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Research Article

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Copper, Zinc levels of newly diagnosed type 2 Diabetic patients are better marker in RBC than in Plasma

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Abstract

One of the greatest medical problems threatening the world today is Diabetes Mellitus. Aims of this study were to compare plasma, RBC Cu and Zn between healthy and newly diagnosed type 2 diabetic patients, also association of hyperglycaemia, dyslipidaemia with plasma and RBC Cu, Zn. Study was carried out in departments of Biochemistry jointly with Endocrine Medicine of Bangabandhu Sheikh Mujib Medical University. Thirty three newly diagnosed type 2 DM, thirty one age and sex matched healthy controls were included. Both Cu and Zn were measured by atomic absorption spectrophotometer. The median value of plasma Cu in healthy controls was 942.00 ppb (ranging 846-1393.50 ppb), in newly diagnosed type 2 diabetic patients, 2739.00 ppb (ranging 1400- 5743.50ppb). Significantly higher level of plasma Cu was observed in cases ($p<.001$). Median value of RBC Cu of healthy controls was 1067.50 (ranging 423-2810.5 ppb) whereas that of diabetic patient was 773.50 ppb (ranging 52.50-2765.00 ppb). RBC Cu was significantly lower ($p<.05$) than the healthy controls. Median values of plasma Zn of healthy controls and type 2 diabetic patients were 777.50 ppb (ranging 621.50- 1018.00 ppb) and 703.00 ppb (ranging 472-930 ppb), whereas that of RBC Zn of both groups were 6984.00 ppb (ranging 5693.50-7796.00 ppb) and 5155.50 ppb (ranging 2820- 6153 ppb) respectively. The plasma and RBC Zn were significantly lowered ($p<.001$) in diabetic group. There was significant positive correlation ($p<.05$) between fasting plasma Cu and glucose, significant negative ($p<.05$) correlation between fasting RBC Zn and triglyceride in type 2 DM. RBC is a better marker to see the trace element status than plasma.

Keywords: Cu, Zn, RBC, Type 2 DM

Abbreviations

RBC: Red Blood Cells
ENT: Ear Nose Throat
DM: Diabetes Mellitus
Cu: Copper
Zn: Zinc
SD: Standard Deviation
OCP: Oral Contraceptive Pills
BSMMU: Bangabandhu Sheikh Mujib Medical University
EDTA: Ethylenediaminetetra Acetic Acid
NaF: Sodium Fluride
SGPT (ALT): Alanine aminotransferase
2HAG: 2 Hrs After Glucose Load
SI: System Internationale
BMI: Body Mass Index
BIRDEM: Bangladesh Institute of Research and Rehabilitation of Diabetes, Endocrine and Metabolic Disorders
TG: Triglyceride
TC: Total Cholesterol
HDL-C: High Density Lipoprotein
LDL-C: Low density lipoprotein

Introduction

The total number of Diabetic patients is projected to rise from 171 million in 2000 to 366 million in 2030 worldwide. In Bangladesh highest numbers of estimated cases of Diabetes for 2000 was 3.2 million and predicted to be 11.1 million in 2030 [1]. According to World Health Organization- Diabetes country profiles 2016, prevalence of diabetes in Bangladesh is almost doubled since 1980 to 2014. Statistics shows 3% mortality only for diabetes (fig 1) among all deaths in all ages in Bangladesh [2]. Moreover mortality in 2016 was in males 6060 whereas in females 4760 within the age of 30-69 years [2]. So Diabetes is a burning health challenge not in only developed country but also developing world like Bangladesh as well. No doubt, Diabetes is a multifactorial disease. Besides genetic consideration, a number of factors contribute in its development.

Zinc is an essential trace element involved in the correct packaging and storage of insulin. The most common form of the Zn-insulin complex is a hexamer containing two zinc ions. Cu^{2+} ions also bind to monomeric insulin. The insulin cannot form complexes with zinc ions in circulation when there is low concentration of free Zn^{2+} [3].

