Study of serum copper and zinc in diabetic retinopathy and its correlation with glycemic status

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Abstract

Background: Diabetes Mellitus (DM) is a global public health problem. It is associated with increased level of free radicals which lead to oxidative stress and dangerous complications like retinopathy. Trace elements have specific role in progress to complications of DM.

Objectives: The study was designed to estimate and   compare the levels of serum copper and zinc among type-2 DM with and without retinopathy and to compare them with matched healthy controls. A correlation of serum copper and zinc was done with glycosylated haemoglobin (HbA1c) in patients of type 2 DM with retinopathy.

Materials and Methods: It was a case control study. The investigation included 50 cases of type 2 DM with retinopathy and 50 cases of type 2 DM without retinopathy and 50 matched healthy controls. Venous blood sample was analysed for fasting blood sugar(FBS), post prandial blood sugar (PPBS), serum copper, serum zinc and glycosylated haemoglobin(HbA1c) in both cases and controls. Statistical analysis was done using student 't' test. Pearson's correlation was performed to establish the relationship between study variables.

Results: There was statistically highly significant increase in serum copper levels in cases of retinopathy compared to controls(p<0001) and significantly decreased levels of serum zinc in cases of DM with retinopathy(p<0.001). There was significant positive correlation between serum copper with HbA1c and highly significant negative correlation between serum zinc with HbA1c in cases of retinopathy.

Conclusion: Diabetic retinopathy is influenced by various factors such as glycemic status, trace elements such as copper, zinc which correlated well with HbA1c. Hence these parameter can be used as a supportive diagnostic tool.

Keywords: Diabetic retinopathy, Type 2 DM, Copper, zinc, glycosylated hemoglobin

Introduction

Diabetic retinopathy is one of the leading causes of acquired blindness in adults. Several risk factors are related to the development and progression of retinopathy such as the duration of DM, poor glycemic control, dyslipidemia, hypertension and trace elements[1,2]. Poor glycemic control plays an important role in the development and progression of retinopathy and nephropathy with associated increase in morbidity and mortality[3]. Several studies in this field have suggested a strong association between level of hyperglycemia and the progression of microvascular complications in diabetic patients[4]. Strong evidence exists that improved glycemic control is effective at lessening the risks of retinopathy, nephropathy and neuropathy in diabetes[5].

The Wisconsin eye study showed that the incidence and progression of retinopathy was related to the glycemic status of patients and also showed significant reduction of progression of retinopathy with improvement in glycemic control[6]. The prevalence of diabetic retinopathy is about 34% in Indian studies[7].

Apart from the elevation of inflammatory markers, direct association of elements in type 2 DM with retinopathy has been observed in many research studies. The metabolism of several minerals has been reported to alter in diabetes mellitus and these elements might have specific roles in the pathogenesis and progress of this disease. Of these minerals copper and zinc are the important ones. Studies on these parameters have been done in many other countries but a very few in India. Hence, a study on these parameters is essential in clinically diagnosed type 2 DM with retinopathy patients to show their role in pathogenesis and to ascertain their role as biochemical marker.

Copper(Cu) plays an important role in our metabolism, largely because it allows many critical enzymes to function properly[8]. It is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Copper can act as both an antioxidant and a pro-

oxidant. When copper acts as a pro-oxidant at times, it promotes free radical damage. Maintaining the proper dietary balance of Cu, along with other minerals such as zinc and manganese, is important[9].

Copper 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[10].

Zinc, an essential element, is useful in 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 zinc metalloenzyme[11]. It plays an important role in the maintenance of several tissue functions. Zinc has been found to enhance the effectiveness of insulin in vitro, and it has been postulated that zinc deficiency may aggravate the insulin resistance in non-insulin dependent diabetes mellitus with complications. Zinc has an important role in modulating the immune system and its dysfunction in diabetes mellitus with retinopathy may be related in part to the status of zinc 12. Many studies have shown that serum zinc levels are lower in type 2 DM patients with retinopathy.

Materials and methods

This was a case control study .The study was carried out on 50 cases of clinically diagnosed type 2 diabetes mellitus with retinopathy, 50 cases of type 2 diabetes mellitus without retinopathy attending the Opthalmology OPD at SNMC and HSK hospital, Navanagar, Bagalkot. Fifty(50) age and sex matched healthy subjects were taken as controls. The study was conducted over a period of one year from Jan 2013 to Dec 2013. Ethical clearance was obtained from the institute's ethical clearance committee. Informed consent was taken from the cases and controls after explaining the procedure. Diabetes Mellitus was diagnosed as per the WHO diagnostic criteria[13]. Retinopathy was diagnosed by fundus examination under mydraisis of both eyes using opthalmoscope and 90 D lens[14].

