Evaluation of Serum Magnesium, Chromium, Vanadium, and Selenium Levels in Type 2 Diabetic Patients in Sulaimania City

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ABSTRACT:

BACKGROUND:

Diabetes mellitus is a metabolic disorder; a clinical syndrome which occurs due to absolute or relative insulin deficiency or insulin resistance. Direct association of trace elements, such as magnesium in both type 1 and type 2 diabetes, alteration in the metabolism of these minerals has been demonstrated in diabetes in many studies.

OBJECTIVE:

To evaluate magnesium, chromium, vanadium, and selenium levels in the serum of type 2 diabetic subjects.

METHODS:

The present study was conducted on fifty type 2 diabetic patients, and thirty age-sex-matched healthy controls. In groups, those having renal failure, lipemic serum, or using diuretics, antibiotics, and the pregnant, where excluded. Samples were analyzed using Inductively Coupled Plasma Spectrometer.

RESULTS:

Serum levels of Mg in type 2 diabetic patients group were significantly lower than in the control group $p< 0.001$; a similar result was observed for serum Vanadium level $p< 0.05$. Serum Cr levels in both diabetic and control groups were below the reference range limits. Analysis of Se in serum of controls and patients indicated no significant difference.

Gender did not significantly affected plasma Magnesium, Chromium, Vanadium, and Selenium concentrations. Duration of the disease did not significantly affect plasma levels of (Mg, V, and Cr), but it significantly inversely affected the plasma level of (Se); decreased with increasing duration of diabetes ($r = -0.3539$) and $p< 0.05$.

CONCLUSION:

Diabetes mellitus can result in low levels of Mg and V especially in long standing and insulin treated patients which may affect their management.

KEY WARDS: magnesium, trace elements, diabetes mellitus, inductively coupled plasma, spectrometer, sulaimani.

INTRODUCTION:

Diabetes mellitus is a clinical syndrome characterized by abnormal carbohydrate metabolism leading to an increased risk for atherosclerosis and development of specific microvascular and neurological complications [(1)]

It has been known that in type 2 diabetic patients, the levels of some elements like Cr, Mg, V, Se, Cu, Mn have a great effect on glucose level or controlling this level in the blood, Some of these elements such as Mg and Cr are very essential for these patients [(2)].

Trace elements are elements, usually metals, required in minute amounts to maintain a healthy body. They are required mainly as components of enzymes and hormones, or are involved in the activation of enzymes [(3)].These elements can be subdivided into four major groupings based on their physiological function [(4)]; Firstly, Essential trace elements for which a recommended daily allowance (RDA) has been established. These elements have been shown to be essential for normal growth, development, and maintenance and their specific biological role have been

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identified. The elements in this group are: zinc, iodine, selenium, and iron.

Secondly; Trace elements for which there is a definite evidence of an essential role in human metabolism but for which an RDA has not yet been established. These include the transition metals such as copper, manganese, chromium, cobalt, and molybdenum and the group VII halogen fluorine.

Thirdly, Trace elements that are consistently found in tissues or biological fluids 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.

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 aluminium, cadmium, mercury lead, and arsenic. Cadmium, arsenic, and mercury are transition elements, whereas aluminium and lead are members of the normal series in group III and group IV respectively.

Determinations of trace elements need 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 which have [5,6]; Lower susceptibility to chemical interferences due to their higher temperatures. Also it has good emission spectra result for most elements under a single set of excitation conditions, consequently spectra for dozens of elements can be recorded simultaneously, and this property is of particular importance for the multi element analysis of very small samples. It permits the determination of low concentrations of elements at part per billion (PPb) ranges that tend to form refractory compounds (highly resistant to thermal decomposition).

Finally, 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).

This study was conducted to determine the levels of trace elements in blood of type 2 diabetic patients even to Submicro Scales (less than 1 ppb). We used a new technique and a new instrument for analysis which was Inductively Coupled Plasma (ICP) - spectrometer, and the results were compared with a comparable non diabetic group.

