

## Determination of Serum Selenium in Patients with Type II Diabetes Mellitus

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**Abstract:** Oxidative stress reduces insulin secretion and increases insulin resistance in some experimental models and may thus play a causal role in the pathogenesis of diabetes. The purpose of this study was to examine the relationship between serum selenium levels in serum of patients with Type II Diabetes Mellitus and healthy control treatments. Total of 47 diabetic patients (27 male, 20 female) with mean age of  $55.6 \pm 6.6$  years were recruited into the study. Control group was composed of 33 healthy volunteers (17 male and 16 female) with mean age of  $47.7 \pm 9.5$  years. In addition to the aforementioned parameters, levels of fasting blood glucose and percentage of HbA1C levels were determined in diabetic patients and controls. The results indicated that selenium levels reduced meaningfully in diabetic patients. Reduction in Se levels was probably due to antioxidant effect of this trace element. Supplementation of Se as an antioxidant into the daily diets of diabetic patients will enhance the power of antioxidant defence systems.

**Key words:** Diabetes Mellitus • Selenium • Antioxidant • HbA1C level

### INTRODUCTION

Oxidative stress is caused by a relative overload of oxidants like reactive oxygen species. This impairs cellular functions and contributes to the pathophysiology of many diseases. Evidence has accumulated suggesting that diabetic patients are under oxidative stress and that complications of diabetes seem to be partially mediated by oxidative stress [1-3].

Diabetes mellitus is a disorder with late complications including cardiovascular disease, nephropathy, neuropathy and retinopathy which severely affect the quality of life [4].

Although there are several reports on complications of diabetes, pathophysiology of these complications are still needed to be deciphered [5].

Free radicals are produced as a result of glycosylation of several proteins including hemoglobin (Hb) by non-enzymatic mechanisms [6, 7].

Since free radical production is increased whereas capacity of antioxidant systems is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compared to healthy individuals [8, 9].

Since effects of free radicals in diabetes are now documented, it has been proposed to use antioxidants to block formation of free radicals and hence prevent development of diabetes [6, 10, 11].

While superoxide radicals are cleaned by enzymatic dismutation, compounds known as antioxidants clean free radicals in organisms. Glutathione is a very important non-enzymatic antioxidant. Selenium, functioning as part of glutathione peroxidase, has been recognized as a cellular antioxidant in addition to its protecting function against heavy metal toxicity [5, 12, 13].

Selenium has an important role in vitamin E metabolism and is required for normal pancreatic functions. It is needed for absorption of lipids and vitamin E. In addition, selenium serves the role keeping vitamin E within lipids [8, 14].

There are intrinsic enzymatic and non-enzymatic antioxidants detoxifying mechanisms that decrease reactive oxygen species concentration in human body. Selenium and glutathione are some of the major non-enzymatic antioxidants in the body [15]. Therefore, the idea of using antioxidant to prohibit development of diabetes as well as its complications and/or treat diabetic patient is getting more attention than ever [9, 1, 16].

Although there are studies reporting serum or plasma level of selenium in diabetic patients, results from different groups are rather contradictory. Studies focusing on involvement of selenium in diabetic patients are rather limited. Therefore, the present study was designed to determine and evaluate changes in level of selenium, in patients with type 2 diabetes and healthy subjects.

## MATERIALS AND METHODS

Total of 67 patients (27 males and 20 females) who were diagnosed with type 2 diabetes mellitus in Central Medical Diagnosis Laboratory of Yazd in Iran Associated with Shahid Sadoughi Medical University participated in the study. The average age of diabetic patients was 55.6 ( $\pm$  6.6) years and who were free of clinical symptoms of neuropathy, retinopathy and nephropathy. Control group consisted of 33 healthy volunteers (17 males and 16 females) whose mean age were 47.4 years ( $\pm$  9.5 years).

All patients who participated in the study signed a consent statement in accordance with the requirements of Shahid Sadoughi Medical University Laboratory research ethics.

Venous blood samples were withdrawn after an overnight fasting from patients and controls. Fasting blood glucose level were determined by a commercial kit (Glucose liquicolor, GOD-PAP Method Germany) by auto analyzer (BT 3000 UK). Percentage of HbA1C levels were determined by a chromatography method auto analyzer (ECOM 6125 UK). The selenium concentration in serum were measured by the fluorometric method of Watkinson [17].

Samples were wet digested overnight by a nitric acid / perchloric acid mixture. 2, 3 diamionaphthalene (DAN) was used as a complexing reagent and cyclohexan as the extracting solvent for the Se-DAN complex formed.

