

Status of Micro and Macro Nutrients in Patients with Type 2 Diabetes Mellitus Suggesting the Importance of Cation Ratios

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Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a chronic polymetabolic disorder characterized by chronic hyperglycemia resulting from resistance to insulin action or inadequacy of insulin secretion. Role of the micro & macro nutrients in the pathogenesis of T2DM has not been studied thoroughly. The aim of this study was to evaluate the status of calcium, magnesium, zinc and chromium in relation to HbA1c in a group of subjects with T2DM patients. **Methodology:** The study comprised of seventy three patients with T2DM, attending the OP of a tertiary care medical college hospital. Thirty four individuals were with HbA1c < 7% (group 1) and thirty nine with HbA1c ≥ 7% (group 2). Cation concentrations were determined using Atomic Absorption Spectroscopy and HbA1c by ion exchange chromatography. **Results:** The individual cation concentrations were not significantly different between the groups. Association of these serum ion concentrations with the glycemic control in group 2 (HbA1c 7%) was noted. Significant association of cation ratios with glycemic control was noted. **Conclusion:** The concentration of magnesium, zinc and chromium were low in subjects with poor glycemic control (HbA1c > 7%). Cation ratios were significantly associated with the glycemic control in T2DM.

Keywords

T2DM, HbA1c, Trace Elements, Cation Ratio

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1. Introduction

Type 2 diabetes mellitus (T2DM) constitutes 90% of diabetic population and also shows increasing prevalence worldwide [1]. Trace elements play important role in glucose metabolism by serving as cofactors or components for enzyme involved in glucose metabolism enhancing insulin action by activation of insulin receptor. Being an integral part of antioxidant system they are also implicated in various complication of T2DM. A delicate balance among micronutrients exists due to the synergistic and antagonistic effects existing among them and studies have shown that the status of many trace metals in T2DM patient differs significantly from healthy individuals suggesting some imbalance in micronutrient status among people with T2DM. Trace elements enhance insulin action by activation of insulin receptor, and serve as a cofactors or compounds for enzymes involved in glucose metabolisms increasing insulin sensitivity and also important component of antioxidants system [2]. Elevated cytosolic free calcium concentration is the primary trigger for insulin release and magnesium is a cofactor in the glucose transporting mechanism of cell membrane. Zinc and chromium are important on insulin action and Zinc also part of antioxidant system. The present study was aimed to evaluate the status of Ca, Mg, Zn and Cr in relation to HbA_{1c} in T2DM patients.

2. Materials and Methods

This case-control study was conducted after obtaining the clearance from the Institutional Human Ethical committee. Seventy three patients with newly diagnosed T2DM were included in this study and they were divided into two groups based on their HbA_{1c} values. Group 1 with HbA_{1c} ≤ 7% (n = 34) and Group 2 with HbA_{1c} ≥ 7% (n = 39) diabetic cases.

Analytical grade acids (Merck), standard certified solutions in nitric acid containing 1000 mg/L (Perkin-Elmer), TritonX-100 (Fisher Scientific), LaCl₃ (Sigma-Aldrich) were used. Water quality was secured by treatment on a Millipore Synergy U.V water treatment system. Working standards of zinc was prepared with 5% (v/v) glycerol and that of chromium using a 0.2% solution of nitric acid containing 0.2% TritonX-100. Metals were estimated as per standard methods [5] [6] using Atomic Absorption AAS (Perkin Elmer AAnalyst 700, with electrographite furnace; 4.11 - 11 fitted with autosampler and deuterium lamp background correction; slit width 0.7 nm, oxidant flow rate; 16.0 L/min and acetylene flow rate 7.8 L/min. Flame AAS was used for Ca and Mg. GFAAS was used for the estimation of Zn and Cr. 0.1% Magnesium nitrate was uses as the chemical modifier in the estimation of Zn. Wavelength [Ca (422.7 nm); Mg (285.2 nm); Cr (357.9 nm); Zn (213.9 nm)]. HbA_{1c} was estimated using the ion-exchange HPLC method (BIO-RAD, D10 Haemoglobin A1c programme).

5 ml of blood samples irrespective of the gender were collected from the out patients of the institute and used for trace metal analysis. The blood was separately collected in tubes containing EDTA which was used for HbA_{1c} assessment. HbA_{1c} determination was based on a 3-min cation exchange chromatography on a special cartridge. The eluted molecules were detected at 415 nm and estimated from their areas. HbA_{1c} was reported in conventional percentage.

