Original Article

Relation between Copper and Duration of Diabetes Mellitus

Copper and Duration of Diabetes

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ABSTRACT

Objective: Diabetes is a heterogeneous disease characterized by relative or absolute insulin deficiency. Reports have shown that trace elements and minerals lead to the pathology of obesity and diabetes due to their participation in per oxidation and inflammation. So the present study is aimed to investigate the relationship between serum copper levels and duration of diabetic condition.

Study Design: Observational / descriptive study.

Place and Duration of Study: This study was conducted at the Department of Biochemistry, Liaquat College of Medicine and Dentistry and Darul Sehat Hospital, Karachi from __

Materials and Methods: For this purpose we have selected participants (n=120) on the basis of their history were divided accordingly into four groups; control group, diabetic duration 0-5years, diabetic duration 6-10 years, diabetic duration 10 years onwards. After monitoring their demographic parameters the blood sample was collected for the estimation of FBS, HBA1c and copper levels.

Results: Results have showed a significant rise in FBS, HBA1c and copper levels in all three diabetic groups compared to control (non-diabetic) individuals. Then correlation analysis has showed that levels of copper have a positive correlation with diabetic parameters indicating that copper levels were increased as the diabetic condition progresses.

Conclusion: Hence, blood copper levels are responsible for incidence and progression of diabetes mellitus. As the copper levels in blood are increasing gradually during diabetic condition.

Key Words: Copper, Diabetes, HBA1c

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INTRODUCTION

Diabetes mellitus is a chronic disease which is characterized by relative or absolute insulin deficiency or the ineffectiveness of the body to utilize the insulin it produces[1]. Many of the researches signifies that there is a direct relationship between macro and trace elements with diabetes.[2,3] Trace elements are essential for human life and they interact with vitamins and macro elements and enhance there effectiveness in the body[4]. It has been reported that the diabetics show impaired insulin release which ultimately affects the indices of trace elements. This study denote that the increased levels of trace elements were observed in diabetic subjects with clinical complications of hypertension, diabetic retinopathy and macro vascular diseases.

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On the contrary, the diabetic subjects with none of these clinical complications showed lower trace elements status. Trace elements and minerals lead to the pathology of obesity and diabetes largely due to their participation in per oxidation and inflammation**.** Significant alterations have been noticed in some of the trace elements in case of diabetes such as iron, copper, magnesium and macro element manganese.[5]

Among all the trace elements, an increase in the Copper (Cu) ion levels with diabetic complications may be credited to hyperglycemia which initiates glycation and in turn releases copper ions as shown in one of the studies which indicates significant rise in Cu levels in diabetics as compared to controls[6]. Another study performed in mice that were given copper supplements showed a decrease in serum blood glucose levels[7]. An increase or decrease in Cu levels is related to different clinical conditions including diabetes mellitus which is a disease that leads to the formation of free radical resulting in oxidative damage. Moreover most of the clinical conditions of excess of copper are due to oxidative damage to membranes or macromolecules.[8]

A study done on diabetes and its complications associated with different trace elements indicate an increase in HbA1c levels with lack of control for diabetes mellitus. An increase in copper levels with an increase in HbA1c levels shows how glucose is regulated and that Cu is a vital indicator of oxidative stress[9]. It is a fact that copper, zinc-superoxide

dismutase liberate copper ion when glucose concentration is raised above the normal levels indicating the reason of an increase in serum copper levels in diabetes. However the exact cause is still unidentified. In one of the studies, three parameters were studied i.e. retinopathy, macro-vascular disease and hypertension in diabetic subjects, their plasma levels of copper were administered and it was noticed that the plasma copper levels were significantly higher as compared to the subjects suffering from diabetes without these clinical conditions[10]. However inconsistent results have been noticed in case of diabetic patients who showed high and low serum and plasma levels of copper, in addition further investigations required to determine the relationship between copper and non-insulin dependent diabetes mellitus. So, the present study was designed to investigate the relationship between incidence of non-insulin dependent diabetes mellitus and plasma copper levels.

MATERIALS AND METHODS

Participants and sample collection: Total 120 participants took part in the study. This study was carried out at Liaquat College of Medicine and Dentistry and Darul Sehat Hospital Karachi; patients with DM type 2 were included in the study. The participants were differently grouped; the diabetics were divided depending upon duration of onset of diabetes and the controls into 4 equal groups (n=30);

Group 1: Controls

Group 2: DM with duration of less than 5 years,

Group 3: DM with duration of between 6-10 years and

Group 4: DM with duration of more than 10 years.

