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Alterations in Serum Zinc and Erythrocyte Reduced Glutathione Status in Newly Diagnosed Type 2 Diabetes Mellitus.

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ABSTRACT

Diabetes mellitus is a multifactorial disorder and it has been suggested that oxidative stress may play a role in the pathogenesis of diabetes mellitus. Alterations in vital antioxidant trace elements such as zinc and low molecular weight antioxidants such as glutathione may also lead to oxidative stress. Fasting blood samples were taken from sixty patients with diabetes mellitus and sixty age and sex matched controls. Serum zinc was analyzed using flame atomic absorption spectrophotometer and erythrocyte reduced glutathione level was measured using spectrophotometer. The level of serum zinc was found to be low in type -2 diabetes patients when compared with the controls ($p<0.05$). Erythrocyte reduced glutathione level was also found to be decreased in type -2 diabetes patients when compared to controls ($p<0.05$). No significant difference in the level of zinc and glutathione were observed with respect to age, sex, diet (vegetarianism/non- vegetarianism) and body mass index between the patient and the control groups ($p>0.05$). Since both zinc and reduced glutathione have antioxidant properties, alterations in their level need to be monitored in individuals with type -2 diabetes patients so that further oxidative stress and biomolecular damage may be prevented by appropriate treatment.

INTRODUCTION

Diabetes mellitus is a chronic metabolic syndrome, that is characterized by hyperglycemia that occur due to biochemical alterations in glucose metabolism, insulin resistance, hyperinsulinemia ,and β –cell dysfunction .Oxidative stress plays an important role in the pathogenesis of diabetes and its complications 1.Under normal circumstances, our body is protected from oxidative stress by both enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase and non enzymatic antioxidants such as reduced glutathione, uric acid, bilirubin, ascorbic acid, vitamin E etc. 2.

Alterations of vital trace elements such as zinc may also result in diabetes mellitus. It has been suggested that serum zinc has antioxidant role by protecting sulfhydryl groups against oxidation 3. Zinc, a metal ion is a component of the enzyme super oxide dismutase, an antioxidant enzyme. It plays a clear role in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin in the hexameric form. The decreased Zn level, affects the ability of the islet cell to produce and secrete insulin, may then compound the problem in Type 2 diabetes. Zinc has been found to affect the components of the insulin intracellular pathway 4,5.

Similarly, glutathione also plays a central role against intra and extracellular oxidative stress. Glutathione is a low molecular weight antioxidant that limit or prevent intracellular damage 2. The low molecular weight antioxidants penetrate specific locations in the cell where oxidative stress may occur and protect against reactive oxygen species 6.

As oxidative stress play an important role in the pathophysiology of diabetes mellitus, determining the physiological status of antioxidants may help to identify the individuals with increased risk of developing diabetes mellitus. Therefore, the present case-control study was conducted to analyze the level of serum zinc, an antioxidant element and the level of erythrocyte reduced glutathione, a low molecular weight antioxidant in patients with diabetes mellitus and controls .

MATERIALS AND METHODS

Study subjects and collection of blood samples

Fasting blood samples of newly diagnosed type 2 diabetic subjects (n=60) were collected from Arthur Asirvatham hospital,Madurai,Tamil Nadu,south India. Similarly, fasting blood samples were also collected from non-diabetic healthy volunteers as control samples(n=60). Serum zinc level was measured by flame atomic absorption spectrophotometry (Elico Ltd,India) and erythrocyte glutathione level was measured by spectrophotometry. Ethical clearance and institutional biosafety approval was obtained for the study.

Preparation of blood serum for the measurement of Zinc.

Blood samples were allowed to clot for one hour and centrifuged at 2500 rpm for 15 minutes.The supernatant was stored in -20° C till analysis.

