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STATUS OF CERTAIN TRACE ELEMENTS IN TYPE 2 DIABETES MELLITUS PATIENTS OF NORTH WEST INDIAN POPULATION

Running Title: Status of certain trace elements in T2DM Patients

Kuldip Singh, Professor, Department of Biochemistry, Government Medical College- Patiala (Punjab) - India

ADDRESS FOR CORRESPONDENCE

Dr. Kuldip Singh, Professor,
Department of Biochemistry,
Government Medical College- Patiala (Punjab) – India
E-Mail: drkuldip08@gmail.com
Mobil: 9417355095

ABSTRACT

Background: Diabetes mellitus have been implicated due to certain body trace elements because they can influence the onset of diabetes in various ways including disruption of normal metabolism of glucose and insulin. ***Aim:*** To evaluate the status of certain trace elements in type 2 diabetic patients of North/West population of India. ***Material and Methods:*** 150 type-2 diabetic and 100 healthy control subjects were recruited in the study after taken prior consent of participants. After overnight fasting, 10 ml of venous blood samples were taken for glucose, HbA1C and certain trace element analysis**.** ***Result:*** The trace elements like such as Ferritin, Magnesium and Copper levels raised by 88.92% (from107.92 ± 9.34 ng/ml to 203.89 ± 15.38 ng/ml), 50.12% (From 12.17 ± 0.12 mEq/dL to 18.27± 2.18 mEq/dL) and 19.83% (from 91.14 ± 7.33 μg/dL to 109.21±8.52 μg/dL) respectively while the levels of zinc and calcium significantly fall by 39.23% and 36.67% respectively in the serum of type -2 diabetic patients with respect to healthy control subjects. ***Conclusion: This*** study showed that serum levels of Ferritin, Calcium and Copper were significantly increased while the levels of Magnesium and Zinc levels were significantly decreased in type-2 diabetic patient's w. r. t. healthy control subjects. This imbalance may affect the evolution and pathogenesis of diabetes considering the roles of these trace elements in glucose metabolism.

Keywords: Type-2 Diabetes Mellitus (T2DM), Glycosylated Hemoglobin (HbA1C), Ferritin, Magnesium, Copper, Calcium, Hyperglycemia, Insulin, Oxidative Stress (OS).

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INTRODUCTION

The explosive increase of Diabetic population worldwide is a major public health concern both in the developed and developing (India) Nations. The fundamental molecular defects in type 2 DM are insulin resistance and impaired insulin secretion results from a combination of environmental and genetic factors[1]. It is one of the four priority non-communicable diseases along with cardio-vascular disease, malignancy and chronic respiratory diseases. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels[2,3]

A minor hemoglobin derivative called glycated hemoglobin (HbA1c) is produced by glycation, the covalent binding of glucose to hemoglobin. In assessment of glycemic status, the percentage of HbA1c represents the integrated values of glucose over preceding 6 to 8 weeks[2] At the present time, the HbA1cis used worldwide as the marker of long term glycemic control and also a therapeutic target in the prevention and delay of the development of hyperglycemic complications[4]

Trace metal elements are naturally occurring inorganic elements and have been identified as potential candidates for improving metabolic disorders, because they are involved in prosthetic groups of many proteins and cofactors of many enzymes[1] Literature reports revealed that the status of several trace elements such as zinc, copper, chromium, selenium and manganese is altered in diabetes mellitus and these imbalances can play important roles in the pathogenesis and progress of diabetes[5,6] On the other hand, the homeostasis of these trace elements is frequently disturbed by diabetes mellitus[7]

Trace elements play important roles in body metabolism and cellular homeostasis[8] This includes production, secretion and insulin activity pathway such as insulin mechanism of action has been shown to be modulated by Mg, Zn, Mn, Cr, Se and others[9,10,11].

To the best of our knowledge, there are relatively a few studies on the relationship between diabetic hyperglycemia and relevant blood chemistry and trace metal elements in North/ West Indians. Therefore, this study is aimed to evaluate the status of serum ferritin and certain selected trace metal elements, such as zinc, magnesium, copper and calcium among type 2 diabetes mellitus (T2DM) patients of North / West Indian population. The study results may contribute to the development of evidence-based decision and intervention strategies to enhance a healthier lifestyles and better diabetes care.

MATERIALS AND METHODS

The present was carried out in the Department of Biochemistry, Govt. Medical College Patiala on 150 confirmed cases of type 2 diabetic mellitus and 150 healthy control subjects of both rural and urban community were considered in the study.

