Correlation of serum zinc, magnesium and copper with HbA1c in type 2 diabetes mellitus patients among Bagalkot population-A case control Study

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Abstract

Background: Diabetes is a major worldwide health problem. It is characterised by chronic hyperglycemia resulting from a diversity of aetiologies and environmental and genetic factors acting together. Type 2 diabetes mellitus is associated with increased metabolic processes and oxidative stress. The trace elements are important co-factors in these events.

Objectives: The study was designed to estimate the serum zinc, magnesium and copper and glycosylated hemoglobin (HbA1c) in patients with type 2 diabetes mellitus and healthy controls. A correlation of serum zinc, magnesium and copper with glycosylated hemoglobin (HbA1c) is done in diabetic subjects.

Material and methods: The study included 50 patients of type 2 diabetes mellitus and 50 age and sex matched controls. Fasting venous blood sample was analysed for fasting blood glucose(FBG),serum zinc, serum magnesium, serum copper and glycated hemoglobin (HbA1c). Statistical analysis was done using student 't' test .Pearson's correlation between the study variables was performed to establish the relationship.

Results: The FBG, serum copper and HbA1c levels were significantly elevated in diabetics compared to healthy controls ($p<0.001$). There was significant decrease in the levels of serum zinc and magnesium levels in diabetics compared to the controls($p<0.01$).There is a positive correlation between serum copper with HbA1c ,while there a highly significant negative correlation between serum zinc and magnesium with HbA1c in diabetic patients.

Conclusion: Diabetic patients showed a positive correlation between serum copper with HbA1c and a negative correlation between serum zinc and magnesium with HbA1c.

Key words: Type 2 diabetes mellitus, glycated hemoglobin, zinc ,magnesium, copper.

Introduction

Diabetes mellitus (DM) is a group of metabolic disease characterised by increase blood glucose level resulting from defects in insulin secretion, insulin action or both[1]. The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and failure of various organs especially the nerves, eyes, kidneys ,heart and blood vessels[2]. Diabetes mellitus is one of the greatest medical problems threatening the world. With the worldwide explosion in its prevalence, type 2 DM has turned into global epidemic[3]. According to the recent estimates the prevalence of diabetes mellitus is 4 % worldwide and that indicates 143 million persons are affected this will increase to 300 million by the year 2025[4]. Many hypothesis have been proposed to explain the pathogenesis of type 2 diabetes mellitus that connects the disease to a state of subclinical chronic inflammation[5]. Metabolically triggered inflammation has been proposed as a key step in the pathogenesis of type 2 DM[6].

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Insulin resistance is the primary event and is followed by increased dysfunction in the type 2 diabetes. Insulin resistance often accompanies excess visceral adiposity, dyslipidemia, hypertension, impaired fibrinolysis, increased platelet aggregation, vascular inflammation, endothelial dysfunction and premature atherosclerosis[7]. The metabolism of several minerals has been reported to alter in diabetes mellitus and these elements might have specific role in the pathogenesis and progress of the disease. Among these the trace elements-magnesium, zinc and copper are important for the growth and biological functions. They act as cofactors for many metabolic reactions and play a vital role in basic cellular reactions required to maintain energy production and life[8]. Magnesium is the fourth most abundant cation in the body and second in the intracellular environment. It takes part in more than 300 enzymatic reactions. Magnesium plays a vital role in glucose homeostasis. It is a cofactor in phosphorylation of glucose and helps in carbohydrate metabolism[9].

Zinc, an essential element is useful in the synthesis, storage and secretion of insulin. Zinc is a component of many enzymes .The function of zinc in the body metabolism is based on its enzymatic affinity, way of a zinc enzyme complex or metalloenzyme[10]. Zinc plays a vital role in maintenance of several tissue function and has an important role in modulating the immune system[11]. Copper is one of the essential trace elements and has a particular role in cytochrome oxidase function at the terminal end of mitochondrial electron transport chain .The loss of this activity may contribute to the characteristic swelling and distortion of mitochondria which can be observed in copper deficiency particularly in metabolically active tissues such as pancreatic acinar cells,enterocytes and hepatocytes[12]. Persistent hyperglycaemia causes glycosylation of the proteins especially hemoglobin[13]. Haemoglobin glycation, estimated by percentage of glycated haemoglobin (HbA1c) was first used clinically 30 years ago to assess the degree of chronic hyperglycaemia among diabetic patients in whom values reflect weighted mean glucose levels over the preceding 3 months period; it is useful for characterizing dysglycemia in population studies because it is simpler to perform than the oral glucose tolerance test (GTT[14]. In Diabetics an increase in HbA1c of 1 percent was associated with a 20 % to 30% increase in mortality associated with cardiovascular events[15].

