Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SELENIUM AND GLUTATHIONE PEROXIDASE IN DIABETES MELLITUS

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ABSTRACT

The incidence of type 2 diabetes mellitus is rising with the majority of the cases being expected in China and India. In Diabetes mellitus oxidative stress mostly seems to be due to an increase in production of free radicals or sharp reduction in antioxidant defense. Diabetic patients may require more antioxidants compared to healthy individuals. Selenium is an important constituent of the antioxidant enzyme glutathione peroxidase. Deficiency of selenium may also lead to a decreased activity of this enzyme resulting in production of free radicals. Hence this study is done to compare the role of selenium (Se) in Glutathione peroxidase(GPX) activity among the type 2 diabetic patients with healthy controls. 50 Type 2 diabetic patients aged between 30-60 and 50 healthy age matched controls who attended Sri Jayadeva Cardiology and Diabetology institute were included in this study. Their clinical history was obtained. A fasting blood sample was drawn and the biochemical parameters - blood sugar, lipid profile, thyroid profile, Se and GPX were estimated. Statistical analysis was done by Student's t test. A significant decrease in Se and GPX was observed in diabetic patients when compared to the healthy subjects. A statistically significant increase in TGL, LDL, AIP and a decrease in HDL was observed among the diabetic patients when compared to the healthy subjects. A normal thyroid status was observed in diabetic patients even at low selenium level. Decreased activity of the antioxidant enzyme, GPX may be due to the low level of selenium. Decrease in selenium along with decrease in GPX, reveals that the antioxidant defense mechanism is impaired in diabetic patients which may lead to further complications. Lower HDL and increased AIP level also suggests that the diabetic patients are at increased cardiovascular risk.

KEY WORDS: Selenium (Se), Glutathione peroxidase (GPX), Type 2 Diabetes mellitus , Atherogenic index of plasma (AIP), cardiovascular risk.

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INTRODUCTION

Diabetes Mellitus is a disease caused by deficiency or diminished effectiveness of endogenous insulin. It is characterized by hyperglycemia, deranged metabolism predominantly affecting the vasculature leading to nephropathy, neuropathy, retinopathy, CVD thus affects the quality of life. The incidence of type 2 diabetes mellitus is rising with the majority of the cases being expected in China and India. The prevalence is approximately 285 million people and is expected to be 440 million by 2025[1].Excess nourishment and a sedentary life style may lead to glucose and fatty acid accumulation in the muscle, adipose and pancreatic cells. This leads to the generation of free radicals such as superoxide, hydrogen peroxide or hydroxyl radicals resulting in oxidative stress. The damage caused by these reactive oxygen species (ROS) can be prevented by the antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase etc. In diabetes mellitus oxidative stress mostly seems to be due to an increase in production of free radicals or sharp reduction in antioxidant defense[2]. It has been proposed that diabetic patients may require more antioxidants compared to healthy individuals[3]. Earlier studies also show the use of antioxidants to block the formation of free radicals[4]. Selenium is an important constituent of the antioxidant enzyme glutathione peroxidase[5]. Deficiency of selenium may also lead to a decreased activity of this enzyme resulting in production of free radicals. Hence this study is done to compare the role of selenium (Se) and glutathione peroxidase (GPX) activity among the type 2 diabetic patients with healthy controls.

MATERIALS AND METHODS

The study involved 50 diabetic patients aged between 30-60 and 50 healthy age matched controls who attended Sri Jayadeva cardiology and Diabetology institute, Bangalore. A detailed clinical history was obtained, including their dietary habits, smoking and alcohol consumption. Anthropometric characteristics like height, weight were taken and blood pressure was measured using standard protocols. A fasting blood sample was drawn and the following biochemical parameters were estimated.

