<0.05$). Data were analysed using the statistical pack Students't' Test and one way Analysis Of Variance (ANOVA).

RESULTS

Level of antioxidants in normal and diabetic patients

Antioxidants work to protect our body from free radicals. Antioxidant means "against oxidation ". Antioxidants are effective because they are willing to give up their own electrons to free radicals. In our present study, the enzymic and non-enzymic antioxidants such as Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPX), and total reduced glutathione (GSH) were analyzed. The levels of antioxidants like SOD, CAT, GPX and GSH in normal and diabetic patients are represented in the (**Table 1**).

Table 1: Level of Antioxidants in normal and diabetic patients

Parameter	Normal (Non-diabetic) (n=30)	Diabetic(n=30)	't' Value
SOD	0.97 ± 0.63	0.86 ± 0.68	0.677 ns
CAT	1.21 ± 2.45	0.83 ± 0.82	0.79 ns
GPX	47.66 ± 13.94	35.82 ± 22.84	2.382*
GSH	14.26 ± 13.47	12.14 ± 7.52	0.740

Units:

SOD- 50% inhibition of nitrite formation / min / mg protein

CAT- µ moles of H_2O_2 decomposed / min / mg protein.

GPX- µg of glutathione utilized / min / mg protein.

GSH - µM / mg

Values are mean ± SD; * - Significant at 5% ($t<0.05$) ns – not significant.

Significantly decreased level of GPX was noted in diabetic, when compared to normal control, whereas all the other antioxidants showed only little reduction in their levels but not significant, in diabetic than the normal control.

Antioxidants destroy the peroxides and play a significant role in providing antioxidant defence to an organism (Cuerda *et al*., 2011). The

functions of all antioxidants are interconnected and a lowering of their activities may result in the accumulation of lipid peroxides and increases oxidative stress in diabetes, as observed in the present study.

SOD is one of the most important enzymes in the antioxidant defense system of the body. The major function of SOD is to catalyze the conversion of superoxide anion radicals (the first product of oxygen radical formation) to H_2O_2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions (Sen and Hanninen, 1994). During diabetes, ROS may be generated through glucose auto oxidation and non-enzymatic protein glycation under hyperglycemic condition (Hunt *et al*., 1990; Wolff and Dean, 1987). Moreover ROS could be generated under diabetic condition at an early stage (Ugochukwu and Cobourne, 2003).

The reason for depletion of antioxidants may be due to the protective mechanism of the body in response to the increased generation of superoxide anion radicals. Under invitro conditions, it has been reported that SOD activity could be inhibited by glycation of the enzyme (Arai *et al.,* 1987b; Bray *et al.,* 1974). Furthermore, H_2 O_2 has been shown to inactivate SOD (Arai *et al.,* 1987a). Therefore, the decrease in SOD activity may be due to inhibition of the enzyme by glycation or by the accumulation of H_2O_2. CAT, which is present virtually in all mammalian cells, is responsible for the removal of H_2O_2 the possible reason for the depletion of this enzyme may be due to the defence mechanism against ROS attack in the tissues. The decreased CAT levels in serum of the diabetic patients may also be related to the concentration of H_2O_2 and /or the location of the enzyme. CAT is localized mainly in the peroxisomal compartment in the mitochondria, and it is effective at excess concentrations of H_2O_2 while GPX is found in the cytosol and can detoxify H_2O_2 at lower concentrations (Sen and Hanninen, 1994).

Free radical-mediated oxidative damage has been observed in the development of both type I and type II diabetes mellitus (Marjani, 2010). Chronic hyperglycemia increases oxidative stress by auto-oxidation of monosaccharides,which leads to production of superoxide and hydroxyl radicals. These radicals cause tissue damage by reacting with polyunsaturated fatty acids in membranes and this leads to increase in lipid peroxidation (Østerud and Elvevoll, 2011).

Hyperglycaemia in diabetes may increase ROS production via changes in the redox potential of glutathione and decreased antioxidant defences due to reduction in total antioxidant capacity in plasma (West, 2000).