Aim of the study. The present study was done to determine

1. The levels of serum magnesium, zinc, copper and HbA1c in patients with type 2 diabetes mellitus and compare with healthy subjects.
2. The correlation of serum magnesium, zinc and copper with HbA1c in patients with type 2 diabetes mellitus.

Material and methods

Study Participants: The present study comprises 50 patients with Non-Insulin dependent diabetes mellitus (NIDDM) reporting to Hanagal Shree Kumareshwar Hospital and Research Centre. The criteria for the diagnosis of DM was according to the criteria of the American Diabetes Association (ADA) 2007 guidelines[16].

50 subjects of similar age, sex and socioeconomic status served as controls. The controls were free from any major aliment which could affect the parameters under study (the clinical history or investigative results showed no involvement of any organ). The exclusion criteria included type 1 DM, Gestational diabetes, other specific causes for diabetes, micro- and macro-vascular complications like frank proteinuria detected by albustix, neuropathy, nephropathy and retinopathy. Patients on diuretics, receiving magnesium supplements, taking drugs that affect blood glucose levels, cardiac, infectious and inflammatory disease were also excluded.

Informed written consent was obtained from all the subjects enrolled for the study. Institutional ethical committee clearance was obtained for the study. The study was conducted from Jan 2009 to Dec 2009. A detailed history was taken to know duration of the disease, treatment history and any complication of the disease.

All the subjects underwent clinical examination including anthropometric measurements. The anthropometric measurements comprised of waist circumference, height, body weight and body mass index (BMI) was calculated as weight in Kg/height in m^2. The waist circumference was

determined by applying a tape measure to the midpoint between the inferior margin of the last rib and the crest of the ileum.

Biochemical analysis-The fasting blood sample, 2 ml in fluoride bulb for sugar estimation and 5ml in plain bulb for the estimation of copper, zinc, magnesium and HbA1c was collected from the cubital vein with aseptic precaution. It was allowed to clot and serum was separated by centrifugation at 3000 rpm for 10 minutes.

The following parameters were studied.

1. Fasting blood glucose –Enzymatic, GOD-POD, end point colorimetric method single reagent chemistry.(Trinder P and Teitz N W by autospan kit method).
2. Serum Zinc-NITRO-PAPS method(kit supplied by Tulip diagnostics).
3. Serum Magnesium- XYLIDYL BLUE method(Kit by Raichem Diagnostics).
4. Serum Copper-Di-Br-PAESA method(Kit by Coral Diagnostics)

   All the parameters read using semi auto analyser (STAT FAX 3300).
5. HbA1c was estimated by Nycocard reader II.

All the values were expressed as Mean ±SD

The statistical analysis was done using student 't' test for comparison between two groups and a p-value of <0.05 was considered statistically significant. Pearson's correlation between the study variables was performed to establish the relationship.

Results

The descriptive characteristics and the glycemic status of the control and diabetics subjects are shown in table 1. There was no statistical significant difference seen in age and height of controls and diabetic patients (p>0.05). The weight, WHR, BMI, levels of fasting blood glucose and

HbA1c in diabetes mellitus patients were significantly elevated in comparison to healthy controls (P<0.001) (Table 1).

The levels of serum copper showed statistically significant elevation in DM subjects compared to control subjects (P<0.01). The level of serum zinc and serum magnesium was statistically significantly decreased in diabetes mellitus patients compared to healthy controls (P<0.001) (Table 2).

In Diabetic patients the levels of serum zinc and serum magnesium showed anegative correlation with HbA1c which was statistically highly significant (r = -0.56 and r= -0.61, p<0.001).While the levels of serum copper showed a positive correlation with HbA1c which was statistically highly significant (r=+0.59, p<0.01)

Discussion

Diabetes is a complex and multifactorial disease**.**

The metabolic dysregulations associated with diabetes causes secondary pathophysiologic changes in multiple organ systems that impose a heavy burden of morbidity and mortality from macro vascular and micro vascular complications[17].