Patients of DM with other microvascular complications, individuals with severe inflammatory diseases, infections, cardiac, hepatic or renal diseases and those on drugs that would affect blood glucose levels were excluded from the study. Patients on diuretics, those with metabolic disorders such as ketoacidosis, those with malabsorption or chronic diarrhea, pregnant and lactating women were also excluded.

Based on the analysis of medical history, clinical examination and investigation results, the patients were grouped into type 2 DM with and without retinopathy.

Biochemical analysis

Venous blood sample was collected from antecubital vein under all aseptic precautions in both fasting and post prandial state. It was allowed to clot and serum was separated to analyse following parameters. All tests were done in stat fax 3300 semi-automated analyser.

a) FBS and PPBS –Glucose oxidase Peroxidase method[15,16].(GOD-POD). (kits supplied by Erba Diagnostics)
b) Serum Zinc-NITRO-PAPS method[17]. (kit supplied by Tulip diagnostics).
c) Serum Copper-Di-Br-PAESA method-colorimetric method[18]. (Kit by Coral Diagnostics).
d) HbA1c was estimated by Nycocard reader II[19].

Data was analyzed by statistical tests by using SPSS package version number 19. Data was expressed in terms of mean ± SD. Chi- square test was applied to estimate the difference between the two groups of population. Unpaired 't'-test was used to study the changes in levels of serum copper and zinc. Pearson correlation between the study variables was performed to establish the relationship. p value < 0.05 was taken as statistically significant.

Results

This was a comparative case control study conducted on 50 cases of type 2 DM (n=50) with retinopathy, 50 cases of type 2 DM without retinopathy (n=50) and 50 healthy controls(n=50). The age and gender distribution is shown in **Table 1** and **Table 2** respectively which show that there is no statistically significant difference in age and gender in between cases and controls.(p>0.05).

The FBS, PPBS and HbA1c levels in the study groups is shown in **Table 3** .The FBS, PPBS, and HbA1c levels showed statistically significant elevations in cases as compared to controls.(p<0.001).

Serum copper: The mean serum copper(μg/dl) in cases of retinopathy was 238.1±27.39, in cases without retinopathy was 166.3±24.35 and in controls was 106.4±19.03 and was highly significant. (p < 0.0001) (**Table 4, Figure 1**).

Serum zinc: The mean serum zinc levels (μg/dL) in cases of retinopathy were 50.5 ± 11.9, in cases without retinopathy were 67.50 ±13.85 and in controls were 90.16 ± 19.4 respectively and it was highly significant. (p < 0.0001)(**Table 4**)

Serum copper and HbA1c: There was positive correlation between serum copper and HbA$_1$c (r = + 0.189, p < 0.0001) and was highly significant. (**Table 5, Figure 2**)

Serum zinc and HbA1c: There was negative correlation between serum zinc and HbA$_1$c (r = - 0.57, p < 0.0001) and was highly significant. (**Table 5, Fig. 3**)

Table 1: Age distribution of cases and controls

Age in years	Type 2 DM with retinopathy		Type 2 DM without retinopathy		Controls	
	N	%	N	%	N	%
≤ 35	1	2	0	0	0	0
36-45	17	28	23	39	17	28
46-55	17	28	17	28	22	37
56-65	18	30	17	28	20	33
≥ 66	7	12	3	5	1	2
Total	60	100	60	100	60	100

DM-Diabetes Mellitus

Table 2: Gender distribution of cases and controls

Gender	Diabetic with retinopathy -n (%)	Diabetic without retinopathy -n (%)	Controls- n (%)
Male	31(52)	33(55)	30(45)
Female	29(48)	27(45)	30(45)

X^2= 4.165, p=0.38, Not significant

Table 3: Comparison of FBS, PPBS, and HbA1c in study groups

Study variables	Cases with retinopathy	Cases without retinopathy	Controls	P value
FBS (mg/dL)	196.50 ± 66.5	187.48 ± 67.9	93.46 ± 13.72	0.001
PPBS(mg/dL)	297.90 ± 80.32	280.90 ± 84.23	122.18 ± 10.20	0.001
HbA1c(%)	8.6 ± 1.3	8.2 ± 1.02	5.30 ± 0.63	0.001

FBS-Fasting Blood Sugar, PPBS-Post Prandial Blood Sugar, HbA1c-Glycosylated haemoglobin

Table 4: Comparison of serum copper (µg/dl) and serum zinc levels (µg/dl) between cases and controls

	Diabetic with retinopathy	Diabetic without retinopathy	Controls
serum copper (µg/dl)	238.1±27.39	166.3±24.35	106.4±19.03
Serum zinc (µg/dl)	50.5 ± 11.9	67.50 ±13.85	90.16 ± 19.4