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Inclusion criteria: The confirmed type 2 diabetic patients in the age range of 25-50 years, both genders (Male *&* Female) having fasting blood glucose ≥140mg/dl and glyosylated hemoglobin (HbA_1C) ≥7% with symptoms of diabetes mellitus, polyuria, polydipsia, fatigue, weight loss were recruited from the rural as well as urban areas of Punjab state and equal number (n=150) of healthy subjects of both sex i.e. male & female in age ranging of 25-50 years were recruited as control group.

Exclusion criteria: A known type 2 diabetics, hypertension, hypothyroidism, renal failure, hepatic diseases, acute illnesses, recurrent myocardial infarction, unstable angina and those not on any weight loss treatment, subjects taking vitamin D supplementation and pregnant & nursing mothers were excluded from the study.

Ethical Issues: All subjects recruited for the study were vegetarian, non-smokers and non-alcoholic, with no positive family history of diabetes, cardiovascular diseases (CVD). Data was obtained from subjects using an interviewer-based questionnaire where the detailed information on their lifestyle, medical history, diet etc. and after obtaining the written consent these were considered for the study. Blood Pressure was measured 3 times every 2 minutes after an initial 10-minute rest period to the nearest of 2 mm Hg in the sitting position, using a mercurial sphygmomanometer and appropriately sized cuffs**.**

STUDY DESIGN: This study was a case control cross-sectional prospective study. This study protocol was approved by the Institution Ethical Committee. A detailed history, physical, and systemic examination, including measurement of height, weight, heart rate, blood pressure in the sitting position, body mass index (BMI), fasting glucose along with HbA_1C and certain minerals such as Ferritin, Zinc, Copper, Magnesium, Calcium and Phosphate was carried out in every subject who entered the study as per a pre-designed performa for assessing the signs of diabetes, heart failure and also the presence of any exclusion criteria.

DATA COLLECTION

Measurements of anthropometric parameters: Height (m) and weight (kg) were measured while subjects were wearing light clothing without shoes. Blood pressure (BP) was measured using an automatic manometer with an appropriate cuff size on the right arm after a resting period of 5 min.

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Specimen Collection, Specimen (Serum/Plasma) Preparation and Storage: A volume of 5 ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 and 9 AM after an overnight fast from both groups (Control and newly diagnosed type 2 diabetic subjects) in potassium oxalate and sodium fluoride vial (2ml) and plain (3ml) vial. The blood of potassium oxalate and sodium fluoride vial was immediately centrifuged at 4000 rpm for 10 minutes for the preparation of plasma and the blood collected in plain vial was kept at 37°C for half an hour after that the blood samples were centrifuged at 4000 rpm for 15 minutes for the separation of serum. The plasma and serum was used for various biochemical assays as under;

Biochemical Assays:

1. ***Estimation of plasma glucose levels:*** Fasting plasma glucose levels were estimated spectrophotometrically using an enzymatic test kit based on GOD-POD method supplied by Transasia Biomedical Private Limited, Mumbai (India).
2. ***Estimation of Glycosylated Hemoglobin (HbA1C):*** HbA1C was analyzed by using kit supplied by Transasia Bio-Medicals Ltd. Solan (HP) in Technical collaboration with ERBA agnostic Mannheim, Germany based onion-exchange resin method in which a hernolyzed preparation of the whole blood is mixed continuously for 5 ruin with a weak binding cation exchanges resin. During this time, non-HbAlc binds to the resin. After the mixing period a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The glycohemoglobin percent is determined by measuring the absorption at 415 nm of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbance's gives the percentage glycohemoglobin.
3. ***Estimation of Trace Elements:*** The trace elements such as Ferritin, Zinc, Copper, Magnesium and Calcium in serum was estimated spectrophotometrically using a standardized test kit supplied by Transasia Biomedical Private Limited, Mumbai (India) by colorimetric method in ERBA-XL-360 fully autoanalyzer.

Statistical Analysis: The data was expressed as Mean ± SD and analyzed with the SPSS 16.0.7 statistical software package. Differences between the obese and control subjects were evaluated using the Student's independent samples *"t"* test. Differences were considered statistically significant at $P < 0.05$.