Preparation of hemolysate

After serum separation, the packed RBCs were washed 3 times with 1mL cold NaCl (0.15mol/L) by centrifuging (5000rpm for 5 minutes) at 4° C and decanting between each addition to remove leucocytes, plasma and platelets. Prior to the assay, double distilled water was added to the washed erythrocytes and centrifuged to obtain a dilute hemolysate. The cellular debris and ghosts were removed from the lysate by centrifugation at 3000 rpm for 15 minutes.

Measurement of Reduced glutathione

Reduced glutathione in the hemolysate was measured as mentioned by Beutler et al.,1963 and Patil et al.,2007 7,8.One milliliter of the blood extract was used for the analysis.The assay is based on the principle that sulfhydryl group of reduced glutathione reduces the disulfide chromogen DTNB [5, 5'-dithiobis (2-nitrobenzoic acid)] to an intense yellow colored complex . The absorbance was read at 412 nm immediately and the results were expressed as micromoles/liter. Standard solutions containing 20 µM,40 µM,60 µM,80 µM -100 µM of reduced glutathione were prepared from a stock containing 1000 µM/mL of reduced glutathione.

Measurement of serum Zinc

Serum was diluted five-fold with de-ionized water. The instrument was calibrated and absorption which is the characteristic of zinc was measured at their specific wavelength (213.9nm). The obtained concentration of zinc was multiplied with the dilution factor 5 to get the final concentration. Standard solutions containing 0.1 ppm (10µg/dL) ,0.2ppm(20µg/dL) ,0.3ppm(30µg/dL) ,0.4ppm(40µg/dL) of pure zinc metal were prepared from a primary stock containing 1000 ppm of zinc.

STATISTICAL ANALYSIS

Percentage, mean, standard deviation and student t test were analyzed using Sigma - stat soft ware.

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RESULTS

Base line characteristics of the study population is shown in table:1. Statistically significant difference in mean levels of serum zinc and erythrocyte glutathione levels were observed between type -2 diabetes patients and healthy controls ($p<0.05$) (Table :2). No significant difference in the level of zinc and glutathione were observed with respect to age, sex, diet (vegetarianism/ non- vegetarianism) and body mass index between the patient and the control groups ($p>0.05$) (Table :2). Among the study subjects, 40% of the patients had family history of type 2 diabetic subjects, whereas such condition was present in 25% of the controls.

DISCUSSION

In the present study, the level of serum zinc and erythrocyte reduced glutathione were determined in type 2 diabetes mellitus patients and in healthy controls. Significant decrease in serum zinc and erythrocyte glutathione were observed in patients with type 2 diabetes mellitus as compared to controls ($p<0.05$).

Zinc has many antioxidant properties and it has been suggested that chronic zinc deprivation may result in increased sensitivity to oxidative stress 3.Similarly, it has been reported that glutathione is one of the most important protective factors against oxidative damage 9.In the present study, 80% of the individuals with zinc deficiency also had lower levels of hemolysate reduced glutathione. Irrespective of age, sex, BMI, glucose level and duration of diabetes, patients with type 2 diabetes mellitus had significantly lower serum zinc and erythrocyte reduced glutathione level when compared to controls ($p<0.05$).

Zinc and diabetes mellitus

The antioxidant zinc is an essential trace element for human nutrition 10.In the present study, significant decrease in serum zinc was observed in patients with type 2 diabetes mellitus as compared to controls. There are studies that have also reported a depletion in the levels of plasma or serum zinc in patients with diabetes mellitus 11,12,13. Jayawardena *et al*.,2012 in their systematic review and meta analysis have reported that zinc supplementation has beneficial effects on glycaemic control and promotes healthy lipid parameters in patients with diabetes 14. The decreased concentration of zinc might lead to increased production of hydrogen peroxide ($H2O2$) and elevated activity of NADPH –dependent reductase and cytochrome P-450, which inturn may cause increased generation of active forms of oxygen 15,16. In the present study, the decreased bioavailability of zinc may increase the generation of reactive oxygen species in patients with diabetes mellitus. Zinc protects against vitamin E depletion, resists endogenous free radical production, stabilizes membrane structure, contributes to the structure of antioxidant enzyme superoxide dismutase and maintains tissue concentrations of metallothionine, a free radical scavenger 3,17,18,19,20,21.