Blood sugar – Glucose oxidase peroxidase method
Cholesterol – Cholesterol oxidase peroxidase method
Triglyceride – Glycerol 3 phosphate oxidase method
HDL – Phosphotungstate method.
LDL & VLDL- Fried walds formula

Thyroid status T3, T4 and TSH were quantitated using ELISA technique. The antioxidant enzyme, glutathione peroxidase was measured by enzyme linked immune assay and Selenium was estimated by graphite furnace atomic absorption spectrophotometer.

Statistical analysis: Statistical analysis was performed using SPSS version 16. Student's t test was used to compare between groups.

Inclusion criteria: Clinically diagnosed diabetic patients receiving oral hypoglycemic drugs

Exclusion criteria: Patients who are smokers, alcoholics and those receiving selenium supplementation.

Ethical clearance: The study was approved by the instituitional ethical committee. The purpose of the study was explained to the participants and an informed consent was obtained.

RESULTS AND DISCUSSION

Table 1
Selenium and GPX in Type II Diabetes Mellitus.

	Parameters	Control (n=50)	Diabetes Mellitus (n=50)	p value
1.	**Selenium**	67.63±14.6	60.7 ± 13.45	**0.016**
2.	**GPX**	271.26±57.04	225.5±44.80	0.000

Table 1 shows the comparison of Se and GPX among the diabetic patients and healthy subjects. A significant decrease was observed in Se and GPX activity in diabetic patients when compared to control. The normal reference for selenium ranges from 60 µg/L to 120 µg/L[6]. Though a significant decrease in selenium was observed in our study, the levels of selenium for control and diabetic group were at the lower normal range. In India there are some selected reports of serum selenium concentration of healthy individual. Mean value reported by Mahalingam etal[7] is72±4 µg/L, Srikumar etal 125±19 µg/L[8], Yadav etal 117 ± 16 µg/L[9]. It was reported that selenium intake may increase the serum selenium, but the activity of selenoprotein concentration and activity are maximized at plasma levels of 70 and 90 µg/L[10].Our present value is lower than the reported which may be due to a low intake. Hence, these findings create a greater concern about the association of Se intake above the recommended daily allowance and diabetic risk. The existing data regarding the relationship between diabetes mellitus and selenium are controversial. A study by Campbell found a statistically significant increase in selenium levels between diabetic and non diabetic individuals[11]. Some studies showed lower selenium levels in diabetics compared to non diabetics[12]. Another recent study in Asia documented no significant difference for selenium levels in type 2 diabetes mellitus and non diabetic individual[13].Studies also show a low activity of GPX in diabetic patients when compared to normal[14]. In the present study, hyperglycemia seen in the study subjects itself can initiate the formation of ROS which is the cause for oxidative stress. Three primary antioxidant enzymes have been demonstrated in antioxidant systems, namely superoxide dismutase, catalase and glutathione peroxidase. These three enzymes prevent tissue damage by detoxifying ROS[15]. Selenium is an essential trace element and is an integral component of the catalytic site of the enzyme GPX[16]. Hence deficiency of selenium in our study causes a profound reduction in the activity of GPX. This may cause increased oxidative stress leading to complications of diabetes.

Table *2*
Comparison of FBS, Lipid profile among Groups

S.No	Parameters	Healthy	Diabetes Mellitus (n=50)	p value
1.	**FBS**	98.8 ± 12.6	178.58±49.4	0.000
2.	**Cholesterol**	169.46±23.9	176.0±45.20	0.365
3.	**TGL**	118.0±36.6	170.0 ±53.5	0.000
4.	**HDL**	45.0 ± 7.8	34.6 ± 5.67	0.000
5.	**LDL**	94.4±19.9	104.9 ± 40.6	0.117
6.	**VLDL**	26.1±13.2	31.3 ± 12.7	0.051

