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ANTIOXIDANT STATUS AND MINERAL LEVELS IN DIABETIC PATIENTS IN NNEWI

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ABSTRACT
The study was designed to determine the serum levels of antioxidants, mineral levels, antioxidant enzymes and total antioxidant status in diabetic patients, and the control subjects. The plasma levels of antioxidants (albumin, uric acid, total antioxidant status) minerals (magnesium, zinc, selenium and copper) and antioxidant enzyme (superoxide dismutase, gluthathione peroxidase and catalase) were estimated in diabetic and control subjects. In the diabetic patients, the mean fasting blood glucose (FBG)(9.374 ±1.166) mmol/L is significantly higher (P<0.005) when compared with the controls (4.818±0.815) mmol/L. Antioxidants uric acid (6.506±1.522) mg/dl, zinc 112.234±18.125) µg/dl and selenium levels (18.0530±2.166) µg/dl were significantly higher (P<0.005) when compared with the control subjects (5.468±1.600) mg/dl, (90.923±14.519) µg/dl and (17.080±2.157) µg/dl respectively. Conversely, magnesium level (306.870±56.803) µg/dl was significantly lower (P<0.005) when compared with the control subjects (342.085±67.409) µg/dl. Age of subjects correlated negatively with their serum selenium levels (r=-0.311). This study demonstrated the presence of oxidative stress in the diabetic patients as an expression of increased free radical production and diminished antioxidant defense. Appropriate supplementation of antioxidants and minerals in these patients will strengthen their immune system and reduce the adverse consequences of oxidative stress. Routine assessment is recommended.

INTRODUCTION
Antioxidants neutralize the cell-damaging effects of free radicals[1], which are atoms with an unpaired number of electrons that can be formed when oxygen interacts with certain molecules[2]. Antioxidant compounds include vitamins A, C and E, transition metals selenium, magnesium, zinc and copper. Other antioxidants are uric acid, glutathione, albumin, etc [3, 4]. Antioxidants abound in nature; they deactivate free radicals that result from exposure to UV light, gamma radiation, environmental pollutants, xenobiotics and cigarette smoking. Free radical oxidation may damage cell membrane and cell contents such as DNA, protein, lipid and carbohydrates resulting in loss of membrane function, inactivation of enzymes and chemical alteration of the DNA, predisposing the body to degenerative diseases[4]. Antioxidant compounds must be replenished since they are used up in neutralizing free radicals. The antioxidant defense system also includes superoxide dismutase, glutathione peroxidase, glutathione reductase and Catalase[5]. Free radicals are molecules that have been chemically damaged by removing a single electron. When free radicals are produced in excess, the natural antioxidant defense system weakens resulting in oxidative stress that leads to oxidative injury and disease.

Major risk factors, like dyslipidemia and smoking habit have been assessed in conditions such as diabetes mellitus, hypertension and in patients with myocardial infarction[6]. Evidence is accumulating that most of the degenerative diseases have their origin in deleterious effect of free radicals[7]. Humans are endowed with antioxidant defenses and deficiencies of these micronutrients may increase susceptibility to these diseases and

associated complication[7]. The body's defenses against oxidative stress are less effective with aging[8]. There is indication that free radicals may be involved in the development of cancer, cardiovascular disease, Alzheimer's disease, immune dysfunction, cataracts and macular degeneration[2,9,10,11]. Consumption of antioxidants is thought to provide protection against oxidative damage and contribute positive health benefits. Diabetes mellitus is one of the diseases common in our society and constitute a major cause of disability or death. Current evidence has shown that free radicals may be implicated in its etiology[12].

The objectives of this study are to determine the total antioxidant status, the level of secondary antioxidants uric acid and albumin in serum of both patients and control; to determine the level of some antioxidant minerals, and the activities of the antioxidant enzymes in both patients and control.

PATIENTS AND METHODS.