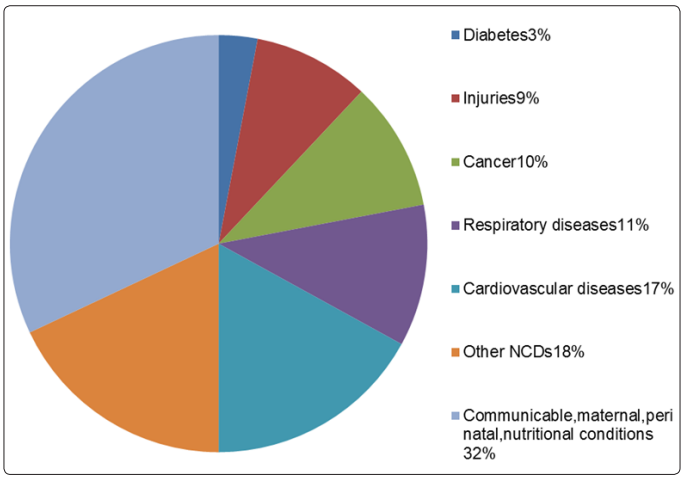


Figure 1: Proportional mortality (%Total Deaths All Ages) (WHO)

Fields et al in 1983 suggested that Cu like Zn may interact with insulin to form a complex which has a greater affinity for a receptor site than does insulin alone and /or increases the stability (decreases the degradation) of insulin [4]. The trace elements take part in a variety of enzymatic processes on a molecular level. Several of the complications of diabetes may be related to increased intracellular oxidants and free radicals associated with decrease in intracellular Zn and in Zn dependent antioxidant enzymes. There appears to be a complex interrelationship between Zn and both Type 1 and Type 2 diabetes [5]. Cu plays an important role in cytochrome oxidase function in mitochondria. Also, Cu deficiency results in subsequent disruption of mitochondria of metabolically active tissues like hepatocytes and pancreatic acinar cells [6]. McCarty and Rubin (1984) found that the presence of micronutrients in food, including Zn and Cu might significantly influence carbohydrate metabolism [7].

A number of studies have been undertaken with Cu and Zn levels in plasma in type 2 diabetic in Bangladesh and abroad. Several studies of Cu and Zn have been done in type 2 diabetic patient at the erythrocyte level abroad but unfortunately, there were no such studies in our type 2 diabetic patients. Therefore, the aims of the study were to see the RBC Cu and Zn in newly diagnosed type 2 DM together with the association of hyperglycaemia, dyslipidaemia with plasma and RBC Cu, Zn in Bangladeshi population.

Materials and Method

This was a case control study jointly carried out by the Department of Biochemistry and Endocrine Medicine of Bangabandhu Sheikh Mujib Medical University. A total of 64 non-smoker, non-alchoholic, normotensive clinically and biochemically free from other systemic illnesses were included in the study. Among them thirty three were newly diagnosed type 2 DM. Thirty one were age and sex matched healthy control. After ethical clearance from concerned authorities, all the subjects were informed details of the study. Only those who had given written consent, included in this study.

Subjects were divided into

Control subjects (Group I): According to WHO those who had no evidence of diabetes mellitus neither impaired glucose tolerance were taken as Control subjects.

Test subjects (Group II): The newly diagnosed untreated non-insulin dependent diabetes mellitus confirmed by oral glucose tolerance test were included in this group.

Inclusion criteria:

- Normotensive
- Non smoker
- Non alchoholic
- Free from acute or chronic systemic illness
- Normal BMI (18-24.99) and overweight (25-29.99) (8)

Exclusion criteria:

- Pregnant and lactating mothers
- Subjects taking drugs like steroids, minerals
- Underweight and obese
- Subjects suffering from Endocrine disorders like hypo/hyperthyroidism, Addison's/Cushing's syndrome.