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RESULTS

1. **Anthropometric Parameters:** The Anthropometric measurements of both type -2 diabetic patients and normal healthy control subjects are summarized in the Table-1. The body weight, height, BP systolic and BP-diastolic was 75.44 ± 5.79 kg, 162.19 ± 5.37 cm, 124.07 ± 2.99 mmHg and 81.91 ± 3.81 mmHg respectively in type 2 Diabetic patients w. r. t. 68.59 ± 5.92 kg ; 165.11 ± 5.59 cm, 82.89 ± 3.93mmHg and 79.19 ± 3.78mmHg respectively of healthy control subjects (Table-1).
2. **Glucose and HbA1c levels:** A significant (P≤0.01) increase was recorded in fasting glucose levels and HbA1c levels by 58.96% (from81.91± 4.91 to180.11± 7.98mg/dL) and 72.32% (from 5.71 ± 0.51 to 9.84 ± 0.34 g%) respectively (Table-2).
3. **Serum Trace elements: Serum Trace elements:** The status of trace elements such as Ferritin, Magnesium, Copper and Calcium in the serum of type -2 diabetic patients and healthy control subjects are summarized in the Table-3. The serum Ferritin, Copper and calcium was 203.89 ± 15.38 ng/ml; 109.21±8.52 and 8.89 ± 0.94mg/dL respectively in type diabetic patients and 107.92 ± 9.34 ng/ml; 91.14 ± 7.33µg/dL and 8.63±0.47 mg/dL respectively in healthy control subjects was observed. A significant increase in the levels of serum ferritin, magnesium and copper by 88.92% (*P* <0.001.), 19% (statistically not significant) and 2.92% (statistically not significant) respectively was recorded in diabetic patients in comparison to healthy subjects (Table-3) whereas the levels of Magnesium and zinc was significantly (P<0.05) decreased by 33.38% (from 18.27±2.18mEq/dL to 12.17 ± 0.12mEq/dL;) and 39.23% (from 89.91 ± 7.39µg/dL to 54.63± 5.03µg/dL) respectively in diabetic patients w. r. t. normal healthy control subjects (Table-3)

Table 1

General characteristics of Type 2 diabetes patients and healthy control subjects.

Anthropometric Assays	Normal Healthy Control Subjects (n=150)	Type 2 Diabetic Patients (n=150)
Subject Number	150	150
Gender (Male / Female)	90/60	85/65
Height (cm)	165.11 ± 5.59	162.19 ± 5.99 (- 1.76)

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Weight (kg)	68.59 ± 5.92	75.44 ± 5.79 (+9.98)
Age (years)	32.29± 5.72	33.91± 6.01 (+5.01)
Blood pressure systolic (mmHg)	82.89 ± 3.93	124.07 ± 2.99 (+49.68)
Blood pressure diastolic (mmHg)	79.19 ± 3.78	81.91 ± 3.81 (+3.43)

Table 2

Alterations in plasma fasting blood glucose and blood HbA_1C levels in Type 2 Diabetics patients and healthy control subjects.

Biochemical Assays	**Normal Healthy Control Subjects (n=150)**	**Type 2 Diabetic Patients (n=150)**
Fasting Blood Glucose (mg/dL)	81.91± 4.91[a]	180.11± 7.98 (+58.96)[b***]
HbA1C (g%)	5.71 ± 0.51[a]	9.84 ± 0.34 (+72.32) [b ***]

a. Values expressed as Mean ± SD of 150 observations.

b. Values in parentheses representing the percentage change w. r. t healthy control subjects.

*** ($P < 0.001$) highly significant

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Table 3

Alterations in serum Ferritin, Magnesium, Zinc, Copper and Calcium in Type 2 Diabetics patients and healthy control subjects.

Biochemical Assays	Normal Healthy Control Subjects (n=150)	Type 2 Diabetic Patients (n=150)
Ferritin (ng/ml)	107.92 ± 9.34	203.89 ± 15.38 (+88.92)***
Magnesium (mEq/dL)	18.27± 2.18	12.17 ± 0.12 (-33.38)**
Copper (µg/dL)	91.14 ± 7.33	109.21±8.52 $(+19.83)^{NS}$
Zinc (µg/dL)	89.91 ± 7.39	54.63± 5.03 (-39.23)*
Calcium (mg/dL)	8.63±0.47	8.89 ± 0.94 $(+2.92)^{NS}$

NS= Not significant ** (P <0.01) Significant; *** (P <0.001) Highly significant

DISCUSSION

1. **Ferritin:** A significant ($p \leq 0.001$) increase in serum ferritin, a reflector of body iron stores was observed in type-2diabtic patients by 88.92% (from 107.92 ± 9.34 ng/ml to 203.89 ± 15.38 ng/ml**)**(Table-3). A significant increase of iron stores may induce diabetes through a variety of mechanisms as; iron deposition in the liver may resist the action of insulin on liver and also there is some evidence that iron overload also affects skeletal muscle.[13,14] Deposited iron in muscle decreases glucose uptake because of muscle damage. Increased accumulation of iron in the pancreas causes decreased insulin synthesis and secretion[15.] Besides insulin resistance, iron may also have a role in DM through oxidative stress. Pancreatic β-cells, due to high iron load might at increased risk of oxidative damage could be responsible for the development of diabetes[16].

