3 may be involved. The chromosomally fragile site, FRA3B, has been linked to lung cancers and more recently has been explored in cervical carcinomas. A large number of smokers and passive smokers showed fragility at FRA3B. FRA3B maps within the fragile histadine triad gene (FHIT), which is a tumor suppressor gene involved in tumorogenesis, including cervical neoplasia.[6]

Health professionals are also target of passive exposure to cigarette smoke which makes them also a focal group essential for creating awareness regarding hazards of passive smoking.[7] In our environment, bidi and huqqa smokers is another addiction that needs to be studied from genetic aspect to find out the multi-factorial polymorphism. With new technology enabling scientists to analyze the interactions between genomic structure and environmental stimuli, researchers should be able to make efforts in clarifying the role that SHS exposure plays in different types of cancers. There should be valid methods for describing the interactions between environmental exposures, pathogens, and genetic composition in the trail to understand the mechanism of all cancers.

REFERENCES

1 California Environmental Protection Agency. Health effects of exposure to environmental tobacco smoke: final report. Sacramento, CA: The office of Environmental Health Hazard Assessment, 1997.

2 Taylor and Francis Health Sciences, What's new in Nicotine and Tobacco Research? Nicotine and Tobacco Research 2003;5,281-287.

3 Amos CI, Caporaso NE,Weston A .Review Host factors in lung cancer risk: a review of interdisciplinary studies.-Cancer Epidemiol Biomarkers Prev. 1992 Sep-Oct;1(6):505-513.

4 Nicole M. Probst-Hensch, Medea Imboden, Denise Felber Dietrich, Jean-Claude Barthélemy. Glutathione S-Transferase Polymorphisms, Passive Smoking, Obesity, and Heart Rate Variability in Nonsmokers. Environ Health Perspect. 2008 November; 116(11):1494–1499.

5 Olivo-Marston SE, Yang P, Mechanic LE, Bowman ED,Childhood exposure to secondhand smoke and functional mannose binding lectin polymorphisms are associated with increased lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2009 Dec; 18(12):3375-83. doi: 10.1158/1055-9965.EPI-09-0986.

6 Stein, C. K., Glover, T. W., Palmer, J. L., & Glisson, B. S.(2002). Direct correlation between FRA3B expression and cigarette smoking. Genes, Chromosomes, & Cancer,34,333-340.

7 Zil-a-Rubab, M. Ata-ur-Rahman. Passive smoking status of students and employees of a private medical university Pak J Med Sci 2007;23:425-428.

ORIGINAL ARTICLE

Zinc and Copper Levels Fluctuate with Altered Glucose Homeostasis

Nudrat Anwar Zuberi[1] Sabeela Noor[2] Fasiha Fatima[3] Khalilullah[4] and Muhammad Iltaf[5]

ABSTRACT

Background: Type 2 diabetes mellitus is becoming one of the major health problems worldwide. Especially in South East Asia, type 2 diabetes has gained critical significance. As pre-diabetes prevalence is increasing worldwide, it has become an important concern to prevent diabetes at an early stage. Trace elements have been gaining attention in improving the glucometabolic conditions like pre-diabetes and diabetes. Zinc and copper are the major trace elements present in the human body and they play a significant role in the pathogenesis of diabetes mellitus and pre-diabetes.

Objectives: The purpose of this study was to compare serum zinc and copper levels in type 2 diabetes and pre-diabetes.

Methods: This study was conducted in department of Biochemistry BMSI, JPMC Karachi. Total 90 subjects were taken out of which 30 were type 2 diabetics, 30 were pre-diabetics and 30 were normal healthy individuals. Serum fasting glucose was measured by glucose oxidase method. Serum zinc and copper were measured by colorimetric method. Statistical analysis was done using SPSS version 16.