A total of 100 subjects aged 40-50 years, made up of 50 diabetic patients and 50 aged and sex matched. Healthy controls were recruited for the study. Informed consent was obtained from each subject before collecting blood samples while ethical approval was obtained through NAU/NAUTH Ethics Committee, before commencement of the study. The study was carried out in the Department of Chemical Pathology in collaboration with some experts in Public Health Medicine in Department of Community Medicine and PHC in the College of Medicine Nnamdi Azikiwe University (NAU) Nnewi Campus. Only patients whose plasma fasting blood glucose level was $\geq$ 7.0mmol/L were included in the study as diabetic patients while patients with values below this were not included in the study. Apparently healthy individuals served as control subjects.

The design was a cross sectional descriptive study. A convenience sampling technique was used to select the subjects that met the inclusion criteria, agreed to take part in the study and whose informed consent was obtained. Questionnaire was used as interview guide for data collection.

Plasma glucose was determined after enzymatic oxidation in the presence of Glucose Oxidase (GOD).[13] The measurement of serum albumin was based on its quantitative binding to the indicator 3,3,5,5-tetrabromo-m-cresol (bromocresol green (BCG).[14] while measurement of uric acid concentration was based on the principle that uric acid is converted by Uricase to allantoin and hydrogen peroxide.[15]

The Superoxide Dismutase (SOD) activity was determined based on the ability of SOD to inhibit the auto-oxidation of adrenaline. Superoxide generated by the Xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome.[16] Glutathione peroxidase activity was determined by the method of Ozdemir et al, 2005 and the principle was based on the oxidation of NADPH to NADP.[17] Total antioxidants assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical[18].

For trace elements: magnesium, zinc, selenium and copper equipment were designed to measure the concentration of elemental metals in solution. It provides integrated measurement in absorbance or emission intensity as well as sample concentration in comparison to standard solution and the readings taken within 0.5 to 10 seconds[19]. Six ml venous blood was collected with minimal venous stasis from each patient into a heparinized tube.

The sample was centrifuged, separated, aliquoted and immediately placed in a freezer until analysis. Quality control sera were run along test in each batch of analysis and this was compared with the reference range of the control material. Then the Standard Deviation

(SD) and coefficient of variation was calculated.

The results were expressed as mean ± SD. Comparisons were made using Student's t test and P<0.05 was regarded as significant. Pearson Correlation Analysis was used to establish possible correlation between antioxidants, trace minerals and antioxidant enzymes.

RESULT

Table 1 shows that there were statistically significant higher mean values of FBG (p=0.000), uric acid (p=0.001), zinc (p=0.000), selenium (p=0.027) and SOD (p=0.001) in the diabetics when compared to the controls. Conversely, there are markedly lower mean values of magnesium (p=0.000) and glutathione peroxidase (p=0.002) in the diabetics when compared to the control. The rest of the mean values showed no significant differences between the diabetics and control.

Table 2 shows statistically significant positive correlations between the FBG of the diabetics and selenium (p=0.014), SOD (P=0.012) and total antioxidants (p=0.001) in their blood. On the other hand, the age of the diabetic subjects is significantly negatively correlated with their blood selenium levels (p=0.02).

Furthermore, while the FBG of control subjects show significant positive correlations with both the selenium level (p=0.001) and catalase (0.000), the same significant relationship is maintained between their age and these variables (p=0.027, p=0.044 respectively). However, a significant negative correlation exists between the age of control subjects and their blood magnesium levels (p=0.000).