Table 5: Correlation between HbA1c, serum Copper and serum zinc in cases of diabetic retinopathy

Correlation between	Pearson's Correlation Coefficient(r)	Significance	
Serum copper and HbA1c	+ 0.189	p < 0.001	Highly significant positive correlation
Serum zinc and HbA1c	-0.57	p < 0.001	Highly significant negative correlation

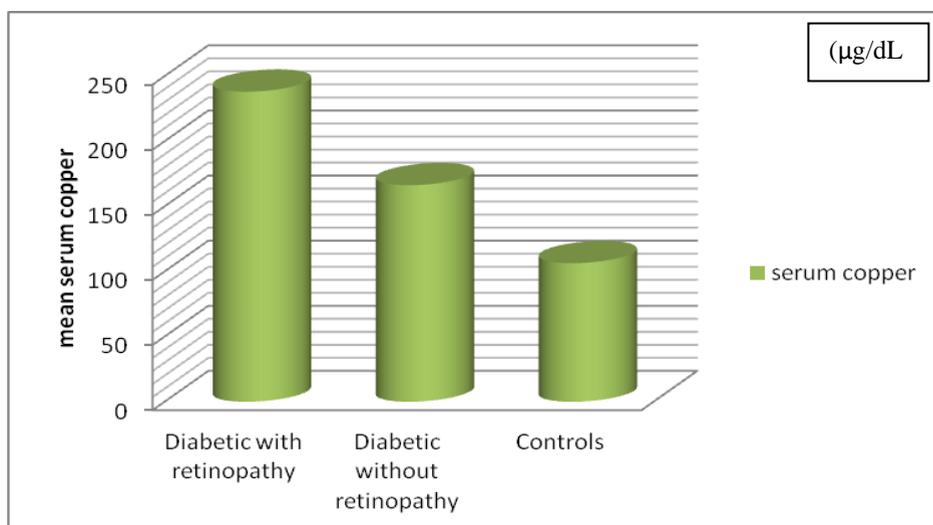HbA1c-glycosylated haemoglobin

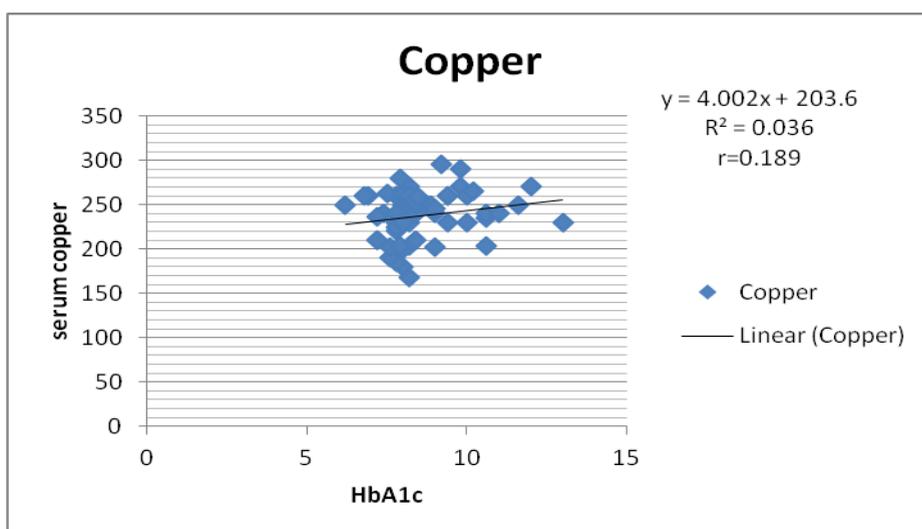**Fig. 1: Comparison of serum copper (μg/dl) levels between groups**

Fig. 2: Correlation between HbA1c and serum Copper in cases of diabetic retinopathy
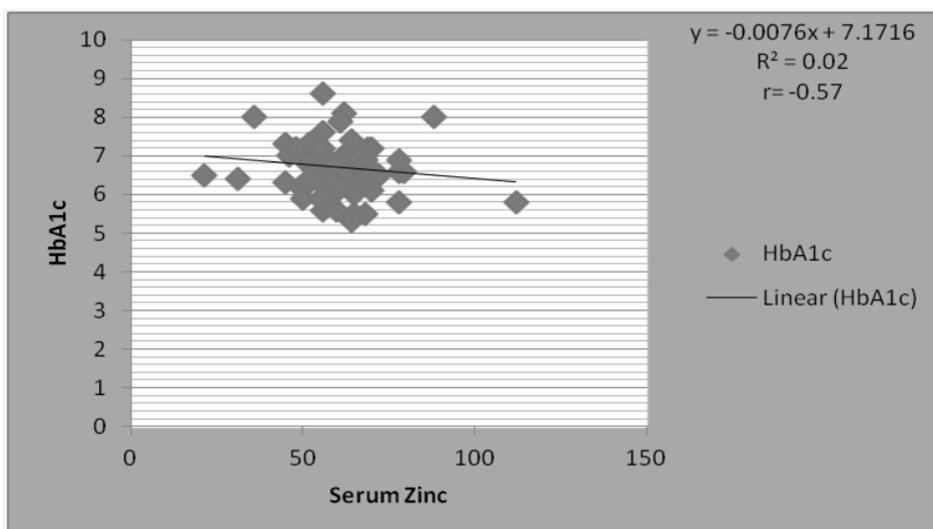