Iraqi studies:

As far as we know, no previous Iraqi study is concerned with determination of (Mg, Cr, V, and Se) in type 2 diabetic patients by ICP method. So this study could be considered of value in this field.

The objectives of the study were to;

Evaluate Magnesium, Chromium, Vanadium, and Selenium levels in the serum of type 2 diabetic subjects by ICP spectrometer, and compare the levels of these elements in diabetic patients and non diabetic individuals; and compare the levels of these elements and their relations with: gender, duration of the disease, and type of therapy.

MATERIALS AND METHODS:

Equipments

- Inductively coupled plasma (ICP) Spectrometer. Perkin Elmer Company, USA.
- Spectrophotometer. Secomam S250.

Reagents

A series of standard solutions of mixture of Mg, Cr, V, and Se at a level of PPb ranges.

Study design

This prospective study was conducted in Sulaimani city from July 2007- Jan2008 using a special forum for studying Fifty samples of non hospitalized patients with type 2-diabetic mellitus, attending diabetic center for follow up. 50 (50%) of them were on insulin therapy and 50 (50%) were on oral anti diabetic drugs. Their age ranged from (40-60) years. For comparison purpose, thirty non diabetic persons having a comparable age and gender were included in this study as control.

Sample collection

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, s. glucose and creatinine performed at the same day. Then the sera were covered and stored at -20 C° and assessed within two weeks, repeated thawing freezing was avoided.

Exclusion criteria;

In both groups, the following patients were excluded from the study;

1) Those who had creatinine level >1.2 mg/dl.
2) Those with lipemic serum.
3) The pregnant.
4) Who used diuretics or antibiotics.

Determination of Mg, V, Cr, and Se in serum by Inductively Coupled Plasma (ICP) Spectrometer

This method is a type of optical atomic emission spectrometry (AES), used primarily for the quantitative analysis of samples that are dissolved or suspended in aqueous or organic liquids.

Principle:

Emission spectroscopy based on plasma sources, and Plasma is an electrically conducting gaseous mixture containing a significant concentration of cations and electrons. The argon plasma frequently used for emission analyses, argon ions and electrons the principal conducting species, although cations from the samples will also be present in lesser amounts. Argon ions, once formed in plasma, can absorb sufficient power from an external source to maintain the temperatures as high as 10,000 K.

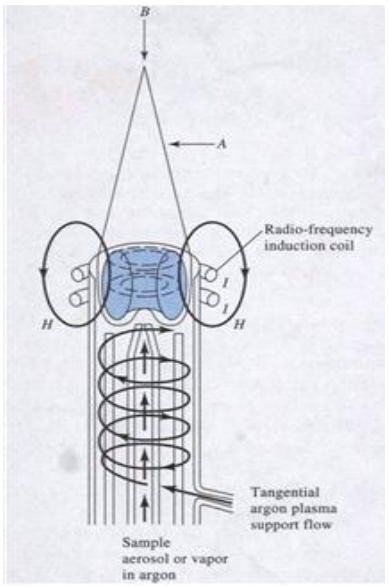

Figure 1. A Schematic of atypical ICP source called a torch.

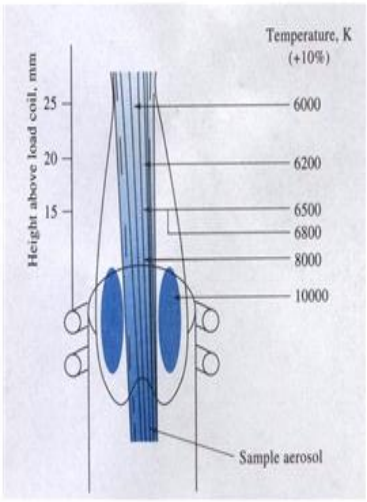

Figure 2. Temperature in ICP source (8)