Results: Serum zinc levels were significantly lower in type 2 diabetics as compared to pre-diabetics and normal individuals (mean differences were 45.17±15.63μg/dl, 59.97±13μg/dl and 86.57±14.34 μg/dl respectively). Serum copper was significantly increased in type 2 diabetics compared to pre-diabetes and control samples (mean differences were 325.55±88.34 μg/dl, 175.53±47.45μg/dl and 126.87±21.57μg/dl respectively).

Conclusion: It was concluded that serum zinc was significantly lower in type 2 diabetics and pre-diabetics and is inversely related to serum fasting glucose while serum copper is significantly higher and positively related with fasting blood glucose.

KEY WORDS: *Type 2 Diabetes, Pre-Diabetes, Zinc, Copper.*

[1] **Nudrat Anwar Zuberi**

Associate Professor, Department of Biochemistry, BMSI, JPMC, Karachi

[2] **Sabeela Noor**

Researcher Department of Biochemistry, BMSI, JPMC, Karachi

[3] **Fasiha Fatima**

Researcher, Department of Biochemistry, BMSI, JPMC, Karachi

[4] **Khalilullah**

Researcher, Department of Biochemistry, BMSI, JPMC, Karachi

[5] **Muhammad Iltaf**

Researcher, Department of Biochemistry, BMSI, JPMC, Karachi

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia causing disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] The prevalence of diabetes and pre-diabetes is increasing globally and becoming a threat to the world population especially South East Asia.[2] In Pakistan the diabetes prevalence is 11.1% while prevalence rate of impaired fasting glucose is 4.2% in males and 2.3% in females.[3]

The role of trace elements for improvement of disturbed metabolic conditions like pre-diabetes and diabetes has been gaining attention.[4,5] Zinc is the second most common trace element profusely distributed in the body after iron.[6] Several studies on humans as well as on animals established that zinc plays a major role in the synthesis and release of insulin. Zinc serves as an effective antioxidant by lowering the oxidative stress and decreasing the insulin resistance and ultimately the progression of diabetes mellitus.[7,8]

Copper is the third most abundant mineral in the human body. Copper is present in the body combined with various enzymes to form metallo-enzymes such as ceruloplasmin, SOD.[9] These enzymes play a major role in redox reactions, such as superoxide dismutase which plays key role in antioxidant defense.[10] Copper is associated with altered glucose metabolism through the stimulation of glycation and release of copper ion enhancing the oxidative damage.[11] Various studies on human subjects demonstrate that diabetic patients have abnormal circulation of copper.[12] Copper and zinc are the major components of antioxidant enzyme SOD.[13] This enzyme inhibits oxidative stress which results from the accumulation of free radicals oxidative stress and plays an important role in the pathological processes ongoing in the diabetic patients.[14] The objective of this study was to determine the serum zinc and copper levels in type 2 diabetics and pre-diabetics and their association with glycemic status.

METHODOLOGY

This is a prospective, cross sectional and comparative study. A total of 90 subjects were selected out of which 30 were pre-diabetics (17 females and 13 male), 30 were diagnosed patients of type 2 diabetes (14 females and 16 males) and 30 were normal healthy individuals (16 females and 14 males) aged 40-65 years. The study was conducted at a diabetes management tertiary care center of Karachi after taking ethical approval from committee of BMSI, JPMC. The required samples were collected from the diabetic patients and normal healthy individuals over a period of three months.

The subjects were selected after procuring written consent, detailed history and examination of the subjects. Patients suffering from endocrinal disorders, hepatic disease, renal diseases, alcoholism or other drug abuse were excluded For female patients, those having pregnancy, on lactation and using oral contraceptive pills were excluded. Both patients and normal healthy individuals were requested to come with 8-10 hours of fasting for sample collection. Samples were collected for blood glucose level, serum copper and zinc level. 7 ml of blood was taken aseptically in 10cc disposable syringes. Out of 7ml, 3ml was placed in sodium citrate tube for FBS estimation; 4 ml of blood was placed in a second test tube and allowed to clot for determination of copper and zinc. Clotted blood in the test tube was centrifuged at 508 Xg for three minutes and serum was separated and stored at -20^{0}C until analysis.

