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Determination of Serum Zinc, Manganese, Copper and Cobalt Traces In Type Two Diabetic Patients in Sulaimaniyah City Using ICP Technique

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Abstract

Direct determination of trace metals Zn, Mn, Cu and Co were performed in serum blood samples of two groups diabetic patient type 2 and non diabetes by ICP spectrometric method. Results show the low levels of these elements Zn, Mn and Co while high level of Cu detected compared with non diabetes according to these results good evidence can be made to control these levels through a special diet containing these metals.

Keywords: Diabetic patients type 2, ICP spectrometer, Serum trace metals

Introduction

Type 2 diabetic is previously known as adult-onset diabetes, maturity-onset diabetes, or non-insulin dependent diabetes mellitus (NIDDM). This disease results from loss of responsiveness (sensitivity) of target tissues to insulin; impaired removal of glucose from blood produces hyperglycemia even in the presence of elevated concentration of insulin [1]. Diabetes is in the top 10, and perhaps the top 5, of the most significant diseases in the developed world. The prevalence of type 2 diabetes increases with age and obesity in all populations and represents a major public health problem causing high economic costs.

Type 2 diabetes (NIDDM) is characterized by insulin resistance in peripheral tissue and an insulin secretary defect of the beta cell. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise [2]. It has been known that in type 2-diabetic patients the levels of some elements like (Zn, Cu) have a great effect on glucose level or controlling this level in human blood.

The trace elements that are important for maintenance of a good health body, they are required mainly as components of enzymes and hormones or are involved in the activation of enzymes [3], can be divided into four major groups based on their physiological function [4] (RDA 1989):

1. Essential trace elements for which a recommended daily allowance (RDA) has been established which are Zinc, Iodine, Selenium and Iron.
2. Trace elements for which there is definite of an essential role in human metabolism but for which an RDA has not yet been established, these include transition metals copper, manganese, cobalt, chromium and molybdenum and the group VII halogen fluorine.
3. Trace elements that are consistently found in tissues or biological fluid in (ultra trace) amounts but that have not been shown to be either essential or detrimental at these levels of concentration. These include Lithium, Nickel, Tin, Silicon and Vanadium.
4. Lastly trace metals that have no known biological function in humans but that, if present at relatively low levels, cause pathological changes. These toxic elements include Aluminum, Cadmium, Mercury, Lead and Arsenic.

Determination of trace elements needs a very specific and sensitive method. Inductively Coupled Plasma (ICP) method, which offers several advantages when compared with the flame and electro thermal absorption methods [5]. Among the advantages is their lower inter element interference, which is a direct consequence of their higher temperature. Second, good emission spectra are obtained for most elements under a single set of excitation condition as consequence; spectra for dozens of elements can be recorded simultaneously. This property is of particular importance for the multi element analysis of very small amount of samples [4].

Flame sources are less satisfactory in this regard because optimum excitations condition vary widely from element to element; highly temperature needs for excitation of some elements and low temperatures for others and finally the region of the flame that gives rise to optimum line intensities varies from element to element.

Generally the ICP method is a more sensitive method for multi element analysis in trace level as in our study and also give a responsible selectivity and its sensitivity can be examined as in the present work by running two standard solutions with the series of sample solutions, then measuring the recovery of both standards, this will give good evidence about the sensitivity of the results during the analysis.

Plasma emission methods usually have concentration ranges of several orders of magnitude, in contrast to a two or three decade range for the absorption methods.

Experimental

1. Instruments

1. Inductively Coupled Plasma, Perkin Elmer USA.
2. Centrifuge type
3. Refrigerator
4. Water bath (GFL 1083)
5. Glass apparatus, like pipettes, beakers, volumetric flask, test tubes, for preparation of samples and standard solutions.
6. Disposable plain tubes.