DISCUSSION

The mean uric acid was significantly higher in diabetic subjects than control. Also, uric acid has been described as a powerful scavenging antioxidant in another study which also reported an increase and a positive correlation between hyper-uricemia and oxidative stress[20] This high uric acid level might be due to rapid cell turnover and muscle wasting as a consequence of oxidative damage to polyunsaturated fatty acid[20]. Moreover, since uric acid binds ions of copper and iron, this may cause increased concentration of copper and thus preventing availability or utilization by tissues.[20]

Superoxide dismutase was significantly higher in diabetics than control, and it is known that reactive oxygen species aggravate disease progression. To counteract their harmful effects, the body produces various antioxidant enzymes. The primary catalytic extracellular defense that protects cells and tissues against lipid peroxidation is glutathione peroxidase and was among the strongest univariate predictor of the risk of cardiovascular event.[21] Glutathione peroxidase was significantly decreased in diabetic subjects and this is consistent with literature and a clear indication of oxidative stress in diabetes.[22,23] A Japanese author in 1993 has however demonstrated a proportional decrease in the antioxidant enzyme activity with declining glycemic control adequacy[24]. Such contradictory findings could be explained as a consequence of insufficient standardization of clinical or analytical procedures utilized in the study.

Trace minerals zinc and selenium were significantly higher in diabetic subjects than in control even though magnesium was significantly lower in diabetics. Magnesium plays an important role in carbohydrate metabolism in that it may influence the release of activity of insulin which helps to control blood glucose levels.[25] Low blood levels of magnesium (Hypomagnesaemia) are frequently seen in individuals with type 2 diabetes[25]. The kidneys possibly lose their ability to retain magnesium during period of severe hyperglycemia. The increased loss of magnesium in urine may then result in lower blood level of magnesium.

In this study, significantly higher level of zinc in diabetic subjects than in control even though previous studies indicated that marginal zinc deficiency is more prevalent among diabetic adults compared to the normal adult population[26, 27]. (Lee et al., 2005; Yoon & Lee, 2007).) The unusual finding in our study can however be explained by the fact that trace minerals are usually linked directly or indirectly with several metalloenzymes having antioxidant activity. Despite the apparently high level of zinc in the diabetics, very little is available for tissue utilization. Zinc plays a key role in the cellular anti-oxidative defense and so if there is insufficient zinc, oxidative stress may damage the cell irreversibly thereby exacerbating some of the complications of diabetes. Hyperzincuria is as a result of hyperglycemia than any specific effect of endogenous or exogenous insulin on the renal tubules.[28] This suggests hyperglycemia as the basis for the hyperzincuria which interferes with the active transport of zinc back into the renal tubule and resulting in the hyperzincuria found in diabetes[26]. If hyperglycemia is the primary etiology, replacement with oral zinc supplementation should provide sufficient treatment. Zinc is a necessary factor in the variety of antioxidant enzymes, particularly superoxide radical (0^{2-}), alkoxyl (R0), Peroxyl radicals (R00) hydrogen peroxide (H202) and lipid peroxides (L00H). Alteration of zinc metabolism such that adequate zinc is unavailable for these enzymes might be expected to contribute to tissue damage observed in diabetes.[29]

From the study, significant increase of selenium in diabetic subject corresponds with the findings of Papp et al in 2000[30]. Another study also found that increased selenium is as a result of hyperglycemia which interferes with the biological functions including protection against oxidative stress.[31]

In conclusion, this study supports the fact that oxidative stress may culminate into deficiency of antioxidant and some micronutrients in diabetes with consequent tissue damage. It also revealed the importance of determining the antioxidant status for early intervention and better management of this disease even as it also suggests lifestyle modification as a preventive measure to reduce the burden of the disease.