Specificity and sensitivity

The ICP has become the most widely used source for emission spectroscopy. Its success stems from its high stability, low noise, low background, and freedom from much interference. Plasma sources produce spectra rich in characteristic emission

lines, which make them analytical for both quantitative and qualitative element analysis (5).
The ICP however is relatively expensive to purchase and to operate. It is widely used to determining trace elements in environmental samples, such as drinking water, waste water, and ground water supplies. It is also used for determining trace elements in petroleum products, in food stuffs, geological samples, and biological materials, and industrial quality control (7), its very high temperature eliminate many problems (9) The correlation coefficient for the for the Cr, Mg, V, and Se were Reference intervals:
In serum, according to American Chemistry Society:
Chromium: 0.12-2.1µg/l, vanadium:3-13µg/l, selenium:85-145 µg/l,
Magnesium: 18-23 mg/l. (2, 10).
Determination of glucose was carried out by enzymatic test GOD and PAP, Trinder method.

Sample preparation:

Two ml of serum samples was transferred to beaker contained 10 ml demineralised water, then the solution was transferred to 25 ml volumetric flask and was completed to the mark; finally it was transferred into a covered plastic container to avoid contamination and interferences. This dilution was performed because the solution should be dilute and clear. All apparatus and tips were disposable.

Procedure:

A series of standard solutions were prepared with concentration of 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 ppb, according to the serum reference intervals of these elements which we wanted to determine. After calibration, we considered two of the different prepared ppb standards as unknown samples and introduced them to the ICP. This operation was also repeated between the introduction of the samples to the instrument and the best recovery was obtained.
The results confirmed the correct calibration. Then all the samples were introduced individually to the ICP spectrometer for the analysis.

Statistical analysis:

Data were translated into codes using a specially designed coding sheet, and then converted to computerized database. An expert statistical advice was sought and statistical analyses were done using SPSS (Statistical Package for Social Science) version 15 and STATGRAGH PLUS version 4 computer software (11, 12). The statistical significant of differences between two variables were assessed by Mann-Whitney test and independent sample t test.
Correlations between variables were assessed by Pearson's (parametric Method) and Kendall tau (nonparametric method). P value less than 0.05 was considered significant.

RESULTS:

There were 50 Samples of non hospitalized patients with type 2-dibaetic mellitus, 23 of them (46%) were male and 27 (54%) were females. Twenty five (50%) of them were on insulin therapy and Twenty five (50%) were on oral anti hyperglycaemic drugs. Their age ranged from 40-60 years.
For comparison purpose thirty nondiabetic persons having the same age and sex were included in the study as control.
The value of serum Mg was significantly lower in diabetic individual as compared to the control group (P< 0.0001).
Serum Cr level revealed no significant difference (p>0.05) between controls and patients, its level was found to be in the below reference range in both groups.
Mean serum V level in diabetic patients showed a significant decrease as compared to controls p<0.05.

Table 1: Comparison of plasma conc. of Mg, Cr, V, and Se between patients and controls.

Element	Mean serum levels ± SD		
	Patient (n=50)	Control (n=30)	P-value
Mg	14.25 mg/l ±4.13	18.97 mg/l ±1.79	< 0.0001
Cr	0.10 µg/l ±0.14	0.11 µg/l ±0.09	> 0.05
V	1.24 µg/l ±0.91	5.7 µg/l ±2.4	<0.05
Se	90.80 µg/l ±12.50	110.10 µg/l ±19.18	> 0.05

The Mean values of Mg, Cr, V, and Se were not significantly different between males and females, as shown in table 2.

Table 2: Gender and plasma elements concentration in diabetic patients.