Table 1. Comparison of the descriptive characteristics and glycemic status between controls and diabetic subjects

Parameters	Control n=50 (mean ± SD) 38/12(M/F)	DM n=50 (mean ± SD) 29/21(M/F)	p value	Statistical significance
Age(years)	48.63±19.51	50.61±23.71	0.1851	NS
Height(cms)	166.58±8.91	167.12±6.34	0.2593	NS
Weight(kg)	77.91±41.72	82.86±38.75	0.0297	SS
WHR	0.92±0.18	1.08±0.30	0.0232	SS
BMI(kg/m2)	26.42±5.02	29.93±6.52	0.0271	SS
FBG(mg/dl)	95.40±6.82	135.60±9.45	0.001	SS
HbA1c(%)	5.82±0.52	7.81±1.39	0.001	SS

BMI-body mass index, WHR-waist hip ratio, FBG-fasting blood glucose, HbA1c-glycosylated haemoglobin, NS-not significant, SS-statistically significant

Table 2. Comparison of serum zinc, serum magnesium and serum copper levels in controls and diabetic subjects

Parameters	Control (mean ± SD) 38/12(M/F)	DM (mean ± SD) 29/21(M/F)	p value	Statistical significance
Serum zinc(µg/dl)	89.61±27.74	67.50±13.85	P<0.001	HS
Serum magnesium(mg/dl)	2.43±0.42	2.01±0.39	P<0.001	HS
Serum copper (µg/dl)	89.92±48.61	136.52±31.74	P<0.01	HS

HS-highly significant

Table 3. Correlation of serum zinc, serum magnesium and serum copper levels with HbA1c in diabetic subjects

Correlation between	Pearson's correlation coefficient (r)	p value	Statistical significance
Serum zinc and HbA1c	-0.56	P<0.001	Highly significant negative correlation
Serum magnesium and HbA1c	-0.61	P<0.001	Highly significant negative correlation
Serum copper and HbA1c	+0.59	P<0.01	Highly significant positive correlation

Oxidative damage due to free radicals is also found to be associated with vascular disease in diabetics[18]. Insulin resistance is a common finding in elderly people and as such ageing is associated with an impaired glucose handling. Reduction in renal function have also been associated with ageing. Decline in the physiological functions with age may influence the absorption, excretion and metabolism of micronutrients[19].

HbA1c reflects average plasma glucose over the previous 8 to 12 weeks. It can be performed at any time of the day and does not require any special preparations such as fasting .These properties have made it the preferred test for assesing the glycemic control in people with diabetes . More recently there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes[20]. The trace elements play a vital role in different metabolic processes in the body. Zinc has many antioxidant properties and has been suggested that chronic zinc deprivation may result in increased sensitivity to oxidative stress. Zinc acts as a cofactor for insulin, although its exact mechanism in carbohydrate metabolism is yet not clear[16]. HbA1c levels rise with the poor control of diabetes mellitus. There was a significant negative correlation of serum zinc with HbA1c. The serum zinc levels are decreased in diabetics may be due to increased urinary excretion due to reduction in renal function associated with disease, gastrointestinal malabsorption or genetic factors or signs of infection during which zinc acts as a defence mechanism[21]. There is concurrent hypozincemia and decrease in the tissue zinc stores[22]. However abnormal zinc metabolism has been suggested to play a role in the pathogenesis of diabetes and its complications[21].

There was a significant negative correlation of serum magnesium with HbA1c. The reason for significant decreased magnesium in diabetic compared to controls may be due to higher urinary losses or impaired absorption of magnesium. The decrease in serum magnesium may also be due to magnesium depletion caused by osmotic diuresis and by indirect hormonal effects[23].It is believed that diabetic state impairs the renal tubular reabsorption of magnesium from the glomerular filtrate[9]. Hypomagnesaemia represents a risk factor in diabetic retinopathy and tends to decrease the hypoglycemiant action of insulin[24]. The levels of serum copper was significantly increased in diabetics compared to controls, there is a strong positive correlation between copper and HbA1c. In diabetics there are several potential sources of

increased free radical production including the auto-oxidation of plasma glucose and increased transition metal bioavailability[25]. The increase in the Cu ion in patients with DM might be attributed to hyperglycemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress so that the formation of AGEs occurs[26]. In addition there is production of highly reactive oxidants that can lead to tissue damage.

Conclusion

In the present study there was a negative correlation in the serum levels of zinc and magnesium with HbA1c in diabetics. The serum copper showed a positive correlation with HbA1c in diabetics. These may be one of the factors for reducing the insulin sensitivity and may increase the risk of secondary complications such as retinopathy, CAD, ketoacidosis and so on. However further studies are needed to be carried out to determine the molecular role of zinc, magnesium and copper in the development of diabetic complications.

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