2. Reagents

Two series of standard solutions at the ranges of concentration between (1-10 ppb for (Mn & Co) and (10-100 ppb for (Zn & Cu) of metal ions prepared from standard solutions given with the ICP instrument (Perkin Elmer)

3. Sample collection

The blood samples were collected in central laboratory in Sulaimaniyah Educational Hospital, at first week of June 2010 with the ages range between (35 – 60) years and divided in to two groups:

Group I: Five non diabetic patients' three females and two males (controls) with normal glucose, range (90-120 mg/dl) and without any other disease.

Group II: Diabetic patients six females and four males without those:

a) Had Creatinine level more than 1.2 mg/dl.
b) With lipemic serum.
c) Pregnant.
d) Who used diuretics or anti bodies.

A fasting venous blood sample (7-10) ml was withdrawn and left in water bath for 15 minutes, then centrifuged at 5000 rpm for 10 minutes. The serum was isolated in a disposable plain tube. The glucose determination was performed at the same day. Then the serum was covered and stored at -15 ^{o}C for analysis of trace metals.

4. Procedure:

1. Two ml of serum sample was transferred to a 25ml volumetric flask containing 10 ml of de-ionized water; after mixing the sample solution was diluted to the mark; finally the solution was transferred into a covered plastic container to avoid contamination and interferences. All apparatus and tips were disposable.
2. Two series of standard solution were prepared according to the serum reference intervals of these elements which we wish to determine as described before in (2) above. During the running of the serum sample for analysis, we consider two of the different prepared std. solution as unknown samples and introduced them to the instrument as serum samples.
3. After employment of the ICP instruments and obtaining a steady state, the series of the standard solutions were determined and when the results confirmed the correct calibration; then all the serum samples were introduced individually to the ICP spectrometer for the analysis; and between these samples, two standard solutions were also introduced in order to examine the analysis process; by this we can also determine the recovery percents for these standards and evaluate the sensitivity of the instrument during the analysis process.

Results

There are 15 samples, 10 samples of them are of non hospitalized patients with type 2-diabetic mellitus and 5 of them are from non diabetic patients (with out suffering from other diseases). Their ages ranged from 35-60 years:

Fig.(1) shows the calibration curves for the metals (Zn & Cu) and (Mn & Co) in the concentration range between (10-100 ppb) and (1-10 ppb) respectively.

The final concentration of the elements was found as (µg/dl) in the serum samples as shown in table (1) below:

Discussion

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and /or insulin action associated with chronic hyper glycemia and disturbances of carbohydrate, lipid and protein metabolism [7]. Metal ions are known to play an essential role in living system both in growt h and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients.

The disturbed body distribution of trace elements is often found in human subjects with diabetes mellitus. Generally the trace elements level (Zn, Mn and Co) in human body is decreased as described in our results for diabetic 2 patients compared with non diabetes, while reversely (Cu) level increases. The element (Zn) is subject of growing interested in the public and in scientific communities. In the present study although the mean serum level of (Zn) was lower, not significantly, in diabetic subjects than in healthy control. The possible explanation of hypozincemia observed in diabetics can be hyperzicuria and/or decreased gastrointetestinal absorption of zinc [8]. Zinc has a great effect on general body activities specially in carbohydrate metabolism and in formation and storage of insulin [9]. Zinc depletion has been found in type 1 diabetic also. So generally Zinc supplements needed for diabetic patients. Antagonistic effect of Zinc on insulin may occur in diabetic patients with low TMA Na/K and Ca/K ratios. Zinc increase glucocoticoid activity, which raises potassium relative to sodium.

Dysfunctional neuro endocrine-endocrine interactions contribute to the disturbances in trace element metabolism and cause severe complications in diabetic mellitus [9]. The present

results showed that the levels of Zinc ,Manganese and Cobalt decreased in the blood of diabetic 2 patients .The loss of theses minerals in urine(glycosurea) in these patients ,which may induce a deficiency or marginal state of these minerals in blood of diabetic patients [10, 11]. The present results also showed the increase in the level of copper in the blood of diabetic 2 patients compared with controls, this might be attributed to hyperglycemia that may stimulate glycation and release of copper ions from copper-containing enzymes, this argument has been supported by Lin [12].