Considering the laboratory fasting blood glucose measurements, participants were categorized into three groups using American Diabetic Association (ADA) guidelines:

Normal (normoglycemic): Where fasting blood glucose level was < 100 mg/dl.

Pre-diabetics: Where fasting blood glucose level was 100-125 mg/dl, and

Diabetics: Where fasting blood glucose level was ≥ 125 mg/dl.

Plasma glucose level was estimated by using glucose oxidase method. The colorimetric randox kit procedure was used for estimating serum copper and zinc estimation. All experimental data were expressed in mean and standard deviation. Data obtained in the study and control group were compared thriugh two-tailed t-tests by using SPSS (Statistical Package for Social Science) version 16. Pearson's correlation coefficient (r) was used to identify trace elements association with glycemic status. P-value <0.05 was considered as significant.

RESULTS

The age, sex, body mass index (BMI) and medication is shown in Table 1. The mean age of the healthy control group was 47.23±6.37, pre-diabetics was 47.40±6.06 and 48.97±6.9 for diabetic patients. There were 49% males and 51% were females in the sample. The BMI of diabetic group was significantly increased (27±3.5) as compared to impaired (25.2±3.03) and control (24.5±4.6). 72% of the patients in diabetic group were taking different hypoglycemic drugs.

Table 2 presents the fasting blood sugar (FBS), copper (Cu) and zinc (Zn) level among study groups. FBS was significantly increased in diabetic group (141±20.53) as compared to control (86.03±8.24) and pre-diabetic group (112±6.7), which is highly significant (p value <0.001). Copper was significantly increased in the diabetic group (325.55±88.34) compared to control (126.87±21.57) and pre-diabetic group (175.53±47.45), which is highly significant (p value <0.001). Zinc was significantly decreased in diabetic group(45.17±15.63) compared to control (86.57±14.34) and pre-diabetic group (59.97±13), which is highly significant (p value <0.001). The serum copper levels were positively correlated with fasting blood sugar, (Fig.1) while the serum zinc levels were negatively correlated with fasting blood sugar (Fig.2).

Table 1: Characteristics of Study Subjects

INDICATORS	CONTROL (N=30)	PRE-DIABETICS (N=30)	TYPE 2 DIABETICS (N=30)
Age (years)	47.23±6.37	47.40±6.06	48.97±6.9
Sex (n, %)			
Male	47	43	57
Female	53	57	43
BMI (kg/m$^{2)}$	24.5±4.6	25.2±3.03	27±3.5
Taking hypoglycemic Drugs (n, %)			
Yes	0	0	21(72.4)
No	30(100)	30(100)	9(27.6)

Table 2: Comparison of Fasting Blood Glucose and Other Biochemical Variables within Study Groups

	CONTROLS	PRE-DIABETICS	TYPE- 2 DIABETICS	p-VALUE
FBS (mg/dl)	86.03±8.24	112±6.7	141±20.53	0.001*
Zinc (μg/dl)	86.57±14.34	59.97±13	45.17±15.63	0.001*
Copper (μg/dl)	126.87±21.57	175.53±47.45	325.55±88.34	0.001*

Values are expressed as mean ± SD, values < 0.05 are considered as significant

Figure 1: Correlation between Fasting Blood Glucose and Serum Copper in Complete Study Population (n=90)

