Evaluation of Serum Selenium Level in Patients With Uncomplicated Diabetes Mellitus, Raipur, India

Usha Joshi,Pd Raut,Sk Agrawal,Pk Patra,Bk Maheshwari,Manu Apurb and Dhirhe Tc.

ABSTRACT

Objective: Free radicals have important role in the pathogenesis of diabetes mellitus. In diabetes, free radical production is increased whereas capacity of antioxidant system is reduced. This study was, therefore designed to determine and evaluate the serum selenium level in patient with uncomplicated diabetes mellitus and a control group of non-diabetic individuals. **Study design**: Hospital based non randomized, multistage, stratified, cross-sectional comparative study. **Material and Method**: 50 uncomplicated diabetic patients (23 male, 27 female) with mean age of 49.10±6.48 years were enrolled in the study. Control group was composed of 50 healthy individual (22 male, 28 female) with mean age of 52.74 ±7.5 years. Serum selenium level was determined by using Hydride generation atomic absorption spectrometry in diabetic patients and controls.**Result**: Mean serum- selenium concentration measured in uncomplicated diabetic patients (51.9±8.23 µg/lit) were significantly lower than those determined in control group (130.66±37.18 µg/lit) ($p< 0.05$).There was significant decrease in mean serum selenium level with increasing age. ($p<0.05$).No significant differences in serum selenium levels were found in relation to the sex of the patient ($p> 0.05$). **Conclusion**: The result of this comparative evaluation confirms the relation between low serum selenium and diabetes. Significant reduction in selenium levels are indicators of metabolic response to oxidative stress in patient with diabetes. Hence, selenium can be used in diabetic patient to prevent the late complications.The present study indicates that since it is a hospital based study, the same study should be conducted in general population (community based) to further evaluate the effect of serum-selenium in diabetes.

INTRODUCTION

Diabetes mellitus is a disorder with late complications including cardiovascular disease, nephropathy, neuropathy, retinopathy which affect severely the quality of life [1]. Recent report indicates that free radicals have important roles in pathogenesis of diabetes and a relationship between oxidative stress and secondary complications of diabetes exists [2], [3].

Diabetes is highly prevalent affecting approximately 150 million people worldwide and this number is expected to be 300 million in the year 2025 with the greatest number of cases being expected in China and India [4].

Oxidative stress in person with diabetes is also related to decreased antioxidant defense [5]. Since free radical production is increased whereas capacity of antioxidant system is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compared to healthy individuals [6],[7].

All aerobic organisms possess some sort of antioxidant defense, with enzymatic and non-enzymatic constituents [8]. Selenium is a trace element that is found in the soil and is absorbed in the food chain. The body needs small amount of it for healthy metabolism. It is an antioxidant and stop cells being damage by oxygen. Selenium is a cofactor for enzyme glutathione peroxidase. Glutathione peroxidase (catalyses the decomposition of reactive oxygen species) is a selenium-dependent enzyme. The substrate, for enzyme glutathione peroxidase is reduced glutathione, which is a specific H donor for reduction of H_2O_2, lipid and non-lipid H_2O_2 and protect the membrane lipid and haemoglobin against oxidation by peroxides [9].

Selenium modulates the cellular response and protects against oxidative stress and the production of reactive oxygen species. [10] In addition, selenium has effect on preventing decomposition, absorption and biological activity of – tocopherol. [11], [12] Selenium and Vitamin E act as complementing each others function against oxidative stress [13], [14]. Recommended daily intake of selenium in adult is 55µg/day [15].

Studies over serum selenium in diabetic population are rather limited. Therefore the present study was designed to determine and evaluate the serum selenium level in uncomplicated diabetic patients and healthy subjects so that early detection of complications, timely and appropriate interventions can be done to decrease the late complications of diabetes mellitus.

MATERIALS AND METHODS

1. **Selection of patients:** The present study was carried out on a group of 50 serum sample from patient with uncomplicated diabetes mellitus attending the OPD / ward of medicine department in B.R.A.M. hospital, Raipur (C.G.)

   The inclusion criteria for the selection of uncomplicated diabetic patients were; diabetic patient of the age between 40-70 years at the time of study, not having any complication of diabetes like diabetes neuropathy, diabetes nephropathy, diabetes retinopathy etc.

   Exclusion criteria were as follows-patients with age below 40 and above 70 years, patients having any complications of diabetes mellitus, pregnant patients, patient taking multi-vitamin and anti-oxidant preparation (containing selenium).