No gender related significant differences were observed in our study, this were with agreement with the studies done before [13, 14] for Zn and other trace metals.

According to these results further evidence can be made for diabetic 2 patients to control the deficiency of those trace elements through taking a special diet that provide a good level of those elements.

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Table (1):Comparison of plasma Zn, Mn, Cu and Co between patient and controls

Element µg/dl	Patients (n=10)	Control (n=5)
Zn	158 ± 13.2	220 ± 7.3
Mn	1.58 ± 0.2	2.3 ± 0.2
Co	5.83 ± 0.4	9.2 ± 0.5
Cu	166 ± 9.3	110 ± 7.8

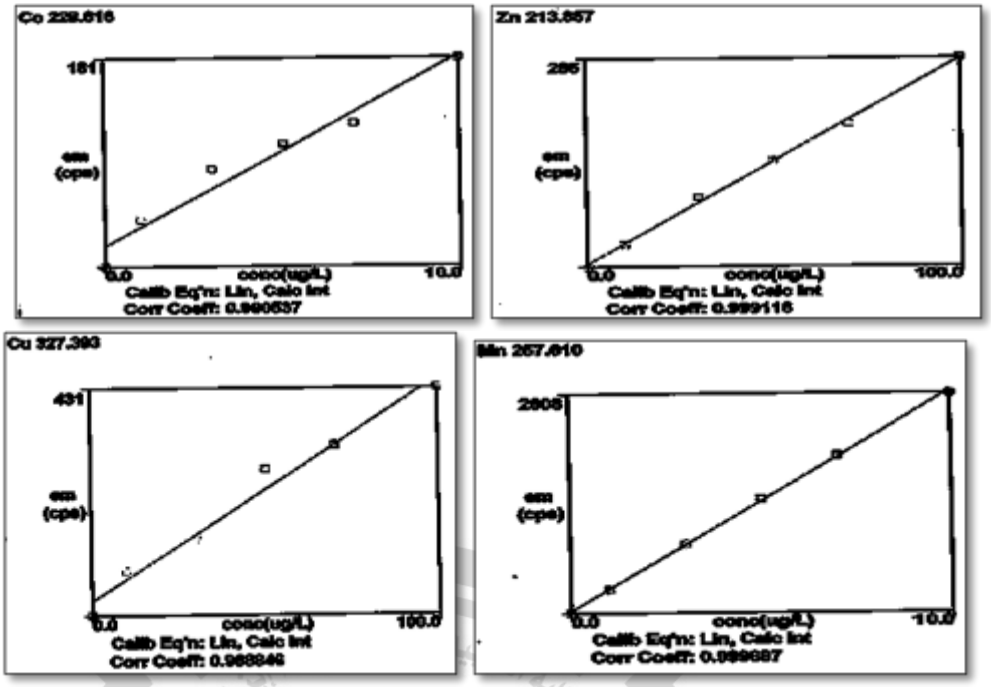

Fig. (1): Calibration curves for the metals (Zn, Cu, Mn & Co)

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تقدير المقادير الضئيلة من العناصر (الخارصين، المنغنيز، النحاس و الكوبلت) في مصل الدم المصابين بداء السكري نوع 2 في مدينة السليمانية بتقنية (ICP)

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الخلاصة

قدرت الكميات الضئيلة من العناصر Zn ,Mn, Cu, Co بطريقة ICP في نماذج مصل الدم لمجموعتين (المصابين بداء السكري نوع (−2−) و (غير المصابين بالسكري تحت السيطرة) لقد اظهرت النتائج انخفاض كميات العناصر(Zn ,Mn, Co) في دم المصابين بداء السكري ،وارتفاع نسبة (Cu) مقارنة مع غير المصابين (تحت السيطرة) بداء السكري. من هذه النتائج يمكن تصحيح مستويات هذه العناصر في المصابين بداء السكري من خلال تنظيم الغذاء و تنوعه و تنظيم الحمية الغذائية.

الكلمات المفتاحية :مرض السكر النوع الثاني ، طيف ICP ،العناصر النادرة