Subjects with DM2 were defined by fasting serum glucose >126 mg/dl or by having taken any hypoglycemic treatment. Studied patients were not taking any bone-active medication, hormone replacement therapy or insulin treatment, and gave their informed consent prior to joining this study. Study protocol was approved by ethics committee of the university. Anthropometric and demographic data including height, weight, age, gender, DM duration, history of coronary heart disease and medications, were recorded. Blood samples were collected following overnight fasting.

Biochemical Estimations: Blood was collected by venu-puncturing the fasting individuals by using an evacuated tube system. Glucose stability in plasma is affected by bacterial contamination, storage temperature and glycolysis. The fasting plasma glucose (FPG), hemoglobin A1C (HbA1c) levels were measured in all samples. Glucose measurements were recorded with glucose oxidase method while HbA1c was determined by automated kit on cobas integra provided by Roche.

- **HbA1c Estimation**

In order to eliminate interference from leukocytes, this process employs TTAB (tetradecyltrimethylammonium bromide) as detergent in the hemolyzing reagent. In order to remove labile HbA1c, pretreatment of sample is not needed. Soluble antigen-antibody complexes are formed by the reaction of anti-HbA1c antibody with glycohemoglobin in the sample. An insoluble antibody-polyhapten complex can be formed by reaction of surplus anti-HbA1c antibodies after the addition of R2 (polyhapten reagents), which can be measured turbid metrically. In the hemolyzed sample, liberated hemoglobin is transformed to a derivative which has characteristic absorption spectrum. It is measured bichromatically during the pre-incubation phase when R1 reagent is added to sample in the above mentioned immunological reaction. Before use, blood specimen and hemolyzing reagent for HbA1c are allowed to equilibrate at room temperature. In order to get a homogeneous mixture of erythrocytes, instantly mix the sample at a reasonable rate and take caution to avoid the formation of foam. The sample is diluted with hemolyzing reagent prior to pippeting. It is then mixed thoroughly after which hemolysate can be used as the solution has changed its color from red to brownish green.

- **Glucose Estimation:**

Ultraviolet testing using enzymatic reference method with hexokinase is performed. The phosphorylation of glucose to glucose-6 phosphate by Adenosine triphosphate is catalyzed by hexokinase. In the presence of NADP, glucose-6 phosphate dehydrogenase oxidizes the glucose to glucose-6 phosphate without oxidation of any other carbohydrate. The rate of NADPH formation is measured photo-metrically and is directly proportional to concentration of glucose.

- **Copper Estimation:**

Copper was estimated by a direct colorimetric test which is based on formation of a stable color complex when copper is released from ceruloplasmin complex and forms complex with 3-5 Di Br-PAESA in buffered medium (At pH=4.70). Serum sample (50 µL) was mixed with 1ml working reagent mixture and absorbance was noted immediately at 582nm then add coloring reagent (3, 5-DiBr-PAESA) (50 µL, 0.4 mmol/L) and incubate for 4-5 min at 37°C. After again read it 582nm and calculate the difference between both readings. Copper standard (100 µg/dL) was used as reference standard.

Statistical Analysis: The results are presented as mean ± SD for n=30 participants in each group. The statistical significant differences were evaluated by Tukey's test following one-way ANOVA using SPSS version 20. Correlation analysis between copper levels and HBA1c was performed using Pearson's correlation test via SPSS. Value of $p < 0.05$ was considered as a significant difference.

RESULTS

The present study investigates the correlation between diabetes and serum copper levels. Demographic data from patients of control Group and different durations of Diabetes mellitus i.e. group 1 (duration of diabetes 0 to 5 yrs.), group 2 (duration of diabetes 6 to 10 yrs.), group 3 (duration of diabetes 10 yrs. onwards) was obtained that includes the age (in years) of patients, body weight (in Kg) and systolic and diastolic blood pressure shown in table 1. Comparative analysis of this data shows that there are no significant differences in age, body weight and systolic and diastolic blood pressure between groups indicating that there is no variations in demographic parameters that can affect the biochemical parameters.

Table No.1: Demographic parameters for participants of control Group (Group 1) and patients of different durations of Diabetes mellitus [group 2 (duration of diabetes 0 to 5 yrs.), group 3 (duration of diabetes 6 to 10 yrs.), group 4 (duration of diabetes 10 yrs. onwards).