3. Statistical Analysis

The statistical analysis of the data was done using (SPSS 19 for Windows). Checked the quantitative variables for normal distribution and summarized variables following normal distribution as mean and standard deviation. Variables not following normal distribution as medians. Spearman's rank correlation was applied to determine the statistical association of the categorical variables. Mann-Whitney U test to compare median values between two categories of outcome variables. Logistic regression was applied to compute unadjusted odd ratio (95% confidence Lt). A significance value (r) > 0.7 and p < 0.05 has been accepted for all cases.

Observations & Results

There were 18 females in group 1 (mean age 45.2 years) and 22 females in group 2 (mean age 45.6 yrs). There was no significant difference with respect to the age and gender distribution of the study participants between the groups. Our study also showed no significant difference in the levels of calcium, magnesium, zinc and chromium among both the groups. But magnesium levels were less than the reference range in both groups (Table 1 and Table 2).

Spearman's rank correlation analysis was performed on the data to find an association between the minerals

Table 1. Comparison of biochemical parameters between the groups.

Parameters	Reference range.	Mean \pm SD in group with A- [(HbA1c \leq 7%) (n = 34)]	Mean \pm SD in group B-with [(HbA1c \geq 7%) (n = 39)]	Significance
Calcium	9 - 1 mg/dl	8.89 \pm 2.72 mg/dl	9.0 \pm 3.02 mg/dl	0.963
Magnesium	1.7 - 2.8 mg/dl	1.3 \pm 1.0 mg/dl	1.2 \pm 0.7 mg/dl	0.808
Zinc	0.1 - 0.3mg/dl	0.15 \pm 0.02 mg/dl	0.13 \pm 0.20 mg/dl	0.168
Chromium	0.1 - 2 μ g/L	0.15 \pm 0.05 μ g/dl	0.14 \pm 0.04 μ g/dl	0.603

Comparison was by done using Mann-Whitney U test.

Table 2. Correlations of calcium, magnesium, zinc, chromium and magnesium/zinc with HbA1c.

Parameters	Group with A-HbA1c \leq 7% (n = 34)		Group B-with HbA1c \geq 7% (n = 39)	
	r value	p value	r value	p value
Calcium	0.235	0.681	-0.124	0.454
Magnesium	0.157	0.374	-0.78	0.042*
Zinc	0.48	0.785	-0.82	0.049*
Chromium	-0.166	0.347	-0.86	0.021*
Magnesium/Zinc	0.244	0.164	0.74	0.04*

Comparison was by done using Mann-Whitney U test. * indicate p value $<$ 0.05, statistically significant.

and glycemc status and there were no significant correlations between Ca^{2+} , Mg^{2+} , Zn^{2+} and Cr^{3+} with HbA_{1c} levels in group 1. In group 2, significant correlations were seen for HbA_{1c} with Mg^{2+} ($r = -0.78$, $p = 0.042$), Zn^{2+} ($r = -0.82$, $p = 0.49$), Cr^{3+} ($r = -0.86$, $p = 0.021$).

We also analyzed the association between ratios of the minerals in both groups by spearman's correlation and found significant association among $\text{Ca}^{2+}/\text{Zn}^{2+}$ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratios and HbA_{1c} levels on both groups ($r = -0.742$ in group 1, $r = 0.83$ in group 2) with slight increase in significance among group 2. Correlation between $\text{Mg}^{2+}/\text{Cr}^{3+}$ and $\text{Ca}^{2+}/\text{Cr}^{3+}$ was seen in both groups ($r = 0.959$ in group 1, $r = 0.87$ in group 2). Negative correlation between $\text{Cr}^{3+}/\text{Zn}^{2+}$ and $\text{Ca}^{2+}/\text{Cr}^{3+}$ ($r = 0.729$, $p = 0.013$) in group 2 was also observed.

4. Discussion

Results obtained from this study indicates that there is no significant change in the levels of the parameters between the groups except a slight increase in the Calcium level and slight decrease in the levels of Zinc and Chromium in group 2. This result is in agreement with previous observations [3]-[5]. We have noticed that all the cases irrespective of the groups had Mg^{2+} levels lower than the reference range and agree with the observations of hypomagnesemia associated with T2DM patients [6].

Niewoener *et al.* reported that only 9% of the T2DM patients under study had low serum Zn concentration than the controls and no correlation was observed between serum zinc and HbA_{1c} levels [7]. We have observed negative correlation for the concentration of Mg^{2+} , Zn^{2+} and Cr^{3+} with glycemc status (HbA_{1c}) in the group 2. Our results in this regard agree with the reported inverse correlations of HbA1c with Mg, Zn and Cr [8]-[10] but against a very recent report in the case of Zn [11]. We have also found a new significant positive correlation between Mg/Zn and HbA_{1c}.