Glutathione and diabetes mellitus

In the present study, glutathione was found to be decreased in patients with diabetes mellitus. Other studies have also reported a decrease in glutathione levels among diabetes mellitus patients 22,23,24,25.Glutathione is an intracellular antioxidant endogenously synthesized in all cells from its constituent aminoacids (γ – glutamyl – cysteinyl – glycine) 26,27. Mckay et al have suggested that supplementation of N-acetyl cysteine stimulates glutathione synthesis 28.

Glutathione is considered as an antioxidant because it has thiol group in its cysteine moiety that acts as a reducing agent and which also can be reversibly oxidized and reduced. 27,29. Oxidation of reduced glutathione (GSH) to glutathione disulfide (GSSG) serves as an indicator of oxidative stress. Glutathione increases the antioxidative defense by enhancing the activities of GSH redox cycle enzymes such as glutathione (GSH) peroxidase, glutathione (GSSG) reductase and also glutathione dependent enzymes such as glutathione (GSH) S transferase. Glutathione minimizes the lipid peroxidation of cellular membrane, which occur due to oxidative stress. A decrease in the level of glutathione in plasma or serum may occur due to increased utilization of it inside the muscle. Quenching of any circulating free radicals might also result in decreased glutathione levels 30. As human blood plasma contains very low amounts of reduced glutathione, in the present study, the level of glutathione was measured in erythrocytes. Increased utilization of reduced glutathione due to quenching of free radicals within the cell might account for decreased level of erythrocyte reduced glutathione in patients with diabetes mellitus.

Irrespective of age, sex , glucose level and duration of diabetes, type 2 diabetes mellitus patients had a depletion of defensive antioxidants such as zinc and glutathione. Further studies should be conducted to find out whether supplementation of zinc in individuals with decreased zinc concentration will lead to significant increase in hemolysate glutathione. As the present study was conducted among newly diagnosed patients, the results of this study supports the hypothesis that oxidative stress play a significant role in the onset of diabetes.

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Table :1 Baseline characteristics of the study population

PARAMETERS	CONTROLS n=60	PATIENTS WITH TYPE 2 DIABETES MELLITUS n=60	P value
GENDER MALE/FEMALE	30/30	30/30	-
AGE (years)	48±4.5	47±6.3	p>0.05
BMI(kg/m^2)	23.6±0.9	26.1±2.2	p>0.05
FAMILY HISTORY (n)	15	24	p<0.05
DIET (n) VEG/NON-VEG	50/10	48/12	p>0.05

Table :2 Level of serum zinc and erythrocyte reduced glutathione in patients with type 2 diabetes mellitus and controls

PARAMETERS	CONTROLS n=60	PATIENTS WITH TYPE 2 DIABETES MELLITUS n=60	P value
ZINC (µg/dL)	92.86	63.88	p<0.05
REDUCED GLUTATHIONE (µM/mL)	70.67	52.96	p<0.05

$p<0.05$ was considered to be statistically significant.

