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# Assessment of trace elements levels in patients with Type 2 diabetes using multivariate statistical analysis

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ABSTRACT

The trace elements metabolism has been reported to possess specific roles in the pathogenesis and progress of diabetes mellitus. Due to the continuous increase in the population of patients with Type 2 diabetes (T2D), this study aims to assess the levels and inter-relationships of fast blood glucose (FBG) and serum trace elements in Type 2 diabetic patients. This study was conducted on 40 Egyptian Type 2 diabetic patients and 36 healthy volunteers (Hospital of Tanta University, Tanta, Egypt). The blood serum was digested and then used to determine the levels of 24 trace elements using an inductive coupled plasma mass spectroscopy (ICP-MS). Multivariate statistical analysis depended on correlation coefficient, cluster analysis (CA) and principal component analysis (PCA), were used to analysis the data. The results exhibited significant changes in FBG and eight of trace elements, Zn, Cu, Se, Fe, Mn, Cr, Mg, and As, levels in the blood serum of Type 2 diabetic patients relative to those of healthy controls. The statistical analyses using multivariate statistical techniques were obvious in the reduction of the experimental variables, and grouping the trace elements in patients into three clusters. The application of PCA revealed a distinct difference in associations of trace elements and their clustering patterns in control and patients group in particular for Mg, Fe, Cu, and Zn that appeared to be the most crucial factors which related with Type 2 diabetes. Therefore, on the basis of this study, the contributors of trace elements content in Type 2 diabetic patients can be determine and specify with correlation relationship and multivariate statistical analysis, which confirm that the alteration of some essential trace metals may play a role in the development of diabetes mellitus.



## 1. Introduction

Patients need at the present time private health care because of the complexities and complications of diabetes that affect the patient's health [1]. Medically, T2D is accompanied by disorders of insulin action and secretion, metabolic disturbance of carbohydrate, fat and protein [2,3]. Recently, many studies have reported that investigation the contents of trace elements in human tissues may be an important factor for treatment or diagnosis of Type 2 diabetes [4]. Trace elements have an important role in synthesis and enhancing insulin action and sensitivity, and in transporting mechanism of glucose across cell membrane [5–8]. In the last decades, many research studies have reported direct correlation between Diabetes mellitus and some trace elements such as Zinc, copper, chromium, manganese [9,10]. Therefore, the disturbance of these essential trace elements may result in the failure both of antioxidant defense and glucose intolerance that affecting on the pathogenesis and progress of diabetes [11]. In addition some studies have reported trace elements imbalance associated with oxidative stress and glycemic control that accompanied the diabetic complications [12,13]. In spite of the biochemical mechanism of these elements for developing Type 2 diabetes is still unclear and complex. However, the recent studies that concerned with describing the inter-relationships between trace elements and diabetes, and concerning with using advanced statistical analysis still limited. Therefore, the objective of this study is to evaluate the levels of 24 trace elements in blood serum of patients. The statistical analyses using the correlation and the multivariate statistical techniques based on cluster analysis and principle component analysis were used for the reduction and grouping the trace elements in patients into clusters and for determine the most crucial trace elements which related with Type 2 diabetes.

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**Table 1**
Quantitative analysis results of three certified reference materials using ICP-MS.

| Element | IAEA-A-13(μg/g) | | Bowen's Kale (μg/g) | | GBWO7601 (μg/g) | |
|---|---|---|---|---|---|---|
| | Specified | This work | Specified | This work | Specified | This work |
| Br | 22.00 | 22.46 ± 0.5 1 | 24.00 | 23.28 ± 0.44 | – | – |
| Ca | 286.00 | 291.43 ± 8.73 | 4.14 | 4.22 ± 0.11 | 2.90 | 2.97 ± 0.07 |
| Cd | 0.07 | 0.07 ± 0.003 | 0.89 | 0.90 ± 0.03 | 0.11 | 0.11 ± 0.003 |
| Cr | 0.26 | 0.25 ± 0.007 | 0.31 | 0.32 ± 0.01 | 0.37 | 0.36 ± 0.01 |
| Cu | 4.30 | 4.39 ± 0.12 | 4.90 | 4.74 ± 0.12 | 10.60 | 10.31 ± 0.28 |
| Fe | 2400 | 2352.78 ± 56.45 | 115.00 | 110.98 ± 2.42 | 54.00 | 55.35 ± 1.49 |
| Hg | 0.16 | 0.15 ± 0.003 | 0.18 | 0.18 ± 0.005 | – | – |
| K | 2500 | 2425.12 ± 65.48 | 2.43 | 2.47 ± 0.07 | – | – |
| Mg | 99.00 | 100.58 ± 2.04 | 0.16 | 0.16 ± 0.005 | 360.00 | 366.84 ± 9.52 |
| Mn | 3.60 | 3.52 ± 0.10 | 15.00 | 15.31 ± 0.38 | – | – |
| Na | 12600 | 12247.21 ± 252.89 | 0.23 | 0.22 ± 0.01 | – | – |
| Ni | 1.00 | 1.03 ± 0.02 | 1.08 | 1.11 ± 0.03 | 0.83 | 0.81 ± 0.03 |
| P | 940.00 | 909.45 ± 25.45 | 0.45 | 0.46 ± 0.01 | – | – |
| Pb | 0.18 | 0.18 ± 0.004 | – | – | 8.80 | 8.58 ± 0.26 |
| Rb | 2.30 | 2.34 ± 0.06 | 52.00 | 50.02 ± 1.52 | – | – |
| S | 6500 | 6292.02 ± 163.59 | – | – | – | – |
| Se | 0.24 | 0.25 ± 0.005 | 0.14 | 0.14 ± 0.003 | – | – |
| Zn | 13.00 | 13.98 ± 0.36 | 31.00 | 31.74 ± 0.65 | 190.00 | 193.06 ± 4.63 |