The fluorescence was measured (excitation at 366 nm and emission at 544 nm) using Hitachi F-4010 fluorescence spectrophotometer. The fluorimetric method is considered to be a very sensitive method [17, 18].

Statistical analysis was carried out using SPSS 13.0. The data obtained are expressed as means  $\pm$  SD. Student's t-test and Pearson test were used to determine whether differences between the means were significant, with  $P < 0.05$  taken as the significance level.

## RESULTS AND DISCUSSION

Demographic features of diabetic patients and controls are summarized in table 1.

Fasting blood glucose, percentage of HbA1C and selenium levels are given in table 2.

Comparing the fasting blood glucose and percentage of HbA1C between control and diabetic patients, there was a two fold increase of the two parameters in diabetic patients ( $P < 0.005$ ). On the other hand level of selenium is observed to be significantly reduced in diabetic patients ( $P < 0.005$ ) compared to the control. Moreover there is a negative correlation between percentage of HbA1C and Se ( $P < 0.005$ ).

Table 1: Demographic features of diabetic patients and controls.

	Controls	Diabetic Patients
Number of subject (n)	33	47
Age (Year)	46.44 $\pm$ 9.5	55.6 $\pm$ 6.6
Sex (M/F)	M:17, F:16	M:27, F:20
Oral antidiabetic usage (Metformin and Glybenclamide)	-----	90%
Insulin usage	-----	10%

Table 2: Comparison of fasting blood glucose, HbA1C and Se by means of t-test (Values are means $\pm$ SD)

	Control n=33	Diabetic Patients n=47
Number of subjects(n)		
Glucose (mg/dl)	90.12 $\pm$ 13.54*	186 $\pm$ 40.78*
Percentage HbA1C	5.51 $\pm$ 1.57*	9.45 $\pm$ 1.23*
Selenium (ppb = ng/ml)	67.9 $\pm$ 5.8*	87 $\pm$ 5.5*

\*Significant at the 5% level compared to the control

Elevated extra and intra-cellular glucose concentration results in an oxidative stress [12, 19]. When diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defence systems is observed [20].

Changes in oxidant and antioxidant systems are related with duration of disease and become more important as complications develop. Finding of several studies demonstrated that overproduction of peroxides along with emaciation of antioxidant defense systems cause oxidative damage and these events in type 2 diabetic patients are observed in an earlier stage, before diabetic complications develop [17, 19, 21].

Results of selenium levels in blood, plasma and serum levels of patients with type 2 diabetes mellitus are contradictory.

Although glucose itself can initiate oxidative stress, deficiency of essential trace elements such as selenium may exacerbate this oxidative stress in diabetic rats [18, 22].

While in the current study selenium levels decreased significantly in diabetic patients, other studies reported serum selenium levels of diabetic patients to have increased, decreased or remained unchanged compared to control [23-25]. Significant reductions in the levels of selenium are indicators of metabolic response to oxidative stress in patients with type 2 diabetes.

The results of the study indicate that there is a direct relationship between fasting blood glucose, percentage of HbA1C and the reverse relationship with selenium levels in diabetic patient.

In conclusion, the diet of diabetic patients should contain recommended daily allowance of selenium to allow non-enzymatic as well as enzymatic antioxidant systems to respond to the oxidative stress observed in diabetic patients.

