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Serum selenium, glycemic control and blood lipoproteins in type 2 diabetes mellitus

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ABSTRACT

The present study determined serum selenium, blood glucose, Glycosylated HbA1, and blood lipoprotein in diagnosed cases of type 2 Diabetes mellitus subjects (T2DM). Department of Medicine, Liaquat University Hospital Jamshoro/Hyderabad from January 2015 to February 2016. A sample of 350 T2DM was selected through non probability purposive sampling according to inclusion and exclusion criteria. Blood glucose, HbA1c, serum triglycerides (TAG), total cholesterol (TC), VLDL, LDLc, and HDLc were estimated. Serum selenium was measured by atomic absorption technique. The Data was analyzed by SPSS 22.0 (USA) using appropriate statistical tests at 95% confidence. Serum selenium deficiency was observed in 229 (65.4%) of cases. Serum selenium in normal (≥70µg/L) and deficient (<70µg/L) was noted as 97.7± 1.29 and 63.9± 4.7 µg/L respectively (p=0.0001). Serum selenium showed negative correlation with TAG, TC, LDLc, VLDL, blood glucose and glycosylated HbA1. The present study reports serum selenium deficiency in majority of type 2 diabetics. Serum selenium showed negative correlation with TAG, TC, LDLc, VLDL, blood glucose and Glycosylated HbA1.

Introduction

The number of people with type 2 Diabetes mellitus (T2DM) is increasing due to increasing population, growing urban population, sedentary life style, and obesity.[1] DM ranks 4th among non communicable disease (NCDs). A global death toll of 1.5 million is caused by DM each year.[2,3] A 2014 study showed worldwide prevalence of 9% among adults.[4] Currently 382 diagnosed cases of DM of age 40-59 are living in the World, as has been estimated by the International Diabetes Federation (IDF).[5] At an estimated 55% rise, this burden will rise to 532 million by the year 2035. Low and middle income countries harbor 80% of diabetic population. Undiagnosed cases of DM are estimated at 175 million which is an alarming situation. The increasing number of diabetic population puts burden on the economy and growth of any country. For example 548 billion US dollars were spent on management of DM in the year 2013.[6] Currently Pakistan is the sixth most populous country of the World with estimated population of 184.35 million.[7] True estimates of DM burden for Pakistan is not known. But previous diabetes surveys by Diabetes Association of Pakistan (DAP) and World Health Organization (WHO) revealed 11.47% of prevalence of DM, ranged from 6.39–16.5%.[8,9] The IDF estimates of 2014 showed 6.9 million DM cases in Pakistan. The prevalence of DM in adults was reported as 6.8%.[10] The projections by IDF by the year 2035 show alarming burden of DM cases for Pakistan. IDF projections reported an estimate 12.8 million diabetics by the year 2035. By that time, Pakistan will rank eighth countries among top ten in the World.[2,6] This diabetic epidemic is true for the Pakistan as it is prevalent.[11] According to WHO estimates approximately 50% of 333 million diabetics will be from Asia by the year 2030. IDF estimates showed that the diabetic's number will be doubled by the year 2030 i.e. from 285 million in 2010 to 439 million by the 2030.[12] A previous estimate reported the Pakistan takes sixth rank in the World for the Diabetics load.[1] A previous study from Pakistan reported 15% Pakistani`s are suffering from DM and millions don't know about their diabetic status.[13, 14] A validated criteria of estimating the glycemic status over previous 3 months comes from the Glycosylated hemoglobin A (HbA1c) levels of a diabetic person. Trace minerals also have role in the glucose homeostasis because they function as coenzyme in the physiological phenomena. Trace minerals such as selenium and chromium are involved in insulin signalling pathways and their deficiency aggravates the glycemic status.[15] However, a previous study reported no association of HbA1c and trace minerals in the blood vessels of diabetic persons.[16] Serum selenium is an essential trace element which plays role in physiological phenomena. Serum selenium concentrations vary with gender, dietary supply, geographical location and aging.[17]

Earlier studies have suggested an inverse linkage of serum selenium concentration and glucose tolerance suggesting the role of selenium in diabetics. Suggested mechanisms propose a direct effect on glucose metabolism, insulin signalling, and insulin release or may be through an independent pathway.