Variables	Group 1: Normal Control	Group 2: Diabetics Up To 5 Yrs Duration	Group 3: Diabetics 5-10 Yrs Duration	Group 4: Diabetics >10yrs
Age	53.56 ± 10.0	53.63 ± 9.30	56.26 ± 9.79	54.80 ± 9.55
Weight	71.23 ± 5.96	64.73 ± 7.36	68.60 ± 7.78	69.29 ± 8.68
Systolic. PR	151.00 ± 8.38	147.6 ± 10.23	149.4 ± 8.16	151.63 ± 6.61
Diastolic. PR	87.33 ± 3.67	88.63 ± 3.81	88.76 ± 3.89	88.56 ± 3.79

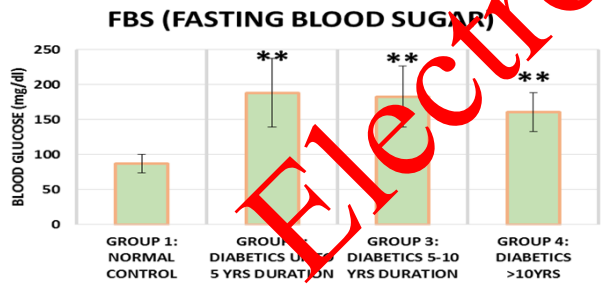

Figure No.1: Fasting blood sugar level in participants of control Group (Group 1) and patients of different durations of Diabetes mellitus [group 2 (duration of diabetes 0 to 5 yrs.), group 3 (duration of diabetes 6 to 10 yrs.), group 4 (duration of diabetes 10 yrs. onwards). Data are means ± SD (n= 30). FBS levels are expressed as mg per dL of blood. Data are analyzed for significant difference by One-way ANOVA followed by Tukey's post-hoc comparisons; **p<0.01 compared with control group.

In addition, the biochemical parameters confirming diabetic status were also determined that includes fasting blood sugar level (FBS) and HbA1c. Analysis of data presented in Fig. 1 shows that FBS was significantly increased (p<0.01) by 115%, 109% and 84% in group 2 (188±49.8 mg/dL), group 3 (182.83±44.75 mg/dL) and group 4 (160.47±28 mg/dL) respectively as compared group 1 (control group, 87.1±13.88 mg/dL). Analysis of data presented in Fig. 2 shows that HbA1c was significantly increased (F=27.472 p<0.01) by 71%, 64% and 78% in group 2 (8.41±1.87 %), group 3 (8.08±1.79 %) and group 4 (8.77±1.44 %) respectively as compared group 1 (control group, 4.91±0.989 %). Post-hoc analysis revealed that these increases are comparable in all three diabetic groups.

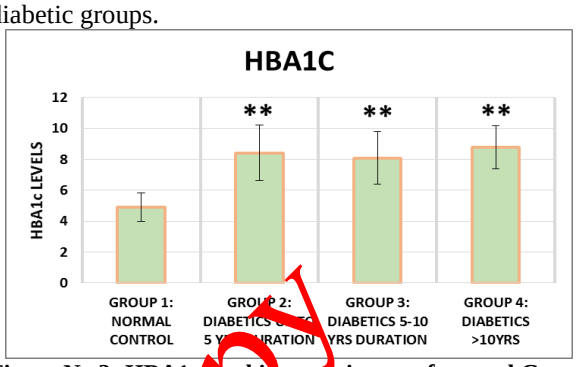

Figure No.2: HBA1c level in participants of control Group (Group 1) and patients of different durations of Diabetes mellitus [group 2 (duration of diabetes 0 to 5 yrs.), group 3 (duration of diabetes 6 to 10 yrs.), group 4 (duration of diabetes 10 yrs. onwards). Data are means ± SD (n= 30). HBA1c levels are expressed as %. Data are analyzed for significant difference by One-way ANOVA followed by Tukey's post-hoc comparisons; **p<0.01 compared with control group.

Copper levels in serum of participants of control group and patients of different durations of Diabetes mellitus [group 2 (duration of diabetes 0 to 5 yrs.), group 3 (duration of diabetes 6 to 10 yrs.), group 4 (duration of diabetes 10 yrs. onwards) are also estimated to find diabetes-induced alterations in serum copper levels. Data for serum copper levels is presented in Fig. 3 which shows that serum copper levels were significantly raised (F=17.588 p<0.01) by 36%, 18% and 38% in group 2 (130.87±26.2 µg/dL), group 3 (114.4±23.75 µg/dL) and group 4 (132.97±20.37 µg/dL) respectively as compared group 1 (control group, 96.2±18.06 µg/dL). Post-hoc analysis revealed that on comparing the three diabetic groups it has been found that this increase was significantly greater (p<0.01) in group 3 and group 4 as compare to group 2.

In order to find out the relation between diabetic profile and copper levels, a correlation test was performed between copper levels and diabetic profile parameters (HBA1c and FBS) to see the effect of duration of diabetes on serum copper levels. Pearson correlation test revealed the existence of a correlation between copper levels and diabetic profile. A significant positive correlation is noticed between Copper levels and FBS (r=0.299, p<0.001) and between copper and HBA1c (r=0.481, p<0.001). For HBA1c, there was a strong positive significance (p<0.01) correlation with copper in group 1, 2 and 3. It also showed a positive

significance correlation for control group ($p<0.05$). In Control group, HBA1c showed a positive significance correlation (0.05 level) while it showed negative significance correlation in group 2 (0.01 level) and a strong positive significance correlation with copper in group 1 and 3 (0.01 level).

