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Estimation of serum copper and magnesium levels in diabetic nephropathy patients

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Abstract

Diabetic Nephropathy is one of the well known complications of diabetes mellitus (DM). It has been reported in several studies that the metabolism of trace elements like copper (Cu) and magnesium (Mg) has altered in diabetes. Aim of the present study is to investigate serum Cu and Mg levels in diabetic nephropathy patients. The study includes 45 diabetic nephropathy patients and 40 controls. Fasting blood sugar (FBS), post parandial blood sugar (PBS) was determined by Glucose oxidase- Peroxidase (GOD POD) method, urinary albumin was estimated by immunoturbidometery and Cu, Mg was done by diethyldithiocarbamate and calmagite method respectively.Mean±SD values of FBS (162.8±21.9 mg/dl), PPBS (267.7±41.6 mg/dl), microalbumin (29.4±5.16 mg/L) and Cu (278.1±25.4 mg/dl) were found high in diabetic nephropathy patients with statistically significant difference ($P<0.001$) when compared with controls. Low MG levels (1.0 ± 0.17µg/dl) were observed in diabetic nephropathy patients than controls (1.97 ± 0.21µg/dl). A positive correlation was observed between serum Cu and FBS, PPBS and microalbumin of patients. While Mg shows negative correlation with FBS, PPBS and microalbumin. Progression of microalbuminuria is associated with poor glycemic control. Continued high levels of serum Cu and low serum levels of Mg lead to more distressing clinical complications, including retinopathy, hypertension and microvascular disease. Evaluation of serum Cu and Mg should be included in routine testing.

INTRODUCTION

DM is characterized by metabolic disorders related to high blood glucose levels. This hyperglycemia leads to various vascular complications like coronary artery disease, neuropathy, retinopathy and nephropathy [1]. Microalbuminuria is a nephropathic condition arises from increased passage of albumin through the glomerular filtration barrier [2]. In type 1 DM prevalence gradually increase from onset of disease while in type 2 DM it can be observed in both newly diagnosed and established diabetes [3].Diabetic nephropathy is the most important cause of death in type 1 diabetes patients compares to type 2 DM [4]. In general (non-diabetic) population, hypertension is found to be the major responsible factor of microalbuminuria. Individuals with essential hypertension who develop microalbuminuria have higher incidence of biochemical disturbances [5].

Chronic complications of glucose metabolism are also found associated with alterations in levels of some trace elements like Cu and Mg [6]. Hyperglycemia results increased cellular antioxidant defense mechanism due to increased reactive oxygen species. Ultimately, hyperglycemia induces the expression of enzymes like superoxide dismutase, catalase and glutathione peroxidase; all these enzymes consist of Cu and zinc [7]. Mg is essential for insulin secretion, insulin receptor interaction, post receptor events (involving tyrosine kinase mediated phosphorylation) and normal carbohydrate utilization by Mg dependent enzymes [8]. Hyperglycemia leads to decreased cellular Mg Levels. Hypomagnesemia leads to collagen and ADP-induced platelet agreeability and also decreased function of Mg dependent enzymes, kinases and oxidative stress [9]. The objective of the study is to estimate the levels of trace elements namely Cu and Mg in diabetic nephropathy patients.

MATERIALS AND METHODS

Study consists of forty five type 2 diabetic nephropathy patients and forty controls from outpatient department. There were 28 males and - 17 female patients age between 40-80 yrs; while 40 age matched healthy volunteers, 25 males and 15 females were chosen as controls. Patients suffering from diabetes at least more than 5 years were included. Informed consent was taken from all the participants, for the additional examination of their samples. Study was carried out at Department of Biochemistry, Chhattisgarh Institute of Medical Sciences, Bilaspur.

Blood samples were collected in the morning after at least a 12-h over night fast for the estimation of FBS, Cu and Mg. After 2 hours of breakfast sample were collected for PPBS. Microalbumin was analyzed by freshly voided urine in a sterile container. For biochemical investigation serum was separated by centrifugation at 3000 rpm for 10 min and was stored at 4°C until analysis.

Diabetic nephropathy patients were selected according to clinical diagnosis and also depending upon the values above reference range of serum parameters like FBS (70-120mg/dl), PPBS< 200 mg/dl, urinary albumin <20 mg/L. Quantitative determination of glucose was done by semi auto analyzer by GOD-POD method [1], estimation kits were provided by Merck. Microalbumin was analyzed using spectrophotometer by immuno-turbidometry [3] at wavelength of 340 nm. Spectrophotometric estimation of Cu was done by sodium diethyldithiocarbamate method [10] at 440 nm (reference value 100-200 µg/dl) and Mg concentrations were determined by calmagite method [11] at 530 nm (reference value 1.6-2.6 mg/dl). Reagents were provided by Sigma Aldrich. Data was statistically analyzed by basic measures (mean ± standard deviation). Significant difference between means of cases and controls was

estimated by student t test at 0.001 level of significance. Karl Pearson's correlation coefficient was done for Cu and Mg with FBS, PPBS and albumin.