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[17-19]Previous studies had suggested role of selenium, copper and zinc in the causation of cardiac and vascular disease in diabetic patients.[17-19]

Hence, the trace minerals deficiency might initiate and progress the vascular endothelial changes which run a precursor role in the atherosclerotic vascular disease.[20, 21] Recently studies had reported on the possible association of serum selenium in the glucose metabolisms in the diabetics. Low serum selenium is reported in diabetics which aggravates the glycemic control. Low serum selenium might play role in the pathogenesis of cardiac and vascular diseases.[18-21]

As the Pakistan is suffering an epidemic of Diabetes mellitus and achieving an ideal glycemic control is very difficult in our population. Selenium might be playing important role in the glycemic control in Diabetic subjects, hence it is worth to evaluate the serum selenium levels in our population whether it is normal, low or high as studies are lacking on the issue, then strategies of selenium supplementation may be implemented for better glycemic controls in diabetics. The present prospective study was planned to determine the serum selenium, blood glucose, Glycosylated HbA1, and blood lipoprotein in diagnosed cases of type 2 Diabetes mellitus Pakistani subjects.

Subjects and Methods

The present prospective study was conducted at the Department of Medicine, Liaquat University Hospital Jamshoro/Hyderabad from January 2015 to February 2016. A sample of 350 diagnosed cases of the type 2 DM were enrolled over the study duration. Study subjects were selected through non-probability purposive sample. Inclusion and exclusion criteria were followed. Subjects of 30- 70 years of at least 10 years duration of Diabetes mellitus of either gender were included. Exclusion criteria were diabetic kidney disease (DKD), chronic kidney disease (CKD), Chronic liver disease (CLD) and bone disease. Patients taking multivitamin pills were also excluded. The study subjects were first examined by a medical officer followed by a Consultant Physician. Medical officer reported on the duration of DM, dietary and drug history, blood glucose checking and education level of study subjects. Only volunteer's subjects were allowed to enter the study protocol. American Diabetes Association (ADA) criteria were used for the diagnosis of Diabetes mellitus. DM was defined as fasting blood glucose (FBG) ≥ 126mg/dl or random blood glucose (RBG) ≥200 mg/dl.[22]

- **Blood sampling**

Patients were called with an overnight fasting. The blood samples were drawn through a Venepuncture under aseptic condition using standard methods of blood sampling. Intravenous cannula 20 G (Johnson and Johnson) was used for blood sampling within vacutainers. Sera were obtained by centrifugation of blood samples at 3000 rpm for ten minutes. Care was taken to prevent hemolysis. Sera were stored frozen at -20^{0}C until used for analysis.

- **Glucose estimation**

The blood glucose was estimated by the glucose oxidase enzymatic method on an automated Chemistry analyzer (Hitachi 902, Roche diagnostics, USA).

- **Glycosylated hemoglobin A1 (HbA1c) estimation**

Glycosylated hemoglobin A1 (HbA1c) was estimated as indicator of Diabetic glycemic control. HbA1c assay was performed according to laboratory criteria set out by DCCT trial assay. Glycosylated HbA1 was estimated on an automated Chemistry analyzer (Hitachi 902, Roche diagnostics, USA). HbA1c ≤7% indicated a good glycemic control and diabetics with HbA1c >7% were defined as uncontrolled Diabetics with bad glycemic control.[23]

- **Serum selenium estimation**

Serum selenium was measured by atomic absorption spectrophotometer. The "*Chemito 201*" spectrophotometer is equipped with a hydride generation system. A hollow cathode lamp for selenium is attached to it. It was operated at 12mA intensity. Selenium compounds of sample were reduced using NaBH4 in NaOH solution. An acetylene flame was used for atomization. Absorbance was estimated as a wavelength of 195 nm and 1.0 nm slit width. Calibration graph measured the serum selenium. Readings were converted from ppm (parts per million) to μg/L of selenium. Procedure was performed for all the samples.[24]

- **Lipids determination**

Dyslipidemia was defined (ATP III) as per criteria; total cholesterol (TC) >200mg/dL, triglycerides (TG) >150mg/dL, VLDL > 30mg/dL, LDLc >130mg/dL and HDL < 40mg/dL. Sera were pipetted into a clean blood sample bottle. Analysis was performed on the day of blood sample collection. Serum TC was estimated by enzymatic (CHOD-PAP) colorimetric method. Serum TG was estimated by an enzymatic (GPO-PAP) method. VLDL was estimated by colorimetric method. Precipitant method was used for the estimation of HDLc. Friedewald's formula [LDL-C = TC - HDL-C – (TG/5)] was used for LDLc estimation.[21]

- **Consent form and ethical clearance**

Volunteer subjects were asked to sign the consent form. Ethical clearance was taken from the ethical committee of the institute. Data was recorded in a pre structured proforma. Confidentially of patient data was secured.