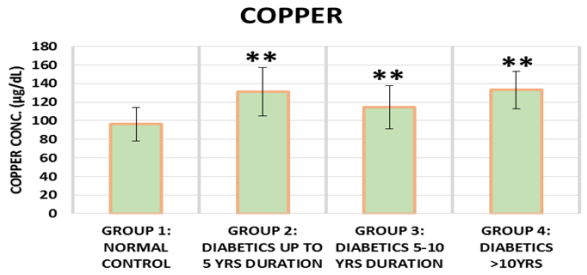

Figure No.3: Copper levels in participants of control Group (Group 1) and patients of different durations of Diabetes mellitus [group 2 (duration of diabetes 0 to 5 yrs.), group 3 (duration of diabetes 6 to 10 yrs.), group 4 (duration of diabetes 10 yrs. onwards). Data are means ± SD (n= 30). Copper levels are expressed as µg per dL of blood. Data are analyzed for significant difference by One-way ANOVA followed by Tukey's post-hoc comparisons; **p<0.01 compared with control group.

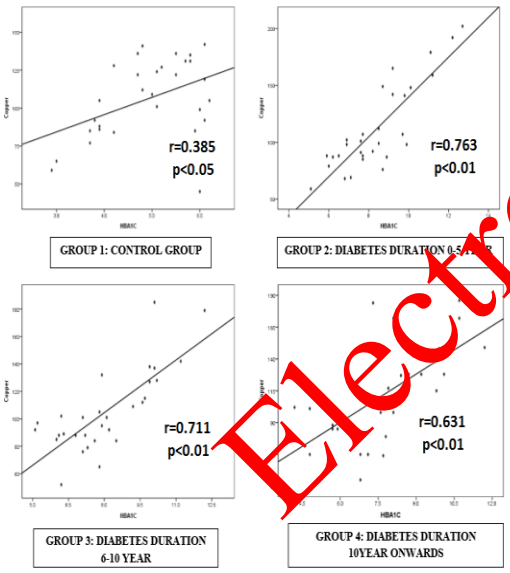

Figure No.4: Correlation between Copper and HBA1c in control Group, and different durations of Diabetes mellitus i.e. group 1 (duration of diabetes 0 to 5 yrs), group 2 (duration of diabetes 6 to 10 yrs), group 3 (duration of diabetes 10 yrs onwards) determined by Pearson correlation test.

DISCUSSION

Present study was designed to investigate the relationship between incidence of diabetes mellitus and plasma copper levels. Previous literature shows that there is a relationship of metal ions with diabetic complications as diabetic complications may be credited to hyperglycemia which initiates glycation and in turn releases copper ions leading to generation of oxidative stress and production of AGEs[11].

Studies have shown that levels of Zn, Mn, and Cr were significantly reduced in blood and scalp-hair samples of diabetic patients as compared to control subjects of both genders while on contrary the urinary levels of these elements were found to be higher in the diabetic patients than in the age-matched healthy controls. In contrast, Cu and Fe levels were highly raised in scalp hair of diabetic patients compared to non diabetic subjects[2]. Present findings revealed that plasma copper levels were significantly raised in diabetic patients as compared to nondiabetic controls. These results are consistent with previous studies that diabetic subjects with retinopathy, hypertension, or microvascular disease had higher plasma copper concentrations compared with both diabetic subjects without complications and with control subjects[10].

Findings of present study shows that diabetic profile parameters (fasting blood sugar and HBA1c levels) were found to be significantly raised in patients of different durations of Diabetes mellitus (0 to 5 years, 6 to 10 years and 10 years onwards) compared to non-diabetic participants but there is no significant difference is found among these groups of different diabetic durations. Along with rise in diabetic profile parameters there is also a significant rise in plasma copper levels in all durations of diabetes mellitus. Then relationship between copper levels and diabetic profile parameters were also found to be significant as there is a significant positive correlation between them. Although the demographic parameters were almost comparable in all diabetic patients as well as in controls having no significant differences among them. So, the chances that rise in plasma copper levels may be due to any other factor are subsided.

Evidence has shown that metabolic complications appear to be associated with alterations in the levels of some minerals, especially copper[12]. Copper ion is involved in the development of type 2 diabetes and thereby a potential therapeutic target for diabetes[13]. Increased Copper content of the lens presumably has a greater association with the development of lens opacification in diabetics than other trace elements[14].

CONCLUSION

From the present findings it can be concluded that Blood copper levels are responsible for incidence and progression of diabetes mellitus from this it may be suggested that the copper levels in blood are increasing gradually during diabetic condition.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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