Level of lipid peroxidation in normal and diabetic patients

Lipid peroxidation (LPO) is a key marker of oxidative stress. It is a free radical induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction. The significant extent of LPO products that was measured as thiobarbituric acid reactive substances (TBARS) has been reported in diabetes (Pari and Murugan, 2005).

Peroxidation of membrane lipids associated with increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity has been implicated in diabetes mellitus. The occurrence of free radical-induced lipid peroxidation causes considerable changes in the structural organization and function of the membrane and makes it leaky (Cuerda *et al.,* 2011).

The level of lipid peroxide (LPO) in normal and diabetic patients are represented in the (**Table 2**).

Table 2: Level of Lipid peroxidation in normal and diabetic patients

Parameter	Normal (Non-diabetic) (n=30)	Diabetic (n=30)	t' Value
LPO	11.71 ± 2.43	14.47 ± 8.94	1.603*

Units:

LPO- nM of MDA formed / min / mg protein.

Values are mean ± SD ; * - Significant at 5% ($t<0.05$) ns – not significant

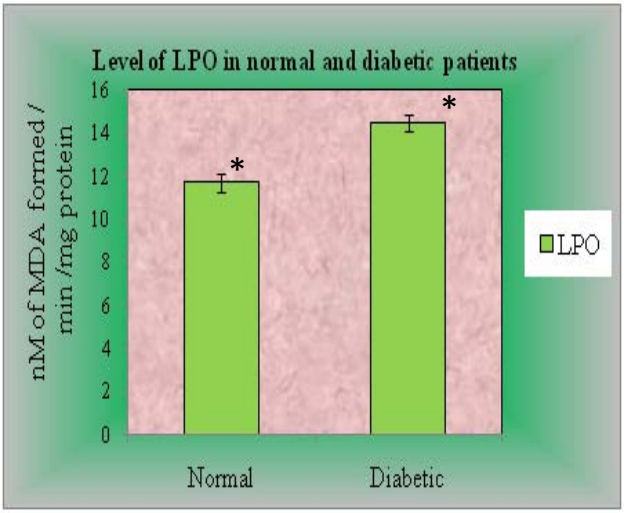
Significantly increased level of lipid peroxide was observed in diabetes as compared to respective control. Free radical induced oxidative stress has been implicated in the etiology of diabetes mellitus. Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes condition (Zhang *et al*., 2010).

Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free-radical generation but also due to nonenzymatic

protein glycosylation, auto-oxidation of glucose (Mullarkey *et al.,* 1990). impaired glutathione metabolism (Ansar *et al.,* 2011), alteration in antioxidant enzymes (Bellamkonda *et al.,* 2011), lipid peroxides formation (Middha *et al.,* 2011) and decreased ascorbic acid levels (Okazaki *et al.,* 2011).

Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications (Gutteridge and Halliwell, 2010). Enhanced oxidative stress and changes inantioxidant capacity, observed in both clinical and experimental diabetes mellitus are thought to be the etiology of chronic diabetic complications (Baynes and Thorpe, 1996). In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications. Free radical may play a pivotal role in the pathogenesis of a number of diseases including diabetes mellitus.

Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes (Freitas, *et al.,* 1997).



DISCUSSION

In recent years, the number of patients suffering from diseases, such as cancer, apoplexy, osteoporosis, hypertension, and diabetes mellitus is increasing worldwide. It is projected that the incidence of diabetes is on the rise (Boyle *et al.,* 2001). The present number of diabetics worldwide is over 150 million and this is likely to increase to 300 million or more by the year 2025 (King *et al.,* 1998; Shaw *et al.,* 2010). Reasons for this increase include increase in sedentary lifestyle, consumption of energy-rich diet, obesity and life span. Type 2 diabetes, a lifestyle-related disease, is recognized as a serious disease. Normally, diabetes is detected by measuring glucose blood levels. However, due to wide deviations in the circulating glucose concentrations, a randomized glucose measurement does not provide clear data for overall glycemic control. A better method to evaluate the level of control is the measurement of the HbA1C value, which is a normally used laboratory test for measuring long-term diabetic control (Andrade-Cetto *et al.,* 2008).