REFERENCE

1. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J Biophys.2008;18: 225–236. | 2. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-Induced Oxidative Stress and Toxicity. J of Toxicol.2012:2012; 13 pages. | 3. Bray TM, Bettger WJ.The physiological role of zinc as an antioxidant. Free Radic Biol Med. 1990; 8: 281-91. | 4. Chausmer AB. Zinc, insulin and diabetes. J Am Coll Nutr. 1998; 17:109–115. | 5. Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr. 2001;131:1414-20. | 6. Psotova J, Zahalkov J, Hrbac J,Simanek V, Bartek J. Determination of total antioxidant | capacity in plasma by cyclic voltammetry. Two case reports. Biomed. Pap. 2001; 145:81-83. | 7. Beutler E, Duran O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med.1963;61: 882-8. | 8. Patil N, Chavan V, Karnik ND. Antioxidant status in patients with acute myocardial infarction. Indian J of Clin Biochem. 2007; 22: 45-51 | 9. Dimascio P, Murphy M, Sies H. Antioxidant defense system : The role of carotenoids , tocopherols and Thiol . Am J Clin Nutr.1991; 53: 1945-2005. | 10. Jeejeebhoy K. Zinc: an essential trace element for parenteral nutrition. Gastroenterology. 2009;137: S7-12. | 11. Walter RM, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. | Diabetes Care. 1991;14:1050-6. | 12. Anetor JI, Senjobi A, Ajose OA, Agbedana EO. Decreased serum magnesium and zinc levels: artherogenic implications in type-2 diabetes mellitus in Nigerians. Nutr Health 2002; 16:291-300. | 13. Al-Maroof RA, Al-Sharbatti SS. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type-2 diabetics. Saudi Med J 2006; 27:344-50. | 14. Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi RLDK, Constantine GR and Katulanda P. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. Diabetology & Metabolic Syndrome. 2012; 4:11 pages. | 15. Girelli D, Olivierio O, Stazial A.M, Azzini M, Lupo A, Bernich P, Menini C, Gammario L,Corrocher R. Low platelet glutathione peroxidase activity and serum | | | | selenium concentration in patients with chronic renal failure. Relations to dialysis treatment, diet and cardiovascular complications. Cli Sci.1993; 84: 611-617. | 16. Kulikowska-Karpińska E, Moniuszko-Jakoniuk J.The Antioxidative Barrier in the Organism. Polish Journal of Environmental Studies. 2004; 13: 5-13. | 17. Marklund SL. Human copper-containing superoxide dismutase of high molecular weight. Proc. Natl. Acad. Sci. U.S.A.1982;79:7634-7638. | 18. Cousins RJ. Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Phys Rev. 1985; 65:238-309 . | 19. Bunk M, Dnistrain AM, Schwartz MK, Rivlin RS. Dietary zinc deficiency decrease plasma concentration of vitamin E. Proc. Soc. Exp. Biol. Med. 1989; 190: 379-384. | 20. Noh S K, Koo SI. Feeding of a marginally low level of dietary zinc lowers the concentration of -tocopherol (-P) in selected organs. FASEB J.1998;12:A217(abs.). | 21. Davis CD, Klevay LM, Milne D B, Nielsen FH. Extracellular superoxide dismutase activity: a promising indicator of zinc status in humans. FASEB J. 1998;12:A346 (abs.) | 22. Pasaoglu H, Sancak B, Bukan N.Lipid peroxidation and resistance to oxidation in patients with type 2 diabetes mellitus. Tohoku J Exp Med. 2004;203:211-8. | 23. Ozdemir G, Ozden M, Maral H, Kuskay S, Cetinalp P, Tarkun I.Malondialdehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. Ann Clin Biochem. 2005;42: 99-104. | 24. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J. 2005;46:322-4. | 25. Prakash S, Sudha S. Lipid peroxidation and antioxidant status in South Indian patients with Type 2 Diabetes Mellitus. Int Res J Pharmacy. 2012; 3: 132-134. | 26. Champe PC, Harvey RA, Vella F. Hexose monophosphate pathway. In lippincot illustrated review : Biochemistry (eds), 2PUnd UP edition, lippincott – Rave,1994; 10: 111-118 . | 27. Sen CK.Glutathione homeostasis in response to exercise training and nutritional supplements.Molecular and Cellular Biochemistry. 1999;196: 31–42. | 28. McKay S, Morlese J, Jackson A, Forrester T, Balasubramanyam A, Reeds P, Jahoor F.Oxidative stress and glutathione synthesis in type 2 diabetes: a stable isotope approach. Diabetes. 2002; 51:A317 | 29. Kidd PM. Glutathione: systemic protectant Against Oxidative and Free Radical Damage. Alternative Medicine Review. 1997; 2 :155–76. | 30. Kerksick C,Willoughby D The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress. Journal of the International Society of Sports Nutrition. 2005;2:38-44 |