– Not reported.

## 2. Subjects and method

### 2.1. Subjects

This study was conducted on 40 Egyptian patients (23 males and 17 females) with T2DM in addition to 36 healthy volunteers (21 males and 15 females). All patients were selected and recruited at different centers at Tanta University Hospitals, Gharbia governorate, Egypt. The healthy volunteers and patients have the same socio-economic status and were diagnosed according to the recommend way. The mean age of the males and females in patients group was 53.41 ± 7.62 and 51.78 ± 8.75 yr, respectively while the mean age of the males and females in control group was 52.47 ± 6.41 and 53.86 ± 5.82 yr, respectively. Both of patients and control group have normal weight, body mass index (BMI) of range 18.5–25 kg/m$^2$. The mean BMI for the males and females in patients group was 22.45 ± 1.0 and 23.70 ± 2.7 kg/m$^2$, respectively while the mean BMI for the males and females in control group was 21.34 ± 1.5 and 22.98 ± 2.0 kg/m$^2$, respectively.

### 2.3. Methods

#### 2.2.1. ICP-MS calibration

To calibrate and test the accuracy of ICP-MS, three certified reference materials, IAEA-A-13, Bowen's Kale, and GBWO7601, were used in this study. The samples of certified reference materials were prepared by recommended method of IAEA, digested in triplicate, and then analyzed using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS: Finnigan element 2). Table 1 summarize and compare the concentrations found in this work together with the specified values.

#### 2.2.2. Samples digestion and treatment

For collecting the samples, 6–8 ml of fasting blood samples were withdrawn from diabetic patient's vein twice; the samples were preserved in sterilized clean tube without using an anticoagulant agent. The samples were centrifuged at 3000 rpm for 10 min to separate serum and red blood cells .After centrifuge, transfer 0.5 ml of serum to a 15 ml plastic centrifuge tube. To digest the sample, a mixture containing 0.5 ml of serum sample and 10 ml of nitric acid (E-Merck, Germany) were placed in a beaker and heated at 120 °C until complete digestion and until the volume of digested sample reach 1.0 ml [14]. (The organic matter is destroyed by wet-acid digestion) and the solution containing the metal in its elemental form is obtained. After cooling to the room temperature and contents were diluted with double distilled water up to 10 ml. After digestion, the trace element analysis was carried out using an inductively coupled plasma mass spectrometry (ICP-MS: Finnigan element 2).

### 2.3. Data analysis

The mutual relation, the correlation, between each pair of trace elements in serum of diabetic patients was investigated using Pearson correlation. The multivariate methods, cluster analysis (CA) using the Word's method and the Euclidean distance for distance calculation, was applied for two reasons, to investigate the similarities and dissimilarities between trace elements in serum of diabetic patients and to group similar trace elements in categories [15]. In addition, the principal component analysis (PCA) was used to determine the most crucial trace elements which related with Type 2 diabetes. The statistical packages SPSS software, version 12, was used to calculate the statistical parameters.

## 3. Results and discussion

### 3.1. Trace elements concentration in healthy and diabetic patients

The number of analyzed trace elements in diabetic patients and healthy volunteers was 24 elements. The analysis of the samples showed that 16 trace elements, Cd, K, Na, Co, Mo, Al, B, Ca, Br, Pb, Ni, P, V, Rb, Hg, and Li had insignificant changes in all cases of diabetic patients in comparison to control group while 8 trace elements in blood serum, Zn, Cu, Se, Fe, Mn, Cr, Mg, and As had significant changes in all cases of diabetic patients in comparison to control group, as shown in Table 2. All of trace elements, Zn, Se, Fe, Mn, Cr, Mg, and As, exhibited a highly significant decrease while Cu exhibited a highly significant increase in all diabetic patients as compared to healthy controls. For all trace elements, there were no significant differences between the patients and control groups with regards to the age, weight and sex.

For Zn level, the mean concentration for patients group, 985.88 ± 43.04 μg/L, is lower than that of healthy group, 809.60 ± 19.34 μg/L. These results were in agreement with that of Marjani, who mentioned that Zn level was significantly diminished in diabetic patients as compared to controls [16]. The study of Sun