Element	Mean serum levels ± SD		
	Female (n=27)	Male (n=23)	P-value
Mg	14.54 mg/l ±3.89	12.91 mg/l ±4.40	> 0.05
Cr	0.08 µg/l ±0.04	0.10 µg/l ±0.09	> 0.05
V	1.12 µg/l ±0.8	1.22 µg/l ±1.0	>0.05
Se	89.9 µg/l ±10.43	92.1 µg/l ±13.37	> 0.05

Duration of diabetes was inversely correlated with serum level of Mg, V, and Se; the longer the duration of diabetes the lower their levels were. This relation was statistically significant for Se P<0.05, but was not significant for Mg, and V (P>0.05) table-3. Serum Cr level was not affected by duration of the diabetes (P>0.05).

Table 3: Relation of the serum levels of element with duration of diabetes.

Element	Correlation coefficient ®	P-value
Mg	0.1736	>0.05
Cr	0.0129	>0.05
V	0.1250	>0.05
Se	-0.3539	<0.05

Table 4 shows the effect of type of treatment with the serum level of Mg, Cr, V, and Se. Serum Mg, V, and Se levels were significantly lower in patients using insulin as a treatment than those using oral hypoglycaemic drugs p-values were (p< 0.001, p< 0.05, p< 0.05) respectively. There was no significant difference for serum Cr level.

Table 4: Plasma element concentration and type of therapy.

Element	Type of therapy Mean serum levels ± SD		P-value
	Oral anti hyperglycemic (n=25)	Insulin (n=25)	
Mg	15.66 mg/l ±5.13	11.30 ±4.05	<0.001
Cr	0.08 µg/l ±0.08	0.09 ±0.05	0.05
V	9 µg/l ±1.0	1.0 ±0.42	< 0.05
Se	120.91 µg/l ±16.98	90.95±6.13	<0.05

The mean years of the duration of illness was 9.5 years for those on insulin therapy but was 4 years for those on oral anti-diabetic drugs.

DISCUSSION:

Diabetes mellitus is a complex disorder affecting the metabolism of carbohydrate, fat and protein [13].

It is generally agreed that disturbed body distribution of trace elements and magnesium are often found in human subjects with diabetes mellitus. In the present study diabetic subjects were found to have lower mean serum level of the mean serum Mg as compared with healthy controls which support the earlier reports (14-17). Mg depletion has a negative impact on glucose

homeostasis and insulin sensitivity in diabetic patients, presenting low Mg status in diabetics; therefore, Mg supplementation may be beneficial in the management of the disease [18].

Hypomagnesaemia in patients with diabetes may result from poor oral intake, poor gastrointestinal absorption, and enhanced renal Mg excretion. Diabetic autonomic neuropathies that may reduce oral intake and gastrointestinal absorption include oesophageal dysfunction, gastroparesis, and diarrhea [19]. This decrease may also be due to renal causes; in the patient with diabetes, the ultrafilterable Mg load may be enhanced by glomerular hyperfiltration, recurrent excessive volume repletion after hyperglycemia-induced osmotic diuresis, recurrent metabolic acidosis associated with diabetic ketoacidosis, and hypoalbuminemia [20,21]. The last two conditions may increase the serum ionized Mg fraction and, hence, ultrafilterable Mg load and subsequent urinary loss.

Aggressive volume reexpansion and glomerular hyperfiltration also may induce renal Mg wasting at the proximal tubule and TAL, independent of the filtered load. High tubular flow through the TAL may reduce Mg reabsorption at this segment [20], so insulin deficiency or resistance in the diabetic state can promote Mg wasting at this nephron segment [22].

Hypomagnesaemia in diabetes may also be due to metabolic disturbances [23,24] hypophosphatemia, metabolic acidosis [25,26], insulin deficiency and/or resistance [27,28].

The more common use of antibiotics and antifungal drugs such as aminoglycosides and amphotericin in patients with diabetes may also contribute to renal Mg wasting [29].