After selecting the subjects, anthropometric measurements and clinical examinations were done at the Department of Biochemistry of BSMMU. Following an overnight fast, blood samples were collected within 8:00-8:30 AM from antecubital vein preferably. Six ml fasting blood samples were taken in a pretreated metal free plastic test tubes containing EDTA. Blood samples were separated into plasma and cellular components. Plasma was preserved at -35°C to test fasting glucose, creatinine, SGPT, lipid profile, Cu and Zn. Cellular components were taken, processed to haemolysate according to Raz and Havivi [9]. The haemolysate was also preserved at -35°C to measure RBC Cu and Zn. The second sample collected after 75 gm glucose load was preserved at -35°C after following same procedure to test glucose level. Cu and Zn level in plasma and RBC were measured by pyro- coated graphite furnace atomic absorption spectrophotometry. Others by biochemical procedures.

Statistical analysis: Parametric results were presented as mean±SD and student's unpaired "t" test was done. Nonparametric values expressed as medians with ranges and Mann-Whitney U tests were done for analysis. Correlation was analyzed between fasting plasma, RBC Cu and Zn with blood glucose and lipid profiles. Cu expressed, as 1ppb in SI unit level will be 0.0157 µmol/L, Zn level of 1ppb is equal to 0.0153 µmol/L in SI unit.

Results and discussion

In this study general characteristics like age, BMI, BP showed no significant differences among groups. Biochemically serum creatinine and SGPT were done to exclude renal and liver diseases besides clinical examinations. Both the parameters among groups showed no significance differences. Plasma glucose both fasting and 2HAG were significantly higher ($p<.001$) in cases than controls showed in Table I. Total cholesterol and total triglyceride in Table II were significantly higher ($p<.001$) in type 2 diabetic subjects than the healthy controls. But HDL-C and LDL-C did not differ significantly among groups. Trace elements levels in Table III showed nonparametric distributions. So the median values with ranges were expressed and Mann-Whitney U tests were done for analysis. Present study revealed the plasma Cu concentration in

healthy controls to be consistent with the reference plasma Cu levels in adults [10, 11]. Whereas RBC Cu were a bit higher than referred level [10]. The plasma and RBC Zinc were a bit deficient than the referred range [10, 11]. Bangladesh Agriculture Research Council did several studies on soil and plant Zn content and extent of Zn deficiencies. A range of Zn deficiencies has been identified in Bangladesh and it is natural that the people of Bangladesh will be in the deficient state [12].

Table I : Plasma glucose level (fasting and 2HAG) in different groups

Variable(Mean±SD)	Group-I(mmol/L)	Group-II(mmol/L)	P-value
F.Plasma glucose	4.53±.46	13.28±4.94	P<.001
Pl. Glucose 2HAG	5.78±.65	18.92±7.24	P<.001

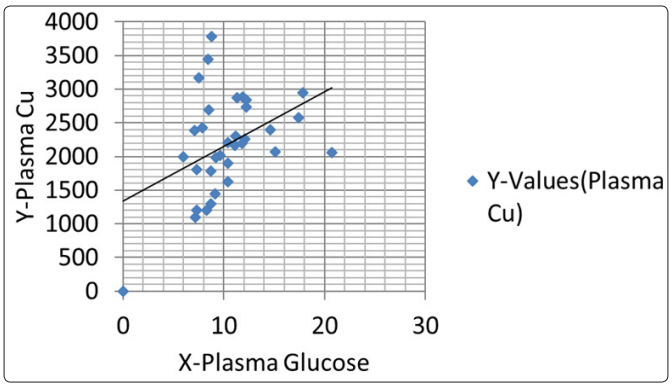
Table II : Serum lipid profile in different Groups

Variable (Mean±SD)	Group-I (mg/dl)	Group-II (mg/dl)	P-value
Total Cholesterol	146.94±23.21	171.64±29.90	P<.001
Total Triglyceride	98.87±39.69	198.90±102.05	P<.001
HDL-C	55.89±11.70	55.80±17.81	NS
LDL-C	71.43±26.63	77.47±26.70	NS

Table III: Trace elements levels in different groups

Variables		Group –I(ppb)	Group-II(ppb)	P-value
Plasma Cu	Median	942.00	2739.00	P<.001
	Range	846-1393	1400-5743.50	
Plasma Zn	Median	777.50	703.00	P<.05
	Range	621.50-1081.00	472.00-930.00	
RBC Cu	Median	1067.50	773.50	P<.001
	Range	423-2810.50	52.50-2765.00	
RBC Zn	Median	6984.00	5155.50	P<.001
	Range	5693.50-7796.00	2820.00-6153.00	