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#### REFERENCES

1. Hayoz, D., T. Ziegler, H.R. Brunner and J. Ruiz, 1998. Diabetes Mellitus and Vascular Lesions. *Metabolism*, 47: 16-19.
2. Rosen, P., X. DU and D. Tschope, 1998. Role of Oxygen Derived Radicals for Vascular Dysfunction in the Diabetic Heart: Prevention With alpha-tocopherol?. *Mol. Cell Biochem.*, 188: 103-111.
3. Szaleczky, E., J. Prechl, J. Fether and A. Somogyi, 1999. Alterations in Enzymatic Antioxidant Defence in diabetes Mellitus-A Rationale Approach. *Postgrad Med. J.*, 75: 13-17.
4. Halifeoglu, I., F. Karatas, R. Colak, H. Canatan and S. Telo, 2005. Tip 2 Diyabetik Hastalarda Tedavi Oncesi ve Tedavi Sonrasi Oksidan ve Antioksidan Durum. *Firat Tip Dergisi*, 10(3): 117-122.
5. Alan, W.S., 2003. The Role of Advanced Glycation in the Pathogenesis of Diabetic Retinopathy. *Exp. Mol. Pathol.*, 75: 95-108.
6. Signal, P.K., A. Bello-klein, F. Farahmand and V. Sandhawalia, 2001. Oxidative Stress and Functional Deficit in Diabetic Cardiomyopathy. *Adv. Exp. Med. Biol.*, 498: 213-220.
7. Mercuri, F., L. Quagliario and A. Ceriello, 2000. Oxidative Stress Evaluation in Diabetes. *Diabetes Technol. Ther.*, 2(4): 589-600.
8. Kar, M. and A.S. Chakraborti, 1997. Release of Iron from Hemoglobin-a Possible Source of Free Radicals in Diabetes Mellitus. *Indian J. Exp. Biol.*, 37(2): 190-192.
9. Jain, J.K. and M. Palmer, 1997. The Effect of Oxygen Radicals Metabolites and Vitamin E on Glycosylation of Proteins. *Free Radic. Biol. Med.*, 22(4): 593-596.
10. Ceriello, A., N. Bortolotti, E. Falletti, C. Taboga, L. Tonutti, A. Crescentini *et al.*, 1997. Total Radical-Trapping Antioxidant Parameter in NIDDM Patients. *Diabetes Care*, 20(2): 194-197.
11. Maxwell, S.R., H. Thomason, D. Sandler, C. Leguen, M.A. Baxter, G.H. Thorpe, *et al.*, 1997. Antioxidant Status in Patients with Uncomplicated Insulin-Dependent and Noninsulin-Dependent Diabetes Mellitus. *European Journal of Clinical Investigation*, 27(6): 484-490.
12. Al-Saleh, I.A. and I. Al-Doush, 1997. Selenium Levels in Wheat Grains Grown in Saudi Arabia. *Bull Environ Contam Toxicol.*, 59: 590-594.
13. Oster, O. and W. Prellwitz, 1989. Are Germans Selenium-Deficient? In *Selenium in Biology and Medicine*, Springer-Verlag Pub., Berlin Heidelberg, New York.
14. Oldfield, J.E., 1987. The Two Faces of Selenium. *J. Nutr.*, 117: 2002-2008.
15. Halliwell, B., 1994. Free Radical Antioxidants in Human Disease. Curiosity, Cause or Consequence. *Lancet*, 344: 721-724.
16. Ceriello, A., D. Giugliano, A. Quatraro, P. Dello Russo and R. Torello, 1988. A Preliminary Note on Inhibiting Effect of Tocopherol on Protein Glycation. *Diabetes Metab.*, 14: 40-52.
17. Watkinson, J.H., 1960. Fluorometric Determination of Traces of Selenium. *Anal. Chem.*, 32(8): 981-983.
18. Whetter, P.A. and D.E. Ullrey, 1987. Improved Fluorometric Method for Determining Selenium. *J. Assoc. Anal. Chem.*, 61(4): 927-930.
19. Soudani, N., I. Ben Amara and M. Sefi, 2010. Effect of Selenium on Chromium (VI) Induced Hepatotoxicity in Adult Rats. *Experimental and Toxicology pathology*, 3: 25-30.
20. Diplock, A.T., 1991. Antioxidant Nutrients and Disease Prevention: An Overview. *Am. J. Chim. Nutr.*, 53: 1895-1935.
21. Sundaram, R.K., A. Bhaskar, S. Vijayalingam, M. Viswanathan and R. Mohan, 1996. Antioxidant Status and Lipid Peroxidation in Type II Diabetes Mellitus with and without Complications. *Clin. Sci. (Colch)*, 90(4): 255-260.
22. Vassort, G. and B. Turan, 2010. Protective Role of Antioxidant in Diabetes-Induced Cardiac Dysfunction. *Cardiovasc Toxicol*, 10: 73-86.
23. Reddi, A.S. And J.S. Bollineni, 2001. Selenium-Deficient Diet Induces Oxidative Stress and Injury via TGF-beta 1 in Normal and Diabetic Rats. *Kidney Int.*, 59(4): 1342-1353.
24. Ashour, M., S. Salem, H. Hassaneen, H. Gadban, N. Elwan, A. Awad, *et al.*, 1999. Antioxidants Status and Insulin Dependent Diabetes Mellitus (IDDM). *J. Clin. Biochem. Nutr.*, 26: 99-107.
25. Uyoyo, D. and J. Awogoko, 2010. Antioxidant Effect of Zinc, Selenium and their Combination on the Liver and Kidney of Alloxan-Induced Diabetes in Rats. *Mediterranean Journal of Nutrition and Metabolism*, 3(1): 25-30.