- **Data analysis**

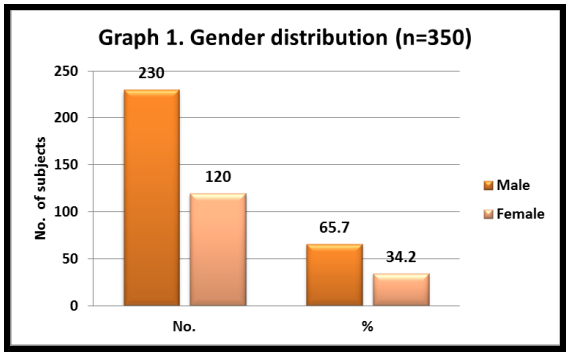
SPSS 22.0 version (IBM Corporation, USA) was employed for data analysis. Shapiro Wilk test was used for normality of data distribution. Student's t test and chi square test were used for numerical and categorical data variables. Pearson`s correlations was used for linear association of serum selenium with HbA1c and lipid subtractions.

Results

Of 350 study subjects 65.7% were male and 34.2% were female. Age (mean ± SD) was noted as 45±13.5 years. Rural and urban areas population comprised 41.4% and 58.5% respectively. Thus majority of study subjects belonged to urban areas. Characteristics of type 2 DM subjects are shown in table 1. Blood glucose, systolic BP, and diastolic BP showed a rise in study subjects as shown in table 1. Systemic hypertension was noted in 62%, dyslipidemia in 55.1% and smoking habit in 21.7%. Approximately 2/3 (66%) showed bad (HbA1c ≥7%) and good (HbA1c <7%) was noted in 34% subjects (p=0.001).

Table 1. Characteristics of type 2 diabetic study subjects (n=350).

Age (years)	**45± 13.5 years**
Blood glucose (mg/dl)	223±60.5
Systolic BP (mmHg)	143±30.5
Diastolic BP (mmHg)	81± 12.5
Duration of DM (years)	16 ± 5.9
Male	230 (65.7%)
Female	120 (34.2%)
HbA1c (<7%)	119 (34%)
HbA1c (≥7%)	231 (66%)
Rural population	145 (41.4%)
Urban population	205 (58.5%)
Systemic hypertension	217 (62%)
Dyslipidemia	193 (55.1%)
Smokers	76 (21.7%)



Normal serum selenium (≥70µg/L) and serum selenium deficiency (<70µg/L) were noted in 121 (34.5%) and 229 (65.4%) of cases respectively (0.0001). Serum selenium in normal (≥70µg/L) and deficient (<70µg/L) was noted as 97.7± 1.29 and 63.9± 4.7 µg/L respectively (p=0.0001) (table 2). Glycosylated HbA1, blood glucose, triglycerides (TAG), total cholesterol (TC), HDLc, LDLc, and VLDL showed statistically significant differences in diabetics with normal selenium and deficiency states (p < 0.05) (table 3). Serum selenium showed negative correlation with TAG, TC, LDLc, and VLDL. HDLc showed positive correlation as shown in table 4. Blood glucose and Glycosylated HbA1 also showed negative correlation.

Table 2. Serum selenium in type 2 diabetic subjects (n=350).

	Mean± S.D	No. (%)
Serum selenium (≥70µg/L)	97.7± 1.29	121 (34.5%)
Serum selenium (<70µg/L)	63.9±4.7	229 (65.4%)
P- value	0.0001	0.0001

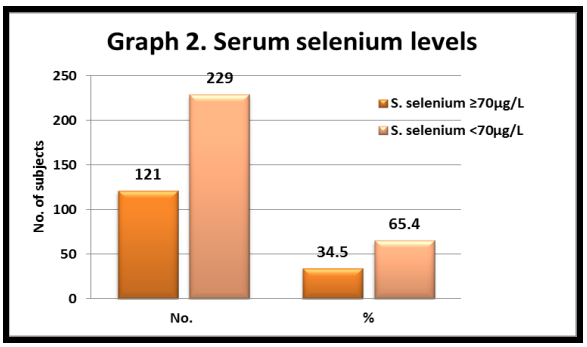


Table 3. Serum selenium, glycemic control and blood lipoproteins (n=350).

	Serum selenium		
	≥70µg/L	<70µg/L	p-value
HbA1c (%)	7.15± 0.71	9.39± 0.81	0.0001
Blood glucose (mg/dl)	229.0± 27.5	265.5± 27.9	0.0001
Triglycerides (mg/dl)	129.5± 35.5	227.5± 25.5	0.003
Total cholesterol (mg/dl)	154.5± 21.6	231.9± 33.5	0.001
HDLc (mg/dl)	39.0± 7.1	34.6 ± 8.35	0.0035
LDLc (mg/dl)	95.5± 15.5	115.5± 25.4	0.0001
VLDL (mg/dl)	41.5 ± 10.0	39.3 ± 5.7	0.0001

Table 4. Pearson`s Correlation of serum selenium with glycemic control and blood lipoproteins (n=350).