The study suggests, the estimation of plasma antioxidants levels with other routine investigations may be useful in the prevention of the diabetic complications, which can be prevented by supplementing the antioxidants rich components of the diet and thus further diabetic events can be avoided.

International researchers observed that antioxidants commonly promoted as being good for our health may speed up early onset of type 2 diabetes by mopping up Reactive Oxygen Species (ROS) that may play a protective role in the early stages of type 2 diabetes by enhancing insulin action (Sarah Akbar *et al.,* 2011).

REFERENCES

1. Kowluru, A.R. and Chan. P.S. (2007). Oxidative stress and Diabetic Retinopathy. Experimental diabetes research. 1-12.
2. U.S. Department of Health and Human Services, National Institutes of Health (NIH). (2008a). *Diabetes overview*. Retrieved January 4, 2010, from http://diabetes.niddk.nih.gov/dm/pubs/overview/DiabetesOverview.pdf.
3. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC). (2009). *Diabetes: Successes and opportunities for population based prevention and control.* Retrieved January 6, 2010, from http://www.cdc.gov/chronicdisease/resources/publications/aag/pdf/diabetes_success.pdf.
4. U.S. Department of Health and Human Services, National Institutes of Health (NIH). (2007). *Monogenic Forms of Diabetes.* Retrieved January 1, 2010, from http://diabetes.niddk.nih.gov/dm/pubs/mody/mody.pdf

5. U.S. Department of Health and Human Services, National Institutes of Health (NIH). (2008b). Insulin resistance and pre-diabetes. Retrieved January 4, 2010, from http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/insulinresistance.pdf
6. Cuerda C, Luengo LM, Valero MA, Vidal A, Burgos R, Calvo FL, Martínez C. (2011). Antioxidants and diabetes mellitus: review of the evidence.Nutr Hosp. Jan-Feb., 26(1): 68-78.
7. Sen, C.K., Hanninen, O., (1994). Physiological Antioxidants. In: Sen, C.K., Packer, L., Hanninen, O. (Eds), Exercise and oxygen toxicity. Elsevier, New York ., PP. 89-126.
8. Hunt, J.V., Smith, C.C and Wolff, S.P. (1990). Autooxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabates., 39 (11): 1420-1424.
9. Wolff, S.P. and Dean, R.T. (1987). Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. Biochemical Journal., 245(1): 243-250.
10. Ugochukwu, N.H. and Cobourne, M.K. (2003). Modification of renal oxidative stress and lipod peroxidation streptozotocin induced diabetic rats treated with extracts from Gongronemalatifolium leaves. ClimicachimicaActa., 36(1-2): 73-81.
11. Arai, K., Iizuka, S., Tada, Y., Olikawa, K. And Taniguchi, N. (1987a). Increase in the glycosylated form of erythrocyte cu-zn superoxide dismutase in diabetes and close association of the non enzymeglucosylation with the enzyme activity. Biochimica et BiophysiaActa., 924 (2) : 292- 296.
12. Arai, K., Iizuka, S., Tada, Y., Olikawa, K. And Taniguchi, N. (1987b). Increase in the glycosylated form of erythrocyte cu-zn superoxide dismutase in diabetes and close association of the non enzymeglucosylation with the enzyme activity. Biochimica et BiophysiaActa., 856 (2) : 345- 784.
13. Bray, R.C., Cockle, S.a., Fielden, E.M., Roberts, P.B., Rotilio, G. And calabrese, L. (1974). Reduction and inactivation of superoxide dismutase by hydrogen peroxide. Biochemical Journal. 139 (1); 43-48.
14. Sen, C.K., Hanninen, O., (1994). Physiological Antioxidants. In: Sen, C.K., Packer, L., Hanninen, O. (Eds), Exercise and oxygen toxicity. Elsevier, New York ., PP. 89-126.
15. Marjani A. (2010). Lipid peroxidation alterations in type 2 diabetic patients.Pak J BiolSci, Aug., 1; 13(15): 723-30.
16. Østerud B, Elvevoll EO.(2011). Dietary omega-3 fattyacids and risk of type 2 diabetes: lack of antioxidants?Am J ClinNutr, Aug; 94(2): 617-8; author reply 618-9.
17. West IC. Radicals and oxidative stress in diabetes. Diabet Med (2000)., 17: 171-80.
18. Pari L, Murugan P. (2005). Changes in Glycoprotein Components in Streptozotocin - Nicotinamide Induced Type 2 Diabetes: Influence of Tetrahydrocurcumin from Curcuma longa. Plant Food Hum Nutr., 62: 25-29.
19. Cuerda C, Luengo LM, Valero MA, Vidal A, Burgos R, Calvo FL, Martínez C. (2011). Antioxidants and diabetes mellitus: review of the evidence.Nutr Hosp. Jan-Feb., 26(1): 68-78.
20. Zhang X, Bao Y, Ke L, Yu Y. (2010). Elevated circulating free fatty acids levels causing pancreatic islet cell dysfunction through oxidative stress. J Endocrinol Invest. Jun., 33(6): 388-94.
21. Mullarkey CJ, Edelstein D, Brownlee M. (1990). Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes.BiochemBiophys Res Commun., Dec 31; 173(3): 932-9.
22. Ansar H, Mazloom Z, Kazemi F, Hejazi N. (2011). Effect of alpha-lipoic acid on blood glucose, insulin resistance and glutathione peroxidase of type 2 diabetic patients.Saudi Med J. Jun., 32(6): 584 - 8.
23. Bellamkonda R, Rasineni K, Singareddy SR, Kasetti RB, Pasurla R, Chippada AR, Desireddy S. (2011). Antihyperglycemic and antioxidant activities of alcoholic extract of Commiphoramukul gum resin in streptozotocin induced diabetic rats. Pathophysiology., 18(4): 255-61.
24. Middha SK, Bhattacharjee B, Saini D, Baliga MS, Nagaveni MB, Usha T. Protective role of