2. **Selection of controls:** 50 subjects were randomly selected from hospital with age group between 40-70 years, and is non-diabetic according to 1999 WHO diagnostic criteria for diabetes.

3. **Study design:** it is a hospital based non randomized, multistage, stratified, cross-sectional comparative study.
4. **Sample collection:** after an overnight fast, venous blood samples were collected aseptically from the diabetic patients and controls via venepuncture.The blood was then centrifuged at 3000 rpm for 10 minutes to obtain serum, taking all precaution to avoid haemolysis. Serum was then frozen at -20'C until analysis.
5. **Serum Selenium Estimation:** Serum selenium was determined by atomic absorption spectrophotometer. A Chemito 201 atomic absorption spectrophotometer equipped with a hydride generation system and a hollow cathode lamp for selenium operating at 12mA intensity was employed.

Reduction of the selenium compounds present on the sample was carried out using 3/. NaBH4 in 1/. NaOH solution. Atomization was performed using an air-acetylene flame. Absorbance was measured using 195 nm wavelength and 1.0 nm slit width. Serum selenium estimation was measured after obtaining the calibration graph. The reading obtained are converted from parts per million to µg/ lit of selenium in all the samples obtained from uncomplicated diabetic patient and controls.

Data		Diabetic patients	Control
Number of Subjects(n)		50	50
Age(in Years)		49.10±6.48	52.74±7.53(28)
Sex	Male	23(46%)	22(44%)
	Female	27(54%)	28(56%)

[Table/Fig 1]: Descriptive data for diabetes and control group used in the study

STATISTICAL ANALYSIS

The results obtained from atomic absorption spectrometer were expressed as mean± SD. Data's were compared using chi-square test. The difference were considered to be significant when P < 0.05. Statistical analysis was done using SPSS for windows statistical software, version 8.0.

RESULTS

50 uncomplicated diabetic patients & 50 normal healthy individuals were selected according to inclusion criteria. In the diabetic group, mean age is 49.10±6.48 years,while in control group, mean age is 52.74 ± 7.5 years. In diabetic group, 46%% were male and 54%% were female, while in control group 44%%were male and 56%%were female. After collection of blood sample, the serum selenium concentration was estimated in all the three groups by Hydride Generator Atomic Absorption Spectrophotometer (HGAAS). The mean serum selenium concentration in diabetic group and in control group was 51.9 ± 8.23 µg/lit and130.66 ± 37.18 µg/lit. There was a significant decrease in mean serum selenium level in diabetic group in comparison to control (P<0.05). The mean serum selenium concentration in diabetic patient in age group 41-50, 51-60yr and 61-70 yr is 57.11±7.05 µg/ lit, 45.79±3.43µg/lit and 42.00 ±1.73 µg/lit respectively. In control group, the mean serum selenium level in the age group 41-50yr, 51-60yr and 61-70yr is 171.17±23.49µg/lit, 117.82±14.70µg/lit and 86.00±8.94µg/l respectively. There was a significant decrease in the mean serum selenium levels in both the groups with increasing age. There was a significant decrease in the mean serum selenium level in 51-60 years age group when compared with 41-50 years age group (p<0.05) and in 61-70 years age group when compared with 51-60 years age group (p<0.05). There was no significant difference in the mean serum selenium level between males and females in the diabetic and control group (p>0.05) [Table/Fig 1].

DISCUSSION

In the present study 50 diabetic patient and 50 controls were analyzed. We determined a significant decrease in serum selenium level of diabetic patients compare to control. The mean serum selenium concentration for the control group in the present study was130.66±37.18 µg/liter [Table/Fig 2-4].

Serum Selenium concentration	Diabetic Patient (n=50)	Control (n=50)
Mean=SD(µg/lit)	51.9±8.23	130.66±37.18

[Table/Fig 2]: Mean serum-selenium in the present study

The mean serum selenium concentration in diabetic group and in control group was 51.9 ± 8.23 µg/lit and130.66 ± 37.18 µg/lit. There was a significant decrease in mean serum selenium level in diabetic group in comparison to control (P<0.05)

Age in Years	Serum selenium level(µg/lit)	
	Diabetic group (Mean±SD)	Control group (Mean±SD)
41-50	57.11±7.05	171.17±23.49
51-60	45.79±3.43	117.82±14.70
61-70	42.00±1.73	86.00±8.94