Metal ion ratios were suggested more informative than the status of individual mineral concentration, because these ratios are predictive of metabolic dysfunctions and indicative of disease trends [12]. $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio is very significant in diabetes as they are very decisive in insulin production. Further, elevated inflammatory response associated with magnesium deficiency. Oxidative stress which is at higher degrees in T2DM cases, due to excess flux of glucose in to the polyol pathway, also noted to worsen hypomagnesaemia [13]. Further, serum magnesium levels depend on the status of calcium [14]. A high $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio as a biomarker for prostate cancer has been suggested. The range of $\text{Ca}^{2+}/\text{Mg}^{2+}$ reported for the entire group of prostate cancer patients was 4.3

to 4.94 in which a subsection consisting of diabetic patients had values between 4.52 and 4.94. In our studies, no significant difference was found in this ratio between the groups 1 and 2 and $\text{Ca}^{2+}/\text{Mg}^{2+}$ was found between 4.4 and 14 with a mean value 0.77 ± 0.29 . The observed correlation between the ratios $\text{Ca}^{2+}/\text{Mg}^{2+}$ and $\text{Ca}^{2+}/\text{Zn}^{2+}$ was found in both the groups, but the correlation is found strengthened in group 2 where $\text{HbA1c} \geq 7$.

In an isolated study, $\text{Cu}^{2+}/\text{Zn}^{2+}$ ratio was shown significantly higher in cases with T2DM with poor glycemic control [15]. Higher $\text{Cu}^{2+}/\text{Zn}^{2+}$ ratio as inflammatory biomarkers for obstructive pulmonary disease [16], autism spectrum disorder [17] and impairment in bone density [18] have been reported. The significant association between the ratios seen in current study throw light in to the relative status of different cations in T2DM. Non redox active metals like Zn, Mg, Ca etc. which often stabilize the structure of protein compete with the redox active metals like Cu, Fe, Cr etc. for binding sites on proteins, cell membranes and DNA for which coordination number, charge to ionic radius are very important [19].

Substitution of Zn^{2+} (CN6, ionic radius 0.74Å) for Cu^{2+} (CN6, ionic radius 0.73Å) in the structure of $\text{Cu}^{2+}/\text{Zn}^{2+}$ superoxide dismutase is reported to maintain 100 reactivity of the enzyme, though results varied with other substitutions [20].

Zn^{2+} needs special mention in the case of mutual substitutions as it can also exhibit CN4 with radius 0.6Å and CN5 with radius 0.68Å. It can be seen that the ionic radii and tendency to form metal-metal bond are very similar for Zn^{2+} and Mg^{2+} in crystal structures and Mg^{2+} caused replacement of Zn^{2+} from its complexes [21].

We have observed that $\text{Mg}^{2+}/\text{Zn}^{2+}$ not correlated to HbA1c in the non-diabetic group but attained significance in the diabetic group. It has been reported that glycemic control in T2DM patients may not correct low Mg^{2+} concentration suggesting other factors to regulate Mg levels [22]. Further, it was suggested that the association between a low blood Mg concentration and insulin resistance was related to abnormalities of other cations [23]. Hence we hypothesise that $\text{Mg}^{2+}/\text{Zn}^{2+}$ need attention in the case of type 2 diabetes.

Chromium being a redox active metal differs from Zn^{2+} , Mg and Ca^{2+} . The role of chromium in the metabolism of sugar is undisputed, but the effect of Cr supplementation in glycemic control is reported conflicting [24]. It is reported recently that Cr^{3+} influence the activity of the enzyme superoxide dismutase which protects the cells from peroxidation in a dose dependent manner [25]. This observation supports the correlation of Cr^{3+} directly and in relation to the other cations with HbA1c .

5. Conclusion

T2DM is associated with imbalance in micro and macro nutrient status. The concentrations of Magnesium, Zinc and Chromium were significantly reduced in subjects with T2DM with $\text{HbA1c} \geq 7$. The ratios of these metal ions were associated significantly with the glycemic status. Monitoring the ratios of these bioactive metal cations is suggested than mere free ion concentration of specific mineral in T2DM patients. Documentation and correction of these micro and macro nutrient imbalances will help in better glycemic control in subjects with T2DM. Regular monitoring of the metal ion ratios will prevent or delay the onset of micro and macro vascular complications of T2DM by alleviating the oxidative stress, systemic inflammation and insulin resistance.

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Conflict of Interest

The authors declare that we have no conflict of interest in the results of the studies.

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