Trigonellafoenumgraceum extract against oxidative stress in hyperglycemic rats.Eur Rev Med Pharmacol Sci. 2011 Apr; 15(4): 427-35.
25. Okazaki T, Otani H, Shimazu T, Yoshioka K, Fujita M, Iwasaka T. (2011).Ascorbic acid and N-acetyl cysteine prevent uncoupling of nitric oxide synthase and increase tolerance to ischemia/reperfusion injury in diabetic rat heart.FreeRadic Res., Oct; 45(10): 1173-83.
26. Gutteridge JM, Halliwell B. (2010). Antioxidants: Molecules, medicines, and myths.BiochemBiophys Res Commun., Mar 19. 393(4): 561-4.
27. Baynes JW, Thorpe SR (1996). The role of oxidative stress in Diabetic complications of diabetes. CurrOpinEndocr., 3: 277- 284.
28. Freitas, J.P., Filipe, P.M. and Radrigo, F.G. (1997). Lipid peroxidation in Type-II normolipidemicdiabeticpatients Diabetes. Res. Practy., 36 (2), 71-75.
29. Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H. ThompsonTJ. (2001). Projection of diabetes burden through 2050: Impact of changing demography and disease prevalence in the US. Diabetes Care, 24., 1936-40.
30. King H, Aubert RE, Herman WH. (1998). Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections.DiabetesCare., Sep., 21(9): 1414-31.
31. Shaw JE, Sicree RA and ZimmetPZ . (2010). Global estimates of the prevalence of diabetes for 2010 and 2030.Diabetes Res. Clin.Pract., 87; 4–14.
32. Andrade-Cetto A, Martínez-Zurita E, Soto-Constantino A, Revilla-Monsalve C, Wiedenfeld H. (2008). Chronic hypoglycaemic effect of Malmeadepressa root on streptozotocin diabetic rats.JEthnopharmacol., Mar 5., 116(2): 358-62.
33. Sarah Akbar, Srikanth Bellary, Helen R Griffiths. (2011). Dietary antioxidant interventions in type 2 diabetes patients: a meta-analysis. British Journal of Diabetes & Vascular Disease; 11: 62-68.