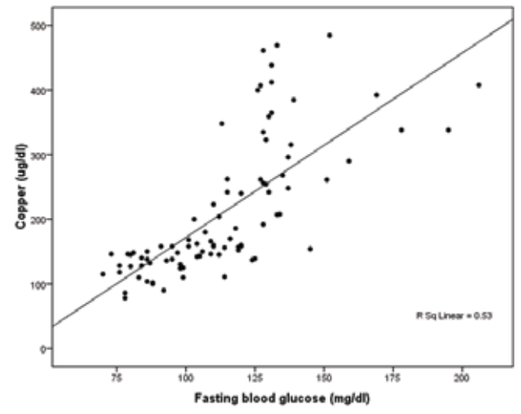
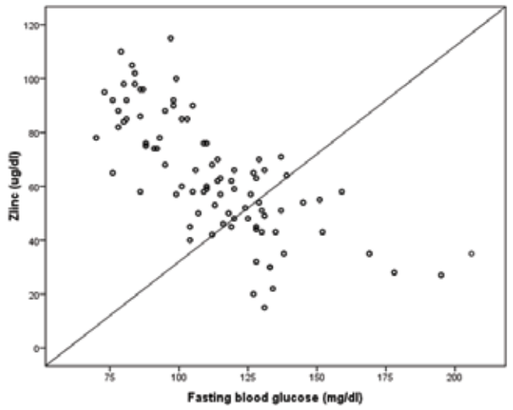


Figure 2: Correlation between Fasting Blood Glucose and Serum Zinc in Complete Study Population (n=90)



DISCUSSION

Trace elements have been investigated as potential preventive and therapeutic agents for type 2 diabetes and for common complications of diabetes. In particular, diabetes is shown to be associated with abnormalities in the metabolism of zinc, chromium, copper, magnesium and manganese.[15] The present study was designed to evaluate serum zinc and copper levels in type 2 diabetes and impaired fasting glycemic individuals while evaluating against a healthy control group.

In the study, it was observed that mean serum zinc level was significantly low in diabetics as compared to the control subjects. Similarly Al-Maroof also reported significantly lower serum zinc level in diabetics than in control subjects.[16]

Lower levels of zinc were observed in impaired fasting glycemic group respectively. Some studies have reported zinc deficiency along with alterations in zinc metabolism in patients with diabetes. Zinc is useful in the synthesis, storage, and secretion of insulin.[17] It effects antigenic properties of insulin which leads to hyperglycemia. Increase in the copper ion levels in patients with diabetes mellitus (DM) may be attributed to hyperglycemia that may stimulate glycation and release of copper ion and this accelerates the oxidative stress.[18,19] The results have shown that copper levels are increased in diabetic patients as well as in impaired fasting glycemic group as compared to control. The increased level of copper in the diabetic patients agrees with other studies.[20]

Copper acts as a pro-oxidant and may participate in metal catalyzed formation of free radicals. The increased production of free radicals is likely to be associated with development of type 2 DM.[21] A decrease in zinc concentrations and an increase in copper concentrations can be additional factors of atherogenicity.[22] Serum copper levels were positively correlated with fasting blood sugar while serum zinc levels were negatively correlated with fasting blood sugar, depicting that both of these trace elements have marked effect on glucose metabolism.

Copper and zinc are the major components of antioxidant enzyme SOD. This enzyme inhibits oxidative stress which results from the accumulation of free radicals oxidative stress and plays an important role in the pathological processes ongoing in the diabetic patient. Excessive oxidative stress has adverse effects on islet survival and function, and accelerates complications in target organs and tissues.[23, 24]

Our study has a number of potential limitations as other trace elements such as iron are not considered even though they may influence zinc status. The sample size was also relatively small, therefore the numbers of individuals in each group were limited to reach definite conclusion for comparison among groups.

CONCLUSION

From the present study, it was concluded that type 2 diabetics and pre-diabetics have significantly lower level of serum zinc as compared to control group and significantly higher level of serum copper. It was determined that zinc deficiency plays an important role in the development of impaired glucose homeostasis. It prospectively might serve as a protective agent against the development of type 2 diabetes. However, in order to better understand the role of these trace elements on diabetes larger clinical trials are required.

REFERENCES

[1] World Health Organization. Part 1: diagnosis and classification of diabetes mellitus. In: Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. 1999Geneva: World Health Organization.

[2] Jayawardena R, Ranasinghe P, Byrne NM, Soares MJ, Katulanda P, Hills AP. Prevalence and trends of the diabetes epidemic in South Asia: a systematic review and meta-analysis. BMC Public Health, 2012; 12.