The element Cr is subject of growing interests in the public and in scientific communities[30]. In the present study although the mean serum level of Cr was lower, not significantly, in the diabetic subjects than in healthy controls, its level was found to be below detectable range in both groups and this is reported in other study[16]. While in the earlier study serum Cr level was significantly lower in patients with type 2 diabetes than control group [31]. This maybe due to many reasons such as: type of the food and its source which can affect the Cr level. Consumption of refined sugar, white flour and lack of exercise [32,33]. These causes not only discard Cr in these foods but further exacerbate Cr deficiency, this is because human body cannot metabolize and transform these highly refined foods into energy without the presence of Cr. It is; therefore, obvious that the more one consumes these highly refined foods, the more Cr is depleted from already marginal body stores [34]. Also Cr excretion, especially by urinary system, may increase 10 to 300 times in the stressful situations or due to diet rich in carbohydrate [35]; observed Cr is excreted primarily in urine, by glomerular filtration [36].

Statistically, significant age-related decrease in serum Cr concentration was observed in 40,872 patients referred by their physicians to a medical research clinic and laboratory [30]. The effect of ageing was observed in both males and females and Hurt RB suggests that dietary Cr requirements increase with age in adult [37]

Demonstrated increases in the urinary Cr concentration in both the diabetes and control group with increasing age. These support the low Cr level in both groups, and in the current study, patients aged between (40-60) years.

Despite all these reasons no reliable method for determination of Cr status is available yet, Anderson suggests that even when Cr analysis is carefully performed, the levels of Cr in serum or urine may not be indicative of the body status [38]. One reason of this is difficulty associated with its accurate measurement in biological fluids because of contamination [39].

In the present study ,the mean serum V level in diabetics showed a significant decrease as compared to controls, this may be due to the glomerular hyper filtration in diabetes 44 (20). In the earlier study V in lymphocytes was significantly higher in diabetic patients as compared to control (31). Although there is limited information about the metabolic effects of vanadium in human [40], It has been studied that Vanadyl sulphate, which is the biologically significant form of V improves hepatic and muscle Insulin Sensitivity in type 2 diabetes [41].

Serum Se level was found to be lower in diabetics than controls but not to a significant level which is similar to other studies [42,43]. But our findings are in contrast with a cross-sectional analysis of the Health Professionals Study, which showed an inverse association between toes nail selenium and diabetes prevalence [44]. Serum Selenium level of diabetic patients was reported to be decreased significantly [45,46] since Se in soil is essential for plant growth, the level of Se in food of plant origin depends on the soil conditions under which they grow. In fact, studies have shown that the Se content of cereal crops between different countries even between regions can vary as much as 1,000 folds [47]. No gender related

significant differences were observed in the serum level of Mg, Cr, V, and Se in diabetes group in the present study. In consistent with our finding, some studies reported that plasma Se concentrations were not significantly different between men and women. [48].

In an earlier study done in Nigeria to study influence of age and duration of diabetes on the serum level of Mg, Cr, and Se [49], no significant differences in serum Mg and Cr levels were observed in both diabetic males and females. The same result was observed for Mg in another study which is in consistent with our findings. [14]

The serum Se level was found to be significantly higher in diabetic females than those for the diabetic males [49], which is against our finding

Duration of diabetes significantly affected the serum level of Se; its level decreased with increasing duration of diabetes which is in consistent with the Serum level of Mg and V decreased with increasing duration of diabetes but not to a significant level, while the results of serum Mg level decreased significantly with increasing duration. Serum Cr level was not affected by duration of diabetes in consistent with the other studies [49].

Type of therapy significantly affected the serum level of Mg, V, and Se, their levels were significantly lower in patients using insulin as a treatment compared to those using oral anti hyperglycemic drugs. In an earlier study, type of therapy did not significantly affect plasma Mg level (14) which is against our finding. This decrease may due to prolonged duration of the disease as those patients using insulin treatment in type 2 diabetes, they might have also received oral antidiabetics for several years before starting the insulin, as the mean years of the duration of illness in the present study was higher in those on insulin therapy that those on oral antidiabetics drugs, and in the present study it has been shown that the duration of the disease inversely affected serum levels of these elements. However, there was no significant difference for serum Cr level.

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