Table 2 depicts the fasting blood sugar and lipid profile among the diabetic patients and healthy subjects. A significant increase in FBS was seen in diabetic patients when compared to control. No significant difference was observed in cholesterol and LDL between the groups. TGL, VLDL showed a significant increase and HDL showed a significant decrease in diabetic patients when compared to control. Decreased Se, GPX activity and significantly decreased HDL in DM compared to the control reveals the existing oxidative stress in DM. Oxidative stress

is implicated in the pathogenesis of diabetic complications. The mechanism by which selenium deficiency causing oxidative stress in kidney was reported to be through TGF beta 1 which is a prooxidant[17]. The inflammatory or proinflammatory properties of HDL are superior to HDL –c concentration in terms of discriminating the complications seen in CHD. Most of the antioxidant properties of HDL is the consequence of the associated enzyme concentration such as paraoxanase, glutathione peroxidase[18, 19]. Hence low HDL- c with low GPX activity suggests more significant clinical role in developing cardiovascular complications in diabetes mellitus.

Table 3
Comparison of Atherogenic risk parameters among Groups

S.No	Parameters	Healthy (n=50)	Diabetes Mellitus (n=50)	p value
1.	**TGL/HDL**	2.7 ± 0.98	4.8 ± 1.5	0.000
2.	**LDL/HDL**	2.1 ± 0.60	3.0 ± 1.1	0.000
3.	**AIP**	0.1 ± 0.1	0.30 ± 0.13	0.000

A significant increase was observed in TGL/HDL and LDL/HDL ratios (Table.3) in diabetic patients when compared to healthy subjects. According to Grover these ratios were considered as predictors of future cardiovascular risk[20]. Recently, another marker for atheogenic risk – Atherogenic index of plasma (AIP) has been introduced by Dobiasova etal[21]. AIP is a measure of log TGL over HDL cholesterol and is inversely related to LDL particle size. The AIP value <0.1, 0.1-0.21, >0.21 suggests low risk, intermediate risk and high risk for atherosclerosis respectively. The use of AIP in predicting atherogenic risk has been studied in hypertensive post menopausal women[22], smokers[23], hypetension[24] and in diabetic patients with metabolic syndrome[25]. The AIP values for control and diabetic patients in the present study are 0.1 and 0.3 respectively. These values along with low Se and GPX activity suggest a decrease in the antioxidant defense mechanism among the diabetic patients. Hence the study reveals that the diabetic patients are at a high risk for cardiovascular disease.

Table 4
Comparison of Thyroid function test among Groups

S.No	Parameters	Healthy (n=50)	DiabetesMellitus (n=50)	p value
1.	**T3**	2.06±0.45	1.80±0.49	0.006
2.	**T4**	115.7 ± 24	99.28 ± 28.9	0.002
3.	**TSH**	2.48 ± 0.84	2.55 ± 1.9	0.800

Table 4 reveals the thyroid profile among the diabetic patients and healthy subjects. Though a significant decrease was seen in T3 and T4 in diabetic patients, it is well within the normal limits. The TSH was also found to be normal. This is because the deiodinase 1 enzyme, seem to convert T4 to T3 even at low concentration of selenium[26]. While liver, kidney, heart, skin and muscle are rapidly depleted from selenium during severe deficiency the thyroid, the reproductive system and brain retain Selenium to a remarkable extent[27].This explains a normal thyroid function among the diabetic patients in our study even at a low level of selenium. It was shown that in adult rats deiodinase 1 and phospholipid glutathione peroxidase (phGPX) are kept at high levels in the thyroid and there is no difference in T3 and T4 levels[28, 29].

CONCLUSION

Low levels of selenium and GPX suggests a decrease in antioxidant defense mechanism in diabetic patients. Increase in AIP attributes to

the cardiovascular risk. Hence, measurement of Se, GPX and AIP as a routine diagnostic test in diabetics may help in preventing the further complications in them. Low selenium level observed in healthy subjects in this study may be due to a low intake. Hence a study on soil content of selenium along with serum selenium in various regions of South India may be useful to understand the level of selenium supplementation in the diet.

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