[Table/Fig 3]: Showing Age-wise distribution of serum selenium in the present study

Sex	Serum selenium level(µg/lit)	
	Diabetic group (n=50)	Control group (n=50)
Male	50.61±7.60	129.00±36.78
Female	53.00±8.73	131.67±31.17

[Table/Fig 4]: Showing Sex wise distribution of Serum Selenium in the present study

Similar observations were cited in the following studies:

In the Nutritional Prevention of Cancer (NPC) Trial, a selenium level of 80 ng/ ml is considered the minimum level of plasma selenium necessary in the blood stream for maximum production of selenoproteins (glutathione peroxidase, thioredoxin reductase, etc.).[16] Safaralizadeh R et al. [10] evaluated the serum concentration of selenium in 184 healthy individuals living in Tehran by hydride generator flame atomic absorption spectrometry. In adults the mean serum selenium was 100.6 ± 13 µg/lit. Navarro M et al. [17] evaluated the serum selenium concentration in 130 healthy individuals living in Spain. The mean selenium concentration in serum was 74.9µg/lit.

Cunha SD et al. [18] evaluated the serum level of selenium in healthy volunteers living in the city Rio de Janeiro. The mean serum

selenium level was 73.18 ± 9.9 µg/lit. Karatas F et al. [19] found the mean serum selenium concentration of the control group to be 85.81 ± 10.84µg/lit. Nsonwu AC et al. [20] found that mean serum selenium concentration of control group in his study was 0.28 ± 0.24 mg/lit.

In India, there are some selected reports of serum selenium concentration of healthy adults. The mean serum selenium concentration reported by Mahalingum et al.[21] is 72 ± 4 µg/lit, Srikumar et al.[22] is 125 ± 19 µg/lit., Yadav et al.[23] is 117 ± 16 µg/lit and by Gambhir and Lali et al. [24] is 133 ± 39 µg/lit.

The mean serum selenium in the study for uncomplicated diabetes mellitus) was 51.9 ± 08.23 µg/liter, which was significantly lower than the control group ($p<0.05$).

Karatas F et al.[19] estimated the mean serum selenium to be 67.17 ± 11.88 µg/lit, which was significantly lower than the control group (85.81 ± 10.84 µg/lit) ($p<0.05$).Findings of several studies demonstrated that overproduction of peroxides along with emaciation of antioxidant defense system cause oxidative damage and these events in Type 2 diabetic patients are observed earlier before diabetic complications developed [25]. Ruiz C et al (1998) [26] also found that the mean plasma selenium concentration in diabetic patients was significantly lower than controls ($p<0.01$). Diplock et al[27] reported that when diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defense system are observed. In diabetes, there occur glycation of glutathione peroxidase and consequently functional changes of the antioxidant enzymes. Thus decreasing antioxidant status of this selenium dependent enzyme leads to more free radical production and more complication of diabetes.

Decline in physiological functions with age may influence absorption, metabolism and excretion of micronutrients Serum selenium level were significantly lower in the age group of 55-75 years than those of the age group of 40-54 years ($p<0.05$) in the diabetic population [20],[28],[29].

In our study, there was no significant difference in the mean serum selenium level between males and females ($p>0.05$).

Navarro M et al [17] found that the mean serum selenium concentration in healthy individuals did not vary significantly in relation to the sex of the subject. Significant reductions in selenium levels are indicators of metabolic response to oxidative stress in patients with diabetes.

Although glucose itself can initiate oxidative stress, deficiency of essential trace element such as selenium may exacerbate this oxidative stress in diabetic rats [30].

CONCLUSION

Although the results of this comparative evaluation after the collection of data and its analysis confirm the relation between serum selenium with diabetes and its complications, the result and conclusion drawn can be extrapolated to general population (community based) with suitable representative samples to further evaluate the effect of serum-selenium in diabetes.

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AUTHORS:

1. **Usha Joshi,Pd Raut:** Department of Pharmacology, Pt. J.N.M. Medical College Raipur & Dr. B.R.A.M. hospital Raipur (C.G.), 492001, INDIA
2. **PD Raut**
3. **Sk Agrawal**
4. **Pk Patra**
5. **Bk Maheshwari**
6. **Manu Apurb**
7. **Dhirhe Tc.**

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr Presenjit Daulat Raut (09039267377), Asst. Prof. (Pharmacology), Pt.JNM Medical College, Raipur, CG-492001

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