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2. **Copper;** Cu, an essential element that is important for energy production as it is a component of the mitochondrial cytochrome oxidative phosphorylation system[16] Therefore, Cu deficiency is expected to cause distortion of mitochondria, particularly in metabolically active cells as pancreatic and liver cells. In the present study, a nominal increase in cu levels found in the type 2 diabetic patients from 91.14 ± 7.33 µg/dL to 109.21±8.52 µg/dL by 19.83% in comparison to healthy control subjects (Table-3). A similar observations was also observed by various other studies[17,18] A raised in serum copper levels in diabetic patients in the present study could be due to the decrease in Cu affinity to ceruloplasmin because of its glycosylation. However; Cu also has insulin-like activity and this deficiency of Cu may be associated with carbohydrate intolerance and insulin resistance.[16] in the present study, Cu levels were found to be slightly increased in type-2 diabetic patient's w. r. t. healthy controls. The literature reported have conflicting results with respect to Cu levels in diabetes patients like many studies from Brazil and Egypt reported a significant increase in plasma Cu levels[16,19,20] whereas studies from USA, Germany, Austria and Sudan reported a significant decrease[12,22,23]. The discrepancy could be due to differences in lifestyles and dietary habits between different populations.
3. **Zinc:** A significant ($p \geq 0.05$) decrease from 89.91 ± 7.39 µg/dL to 54.63± 5.03µg/dL by 39.23% in Zn levels was observed in T2DM patients in comparison to healthy control subjects (Table-3). Zn is essential for the storage & release of insulin and regulation of its receptor synthesis. Zn being a component of several metabolic enzymes, regulation of immunity and suppression of inflammation. A significant fall in Zn levels in type-2 diabetic patients suggesting the initiation of oxidative stress might be responsible for the destruction of pancreatic β cell. Therefore could be responsible for the etiology of diabetes and its complications. Our results of a significant fall in diabetic patients w. r. t. healthy control subjects consistent with various literature reports.[24-28]
4. **Magnesium:** A significant ($p \geq 0.05$) fall in serum Mg levels was observed in diabetic patients in comparison to healthy control subjects (Table-3) Magnesium is widely distributed in all the human body cells. The role of Mg in glucose metabolism, transport and homeostasis is well documented and hence required for the normal insulin synthesis and its secretion[29,30]. Factors that might contribute to the low levels of Mg (also known as hypomagnesaemia) in diabetes include impairment of tubular re-absorption of Mg by the action of glycosuria and hyperglycemia.[31] In addition, disturbance in insulin levels affects cellular Mg uptake.[32,33]
5. **Calcium:** A nominal rise in the levels of serum calcium by 2.92% was recorded in the T2DM patient's w. r. t. healthy control subjects (Table-3). Insulin secretion is a calcium dependent process as the blood glucose levels increased then glucose transported inside

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with help of GLUT-4 transporters. This glucose is converted to glucose-6-phosphate with aid of glucokinase. This is further oxidized to yield increased ATP which causes closure of potassium channels and hence depolarization of the cell membrane. Depolarization causes increase of calcium flux through calcium channels, which causes docking of vesicles containing insulin to fuse with the cell membrane. Insulin is then secreted by exocytosis. Therefore, calcium is important in insulin responsive tissues such as adipose tissue and skeletal muscle for insulin mediated intracellular processes[34,35]. Further calcium is necessary for insulin receptor phosphorylation and proper signal transduction and thus optimal GLUT-4 transporter activity[36]. In the present study, significant difference was not observed in serum calcium in both type 2 diabetes and non diabetes study subjects.

CONCLUSION

All the formentioned observations of increase in ferritin, Copper, Calcium while a significant fall in Magnesium and Zinc in T2DM patients in comparison to healthy controls subjects suggested that alterations in the level of trace elements like ferritin, Zn, Mn, Cu and calcium may affect the evolution and pathogenesis of diabetes could play a role in glucose metabolism. Hence, determination of selected trace elements in diabetic patients and dietary modification might be advice.

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CONFLICT OF INTEREST: None

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