[3] Shera AS, Rafique G, Khwaja IA, Baqai S, Khan IA, King H, Ahmed KI: Pakistan National Diabetes Survey prevalence of glucose intolerance and associated factors in North West at Frontier Province (NWFP) of Pakistan. J Pak Med Assoc1999;49: 206–211.

[4] Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. J Biomed Biotechnol 2010; 47:62-79.

[5] Henriksen EJ: Dysregulation of Glycogen Synthase Kinase-3 in Skeletal Muscle and the Etiology of Insulin Resistance and Type 2 Diabetes. Curr Diabetes Rev 2010; 6:285-293.

[6] King JC. Zinc. In: Shils ME, Shike M, eds. *Modern Nutrition in Health and Disease.* 10th ed. Philadelphia, Pa.: Lippincott Williams & Wilkins; 2006:271-285.

[7] Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA: Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010; 45:38-92.

[8] Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes Metab 2003; 29:579-585.

[9] Pedrosa., Lucia de Fatima Campos.,Cozzolino., Silvia .,Maria., Franciscato. Metabolic and functional alterations of copper in diabetes mellitus. Brazilian J .Nutr, 1999;12(3): 213-224.

[10] Michael L., Bioship Edward P., Fody larry Schoeff. -Clinical chemistry principle, procedures, correlations. Fifth edition, Chapter6 1998: 60-80.

[11] Isbir T. Tamer L., Taylor A., Isbir M. Zinc, copper and magnesium status in insulin-dependent diabetes. Diabetes Res, 1994, 26(1): 41-51.

[12] KinlawWB ., Levine AS., Morley JE., Silvis SE., Abnormal zinc metabolism in type II diabetes mellitus. Am J Med,1983; 75 (2):273-277.

[13] Haskins K., Kench J., Powers K., Bradley B., Pugazhenthi S., ReuschJ. Role for oxidative stress in the regeneration of islet beta cells . J Invest Med, 2004; 52:45-49.

[14] Halliwell B., -Free radicals, antioxidants and human disease:cause or consequence? Lancet, 1994; 344:721-724.

[15] Akhuemokhan KI, Eregie A and Fasanmade OA. Trace mineral status and glycaemic control in Nigerians with type 2 diabetes. African Journal of Diabetes Medicine. 2010;20-22.

[16] Al-Maroof RA, Al-Sharbatti SS. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type-2 diabetics: Saudi Med J 2006; 27:344-350.

[17] Diwan AG, Pradhan AB, Lingojwar D, Krishna KK, Singh P, Almelkar SI. Serum zinc, chromium and magnesium levels in Type 2 diabetes. Int J Diab Develop Ctries 2006; 26:122-123

[18] Kazi TG, Afridi HI, Kazi N, JamaliMK, Arain MB, Jalbani N et al. Copper, chromium, manganese, iron,nickel, and zinc levels in biological samples of diabetes mellitus patients.Biol Trace Elem Res 2008;122:1-18

[19] Mosaad A., Abou-Seif., Abd-Allah Youssef. Evaluation of Some Biochemical Changes in diabetic patients. Clinica Chimica Acta, 2004-346: 161-170.

[20] Zheng Y, Li XK, Wang Y, Cai L.The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators. Hemoglobin 2008; 32:135-145.

[21] Viktorínová A, Toserová E, Krizko M, Duracková Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism. 2009;58:1477–1482.

[22] Walter RM., Uriu-Hare JY., Olin KO., Oster MH., Anawalt BD., Crichfield JW., Keen CL., -Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. Diabetes Care, 1991; 14:1050-1056.

[23] Disilvestro RA.,Zinc in relation to diabetes and oxidative disease. J Nutr , 2000; 130 (5):1509-1511.

[24] Zelko IN., Mariani TJ., Folz RJ ., Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med, 2002; 33:337-349.