REFERENCES

1. Sies H. "Oxidative stress: Oxidants and Antioxidants Exp. *Physical* 1997; 82 (2): 291-5.
2. Valko M., Izakovic M., Mazur M., Rhodes C. and Telser J. "Role of Oxygen Radicals in DNA Damage and Cancer Incidence" *Mol cell Biochem.* 2004; **266**(1-2): 37-56.
3. Halliwell B. and Gutteridge J.M.C. (1990). The Antioxidants of Human Extra Cellular Fluids. *Archives of Biochemistry and Biophysics* 1990; **280**: 1-8.
4. Mary Khakoni Wailing. Efficacy of Antioxidants in Human Health. *African Journal of food, Agriculture, Nutrition and Development.* 2004; 4: 2.
5. Diaz M.N., (1997). Antioxidant and Atherosclerotic Heart Disease. *New Engl. J. Med.* (337): 408-416.
6. Baharan T. and Soobratte M.A. Free Radical and Antioxidant in Cardiovascular Health and Disease Internet Journal of Medical Update E. Journal 2006; 1:2.
7. Muhammed A., Mansur L. Serum Antioxidant in Diabetes Mellitus Subjects: *Biokemistri* 2005; **17**(2): 107-114.
8. Knight J.A. (1995). Diseases Related to Oxygen-Derived Free Radicals. *Ann Clin. Lab Sci.* 1995; **25**: 11-121.
9. McCall M.R and Frei B. Can Oxidants Materially Reduce Oxidative Damage in Humans? *Free Radical Biol Med.* 1999; **26**(7): 1034-53.
10. Halliwell B. Oxygen and Nitrogen are Procarcinogens. Damage to DNA by Reactive Oxygen, Chlorine and Nitrogen Species, Measurement, Mechanism and Effects of Nutrition. *Mutat Res* 1999; **443**: 37-52.
11. Aslan M., and Ozben J.(2004) Reactive Oxygen and Nitrogen Species in Alzheimer. *Res* 2004; **1**: 111-119.

12. Lakhanpal P. and Rai D.K. Role of Quercetin in Cardiovascular Disease. *Internet Journal of Medical Update. E-Journal* 2008; **3**:1.
13. Trinder P. Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. *Ann Clin Biochem* 1969; **6**: 24-27.
14. Doumas B.T, Watson. W. A, Biggs H.G. Revised Method for Albumin Estimation. *Clin. Chim Acta.* 1971; **31**: 87.
15. Fossati P; Prencipe, Berti G; Revised method for uric acid estimation. *Clin. Chem* 1980; **26**(2): 227-23.
16. Misra H.P, Fridovich I. The Role of Superoxide Anion in the Autooxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *J. Biol Chem* 1972; **247**: 3170-3175.
17. Ozdemir G., Ozden M., Maral H., Kuskay S., Celtinalp P. Tarkun I. Malonaldehyde, Glutathione, Glutathione Peroxidase and Homocystein levels in type 2 diabetic patients with and without Microalbuminuria. *Ann Clin Biochem* 2005; **42**: 99-104.
18. Yokazawa. Evaluation of antioxidants activity scavenging of DPPH radical in *Afr J. Trad.* 1998; **2**(1): 70-85.
19. Gerald J. De Menna Buck. 210VGP System Model, Atomic Absorption Spectrophotometer: 321-326.
20. Perlstein TS, Gumieniak O, Hopkins PN, Murphey LJ, Brown NJ,Williams GH, et al. Uric Acid and the State of the Intrarenal Renin-Angiotensin System in Humans. *Kidney Int* 2004; 66: 1465–1470.
21. Stefan B, Hans J.R, , Christoph B, Michael T, Gerd H, Laurence T, et al. Glutathione Peroxidase 1 Activityand Cardiovascular Events in Patients with Coronary Artery Disease. *N Engl J Med* 2003; 349:1605-13.
22. Mohammed, A.K., Bierrhaus A, Schiekofer A, Chiekofer S, Tritschler H, Ziegler R, et al. The Role of Oxidative Stress and NF-Kappa B Activation in Late Diabetic Complications, *Biofactors*, 1999; **10**: 157–167.
23. Martin, A.C., Sanders RA, Watkins JB. Diabetes, Oxidative Stress and Antioxidants:a review, *Journal of Biochemical and Molecular Toxicology*, 2003; **17**: 24–38.
24. Asayama, K., N. Uchida, T. Nakane, H. Hayashibe and K. Dobashi *et al.* Antioxidants in the Serum of Children with Insulin-Dependent Diabetes Mellitus. Free Radical Biol. Med., 1993; 15: 597-602.
25. Kobrin, M. S., Somsoondar, J. & Kudlow, J. E. (1986) J. Biol. Chem. 1986; 261: 14414-14419.
26. Lee JH, Lee HJ, Lee IK, Yoon JS. Zinc and Copper Status of Middle- and Old-Aged woman in type 2 diabetes. The Korean Journal of Nutrition. 2005;38:56–66.
27. Yoon JS, Lee JH. A Suggestion tolimprove Zinc Status of type 2 Diabetic Women: Relation Among Zinc, Protein and Phytate Intake. Journal of Korean Diebetic Association. 2007;13:301–310.
28. El-Yazigi, A., N. Hannan D. Raines. Effect of Diabetic State and Related Disorders on the Urinary Excretion of Magnesium and Zinc in Patients. Diabetes Res., 1993; 22: 67-75.
29. Sumovski, W., Baquerizo H., Rabinovich A. Oxygen Free Radical Scavenger Protect Rat Islet Cells from Damage by Cytokines. Diabetelogica 1992; 32: 792-796.
30. Papp, L.V., J. Lu, Holmgren A., Khanna, KK. From Selenium to Selenoproteins: Synthesis, Identity and their Role in Human Health. Antioxidant Redox Signaling. 2007; 9: 775-806.
31. Rayman, M.P. The Importance of Selenium to Human Health. Lancet. 2000; 356: 233-241.