	Correlation coefficient (r-value)	p-value
Triglycerides (mg/dl)	-0.423	0.003
Total cholesterol (mg/dl)	-0.387	0.002
HDLc (mg/dl)	0.541	0.003
LDLc (mg/dl)	-0.341	0.0001
VLDL (mg/dl)	-0.231	0.001
HbA1c (%)	-0.232	0.001
Blood glucose (mg/dl)	-0.293	0.001

Discussion

Selenium (Se) is one of the essential trace elements for the prevention of disease in needed in human diet. A review of serum selenium in medical literature is a topic of dispute and debate in diabetic and non-diabetic subjects.[25-31] Several epidemiological studies have reported positive association of Se deficiency with the increased risk of cardiovascular disease, and carcinogenesis in the human body.[32,33]

The present study reports a high frequency of serum Se deficiency in type 2 diabetics. Normal serum selenium (≥70µg/L) and serum selenium deficiency (<70µg/L) were noted in 121 (34.5%) and 229 (65.4%) of cases respectively (0.0001). Serum selenium in normal (≥70µg/L) and deficient (<70µg/L) was noted as 97.7± 1.29 and 63.9± 4.7 µg/L respectively (p=0.0001). The findings are in agreement with previous studies.[21-23] However, other previous studies had reported contrary results.[25-27] A recent study has reported no differences of serum selenium in type 2 diabetics compared to normal healthy subjects.[34] Contrary to this, previous studies had reported mean serum selenium was low in type 2 diabetics compared to controls.[36,37]

The findings of above studies are in agreement with the present study as low serum selenium was noted in 121 (34.5%) of study subjects. Most probable reasons of controversial results of serum selenium in type 2 diabetics may be difference detection methodologies of serum selenium, sampling errors, different study designs, different geographical areas, and research bias. On the basis of evidence based findings of present study, serum selenium was found low in type 2 diabetics.

In addition, Pearson`s correlation showed negative correlation of serum selenium with glycemic control and blood lipoproteins except for HDLc. In present study, the Glycosylated HbA1, blood glucose, triglycerides (TAG), total cholesterol (TC), HDLc, LDLc, and VLDL showed statistically significant differences in diabetics with normal selenium and deficiency states (p < 0.05) (table 3). Serum selenium showed negative correlation with TAG, TC, LDLc, and VLDL. HDLc showed positive correlation as shown in table 4. These findings are in agreement with a previous study.[28]

A few of previous studies had reported elevated serum selenium in diabetics compared to controls.[30,31,37] A previous study reported high serum selenium levels were positively associated with the prevalence of diabetes.[38] These controversial reports are highly misleading and reveal serious research bias which needs to be confirmed in large sample size studies.

Serum TAG, serum TC, VLDL, LDLc, and HDLc showed statistically significant differences between normal and deficiency serum selenium type 2 diabetic subjects in the present study. Significant differences were also found for the Glycosylated HbA1 and blood glucose in normal and deficient serum selenium subjects. Serum selenium showed negative correlation with TAG, TC, LDLc, and VLDL. HDLc showed positive correlation as shown in table 4. Blood glucose and Glycosylated HbA1 also showed negative correlation. These findings of correlation are in agreement with a previous study.[21]

A previous study had reported serum selenium was negatively associated with fasting blood glucose and Glycosylated HbA1.[30] The finding of HbA1c of present study is in agreement with above study. The negative association of serum selenium may be explained by being integral part of

enzyme system Glutathione peroxidase (GPX). Diabetics are loaded with oxidative burden; hence enzyme systems utilize selenium at more speed resulting in its deficiency. GPX neutralizes the hydrogen peroxide and organic hydroperoxides.[39] In summary, serum selenium was found low in type 2 diabetics in present study. Serum selenium showed negative correlation with TAG, TC, LDLc, VLDL, blood glucose and glycosylated HbA1.

Conclusion

The present study reports serum selenium deficiency in type 2 diabetics. Serum selenium was negatively associated with blood glucose and Glycosylated HbA1. Serum triglycerides, total cholesterol, VLDL and LDLc showed negative correlation. Serum selenium may be estimated in diabetics for better glycemic control and prevention of dyslipidemia.

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