RESULTS

Table 1 reveals Mean ± SD levels of FBS, PPBS, microalbumin, Cu and Mg levels in both patients and controls. Levels of FBS (162.8±21.9 mg/dl), PPBS (267.7±41.6 mg/dl), microalbumin (29.4±5.16 mg/L) and Cu (278.1±25.4 mg/dl) were increased in diabetic nephropathy patients and differ significantly from controls. Significant decrease was observed in Mg concentration (1.0±0.17 µg/dl) in patients when compared to controls (1.97±0.21 µg/dl). Table 2 shows Cu level correlation with FBS, PPBS and microalbumin in diabetic nephropathy patients. Cu shows positive correlation with FBS, PPBS and microalbumin. Table 3 shows the negative correlation of Mg with FBS, PPBS and microalbumin in diabetic nephropathy patients.

DISCUSSION

Altered metabolism of trace elements in DM was found in several studies [12]. Altered glycemic control, osmotic diuresis were found as contributing factor for worsening the situation [13, 14]. In the present study it was observed increased Cu and decreased Mg concentration in serum of diabetic nephropathy patients. A correlation was established between various parameters of diabetic nephropathy patients and trace elements. A positive correlation was seen between Cu concentration and FBS, PPBS & microalbumin of patients. On the other hand Mg shows negative correlation with FBS, PPBS & microalbuminuria of patients.

Microalbuminuria is known for its mildly elevated levels of albumin in urine. The increased level of albumin in urine is proportional to the duration of hyperglycemia, which results to nephropathy [15, 16]. The onset of clinical phase of diabetic nephropathy is signaled by the presence of persistent proteinuria and said to be overt nephropathy [4]. Diabetic nephropathy accounts for 30-40% patients with type 1 and type 2 diabetes [17]. Microalbuminuria is strongly associated with vascular disease in hypertensive patients, suggesting it as a marker of vascular or endothelial damage in this condition [18]. Several studies reported that the vascular complications of DM are dependent on hyperglycemia [4]. Clinical research suggests that the homeostasis of trace elements can be disrupted by DM [19]. Conversely early imbalances of certain elements also disturb normal glucose and insulin metabolism [20].

Plenty of literature showed Cu as cofactor and a dangerous reactant that generates hydroxyl radical [21]. All cells in human body require Cu for their full metabolic needs and the potential toxicity of Cu demands an exquisite level of transport and homeostatic control [7, 21]. Abnormal Cu metabolism can lead to several pathogenesis such as diabetes or its further complications. Similar to the studies of Cooper et al. and Walter et al. [22, 23], our findings of increased copper levels in patients when compared with controls were found significant (P<0.001) with a positive correlation between all parameters of diabetes. The concentration of Cu tends to rise as the illness progress towards macrovascular disease, retinopathy and hypertension. Also high plasma Cu levels have been found in other diseases such as lymphatic

Table I. Mean ± SD levels of serum sugar, trace elements and urinary albumin in diabetic nephropathy patients and controls.

Parameters	Diabetic Nephropathy	Controls	P value
Age	60.2 ± 12.8	56.5 ± 12.8	>0.19
Fasting Glucose (mg/dl)	162.8 ± 21.9	98.3 ± 7.4	<0.001
Post parandial Glucose (mg/dl)	267.7 ± 41.6	156.2 ± 21	<0.001
Microalbumin (mg/L)	29.4± 5.16	16.0 ± 1.17	<0.001
Copper (mg/dl)	278.1± 25.4	156.43 ± 20.2	<0.001
Magnesium (µg/dl)	1.0 ± 0.17	1.97 ± 0.21	<0.001

Table II. Cu levels correlation with fasting blood sugar (FBS), post parandial blood sugar (PPBS) and microalbumin.

	FBS	PPBS	Microalbumin
Correlation Coefficient	0.22	0.24	0.34
t value	1.49	1.64	2.38
Interpretation	(+) Correlation	(+) Correlation	(+) Correlation

Table III. Magnesium levels correlation with fasting blood sugar (FBS), post parandial blood sugar (PPBS) and microalbumin.

	FBS	PPBS	Microalbumin
Correlation Coefficient	-0.05	-0.16	-0.45
t value	-0.35	-1.07	-3.38
Interpretation	(-) correlation	(-) correlation	(-) correlation

leukemia, inflammation, atherosclerosis [23].

Mg is the fourth abundant cation in human body which involves in many fundamental and biological processes. Mg deficiency may cause endothelial dysfunction, inflammation, oxidative stress [24] also it predispose a person to glucose intolerance and to promote the development of diabetic complications [25]. Like other studies [8, 26], it observed that serum Mg levels were significantly lower in diabetic nephropathy patients as compared to controls ($P<0.001$).

Diabetes is associated with macrovascular and microvascular complications. Continuous high serum Cu levels and low serum Mg levels lead to more worsen the clinical complications. Estimation of the serum levels of Cu and Mg should be done as the routine test in diabetic patients for an early prevention to minimize the risk of developing micro- and macrovascular complications.

CONCLUSION

Progression of microalbuminuria is associated with poor glycemic control. Continued high levels of serum Cu and low serum levels of Mg lead to more distressing clinical complications, including retinopathy, hypertension and microvascular disease. Evaluation of serum Cu and Mg should be included in routine testing.

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