Table 1: Mean Levels of Antioxidants, Fasting Blood Glucose, Trace Minerals, in Control, and Diabetic Subjects.

Parameters	Control (N=50) Mean±SD	Diabetics (N=50) Mean±SD	p-value
FBG(mmol/L)	4.818±0.815	9.374±1.166	*0.000
Uric acid (mg/dl)	5.468±1.600	6.506±1.522	*0.001
Albumin (g/dl)	4.474±0.649	4.394±0.617	0.526
Magnesium (µg /dl)	342.085±67.409	306.870±56.803	*0.004
Zinc (µg /dl)	90.923±14.519	112.234±18.125	*0.000
Selenium (µg/dl)	17.080±2.157	18.053±2.166	*0.027
Copper (µg /dl)	195.129±2.157	204.500±34.300	0.152
Superoxide dismutase (µg /mg protein)	0.791±0.281	0.998±0.335	*0.001
Glutathione peroxidase (units/ml)	307.346±107.538	247.200±81.041	*0.002
Catalase (k unit/ml)	263.006±36.222	265.700±55.249	0.777
Total antioxidant status (%)	11.287±1.580	11.214±3.205	0.885

*Statistically significant

Table 2: Correlation of Fasting Blood Glucose Level and Age with the Antioxidants, Trace Minerals, Antioxidant Enzymes in Diabetic and Control Subjects.

Variables	Diabetics				Control			
	FBG		Age		FBG		Age	
	R	p-value	R	p-value	R	p-value	R	p-value
Uric acid	-0.098	0.498	0.127	0.380	-0.016	0.912	-0.033	0.820
Albumin	-0.216	0.133	-0.074	0.58	-0.153	0.290	-0.163	0.259
Magnesium	0.254	0.075	0.058	0.61	-0.300	0.034*	-0.641	0.000*
Zinc	0.074	0.609	0.131	0.364	-0.099	0.493	-0.052	0.720
Selenium	0.344	0.014*	-0.311	0.02*	0.441	0.001*	0.312	0.027*
Copper	-0.254	0.075	-0.119	0.412	-0.191	0.184	-0.012	0.935
SOD	0.351	0.012*	-0.036	0.805	-0.073	0.613	-0.073	0.615
GP	0.098	0.449	-0.124	0.390	-0.023	0.876	-0.019	0.895
Catalase	-0.254	0.075	-0.202	0.16	0.528	0.000*	0.286	0.044*
TA	0.443	0.001*	0.254	0.075	0.127	0.378	0.537	0.000*

*= significant correlation

Keys: FBG= Fasting Blood Glucose; SOD= Superoxide Dismutase; T.A= Total antioxidants