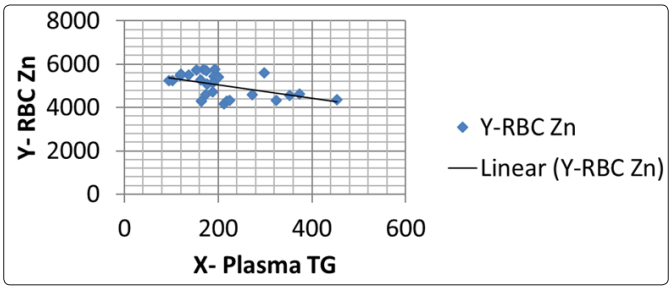
In type 2 diabetic subjects (group II), fasting plasma Cu was significantly (p<.001) higher than the group I individuals. This finding was consistent with previous work done by Mortuza in 1993 and consistent with other findings abroad [13-15]. The RBC Cu showed a wide range of distribution in-group II subjects with a statistically significant lower level (p<.05) than group I. This finding was consistent with the finding abroad [16]. These variable levels of Cu in RBC of diabetic patients despite the elevated plasma Cu levels were probably due to adaptive depletion and mobilization of Cu to peripheral tissues. All these derangements of trace elements lead to glucose intolerance. We found a statistical (p<.01) significant positive correlation between fasting plasma glucose and plasma Cu (fig 2). Martin Mateo et al in 1978 observed a correlation between blood glucose and fasting plasma Cu [16]. The level of plasma Zn in group II subjects was significantly (p<.001) lower than that of group I. This finding was consistent with previous work of Murtoza in 1993 and with the findings of abroad [16-18]. The RBC Zn of group II patients had significantly (p<.001) lower Zn level than group I which was again consistent with the findings of Chen MD et al [17]. Lowered Zn levels may be due to decreased GIT absorption and tissue specific deposition, moreover hyperzincuria in diabetic patients [17]. In a review of experimental studies, H.P. Roth and M. Kirchgessner showed zinc deficient rats had a diminished glucose tolerance, lowered insulin content and an elevated total insulin like activity. The reduced serum zinc concentration of the deficient animals did not change during glucose stimulation whereas it rose in case of the pair – fed controls. These studies showed clearly that nutritional zinc deficiency influences insulin metabolism and action [19]. Thus, Zn and Cu deficiency lead to glucose intolerance and insulin resistance as a fact hyperglycaemia results.



P = .0382
r = .3624

Figure 2: Correlation between Plasma Cu and Fasting plasma glucose

Study showed significant negative correlation (fig 3) between fasting serum TG and RBC Zn. It has been seen that Zn deficiency exerts a small stimulatory effect on hexose transport, which ultimately leads to stimulation of TG production. Moreover, Zn deficiency decreases all pathways of glucose metabolism influenced by insulin [20]. Thus, Zn deficiency aggravates insulin resistance in type 2 DM, together with alteration in lipid profiles [21].



P = .006
r^2 = .226

Figure 3: Correlation between RBC Zn and TG

The contents of plasma Zn did not correlate with the corresponding concentrations in skeletal muscle or circulating blood cells in diabetics seen in number of studies. Thus Sjogren et al concluded that plasma concentrations are not useful in the assessment of these electrolyte statuses [22]. Chen MD et al investigated the diabetics had lower Zn and higher Cu in their plasma and in erythrocytes both the Zn and Cu concentrations were lower [17].

Conclusion

Thus from the above study it is seen that trace elements deficiency leads to decreased insulin synthesis, secretion and resistance as

well with altered lipid profile levels which may lead to earlier development of diabetic complications like cardiac disorders. In conclusion, we can say that altered levels of trace elements Cu and Zn in both plasma and RBC found in diabetes mellitus, which may ultimately complicate its outcome. Moreover, RBC Cu and Zn levels give reliable information than plasma Cu and Zn levels.

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