Fig. 3: Correlation between serum zinc and HbA$_1$c levels in cases.

Discussion

Diabetic retinopathy is a important micro-vascular complication of uncontrolled diabetes mellitus and is one of the leading causes of acquired blindness. It is caused by microangiopathy affecting retinal arterioles, venules and capillaries by both microvascular leakage and occlusion. Oxidative damage due to free radicals is associated with vascular disease in people with Type 1 and Type 2 diabetes mellitus[18]. There are several potential sources of increased free radical production in DM including autooxidation of plasma glucose and increased transition metal biovailability. The radical – scavenging anti-oxidant activity of the serum of people with either Type 1 or 2 DM is lower than that of age-matched controls. This may be attributed to the trace elements[19].

In our study, serum copper levels were significantly increased in cases of retinopathy than controls. A high level of copper enhances the toxic effect of metal dependent free radicals. Moreover the increase in copper levels in patients with type 2 DM with retinopathy might also be attributed to hyperglycaemia, which stimulates glycation and causes release of copper ions from copper binding sites of proteins. The release of copper ions into blood further accelerates the oxidative stress. Ceruloplasmin and serum albumin are the main Cu binding proteins in plasma and there is some evidence that chronic hyperglycemia can damage the Cu binding properties of both[20,21]. Copper, bound to glycated proteins, may blunt normal EDRF dependent relaxation of diabetic arteries and provide a rationale for the use of transition metal chelators in therapy of diabetic vasculopathy and neuropathy[22].

In the current study, we found highly significant positive correlation between serum copper and HbA$_1$c levels.(r= +0.189, p<0.0001). Studies by Meenakshi P, Uma G et al observed that the glycated proteins seen in type 2 DM patients with complications have an increased affinity for copper leading to hypercupremia.

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 [23]. In our study the mean HbA$_1$c values were 8.6±1.31 in cases of diabetic retinopathy, 8.22 ± 1.02 in cases of diabetes without retinopathy and 5.5 ± 0.63 in controls respectively which is statistically highly significant (p<0.001). In cases of type 2 DM, HbA1c values were higher which correlated well with the clinical diagnosis. The values in this study are in accordance with the several studies[24,25].

In our study, serum zinc levels were significantly decreased(p<0001) in cases of retinopathy than controls. Previous studies by others[26,27,28,29] also found similar results. There was highly significant negative correlation between serum zinc and HbA$_1$c levels in our study with r=-0.57,p<0.0001.

The decreased zinc, which affects the ability of the islet cells of pancreas to produce and secrete insulin, might also lead to the development of insulin resistance compounding the problem, particularly in type 2 DM[30,31]. Zinc is an activator of fructose-1-6-bisphosphate aldolase, and an inhibitor of fructose-1-6-biphosphatase[32]. Zinc affects the antigenic properties of insulin and the binding of insulin to hepatocyte membranes and a deficiency can lead to increased insulin resistance and hyperglycemia. Elevated glucose in turn produces hyperzincuria. There is concurrent hypozincemia and decrease in the tissue zinc stores[33]. The predominant effect of diabetes on zinc homeostasis is hypozincemia, which may be the result of hyperzincuria or decreased intestinal absorption of zinc or both[34].

Conclusion

Serum copper levels were significantly increased and serum zinc levels were significantly decreased in diabetic retinopathy thus suggesting the role of inflammation, oxidative stress in the development and progress of complications of diabetes. Elevated serum copper correlated significantly with HbA1c in Diabetic Retinopathy as compared to control diabetic group. Consequently, considering the possible modulating effects of zinc on insulin sensitivity and its antioxidant functions, it was postulated that a restored zinc status in individuals with type 2 DM with retinopathy might counteract the deleterious effects of oxidative stress and help to prevent further progress of complications associated with diabetes.

Further studies on a larger sample are needed to substantiate our findings before firm conclusion can be drawn on the utility of these parameters for the diagnostic assessment

Acknowledgement

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