Diabetes & Metabolic Syndrome: Clinical Research & Reviews 10S (2016) S126–S129

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Correlation of serum chromium, zinc, magnesium and SOD levels with HbA1c in type 2 diabetes: A cross sectional analysis

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ARTICLE INFO

Keywords:
T2DM
SOD
HbA1c

ABSTRACT

The loss of dynamic integrity between homoeostasis of free radicals and antioxidants causes the development of complications like retinopathy, nephropathy, neuropathy, atherosclerosis and cardiovascular diseases in T2DM.

Aims and Objectives: To assess the concentrations of serum chromium, zinc, magnesium and SOD in subjects of T2DM and control and to investigate the effect of these variables versus HbA1c.

Results: Insignificant difference ($P = 0.493$) was reported in age (50 ± 4.7 year compared with 50 ± 7.2 year), while body mass Index (23 ± 2 kg/m^2 compared with 26 ± 4.5 kg/m^2) between the T2DM subjects and control subject showed significant difference (<0.0001). Inverse Pearson correlation coefficient, r (−0.376), (−0.689), (−0.05), (−0.05), (−0.40), (−0.14), (−0.342) and (−0.548) were established when HbA1c of control and T2DM patients were compared with control and T2DM patients of serum Cr, Zn, Mg and SOD variables in that order. The overall "p"-value demonstrated highly significant result at $p < 0.0001$ between the T2DM subjects and controls.

Conclusion: Strong association between serum chromium and SOD in relation to HbA1c in this study gives a strong point that these variables could be used as markers of cell injury with the intention in further part of life en route to progressive complications in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disorder characterized by insufficient production of insulin or deficient in cellular uptake, culminating the homoeostasis of glucose which leads to complications like retinopathy, atherosclerosis, cardiovascular diseases, nephropathy and peripheral neuropathy. Insulin resistance (IR) is defined when insulin sensitive tissues become numb to insulin reception. IR could contribute by deficiency of trace elements [1] and further in development of diabetic complications and progression of the disease also leads to perturbations in trace element metabolism and vice versa.

Chromium (Cr) is a trace element useful in carbohydrate and lipid metabolism [2]. Trivalent chromium (Cr^{3+}), a physiological state binds with an intracellular chromodulin (complex of glutamic acid, cystein, glycine, nicotinic acid and chromic ions) peptide and transmits reception of insulin signal. Cr^{3+}, known to play a role in insulin signal amplification, activates insulin receptor kinase activity [3,4]. Low amount of chromium in the body leads to decrease in insulin signal cascade mechanism and insulin resistance [5].

Another element Zinc (Zn) known to synthesise insulin [6], helps in stabilising the insulin that has been stored in the beta cells of pancreas. Zn is an essential micromineral which has an important role in the secretion of insulin by the pancreatic beta cells [6]. In a study, it has been reported; lower Zn levels when compared between diabetics and control subjects. Elements like Cr and Zn has a role in insulin signalling because Zn-deficient laboratory animals are much less sensitive to insulin [7,8].

Notably, Magnesium (Mg) is responsible for the uptake of glucose in insulin dependent tissues. There were also reports of decreased Mg among those with diabetes [9,10]. Individual studies suggested, supplementation of Cr, Zn and Mg that intake may protect against the development of T2DM [2,8,11].

Insulin resistance is the central dogma in the persistence of chronic extracellular hyperglycemia in T2DM. As a result, glycated haemoglobin (HbA1c) predominates by the non-enzymatic reaction of glucose due to auto-oxidation [12]. Some studies [13,14] in their report reciprocated that the impact of membrane lipid peroxidation is directly proportional to the glucose concentration in vitro and also by the HbA1c. HbA1c is currently used as the

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http://dx.doi.org/10.1016/j.dsx.2015.10.008
1871-4021/

biomarker of glycaemic control in subjects with diabetes and is highly prognostic for long-term diabetes-related complications [15,16].

Hyperglycaemia induces oxidative stress by producing increase amount of reactive oxygen species (ROS) [17]. The possible background of diabetic complications is oxidative stress [18], a phenomenon in which there is loss of dynamic integrity between free radical production and antioxidant generation in the mortal body. IR is exaggerated and also one of the aftermath of ROS due to increase in serine moieties phosphorylation of IRS-1 receptor. The most important ROS is superoxide during persistent hyperglycaemia [17]. This occurs due to increase rate of oxidation of fatty acids and proteins at the expense of glucose in mitochondria. In T2DM, complications arouse due to excessive generation of superoxide [18], apparent increase in the activation of polyol, protein kinase-C, AGEs and DAG pathways [16]. Superoxide dismutase (SOD), an enzyme that nullifies the effect of superoxide by converting it to hydrogen peroxide. Zn is the cofactor for the isomers of enzyme SOD. Based on perceptible association of trace element deficiency in insulin signalling and increase effect of oxidative stress on non-enzymatic glycation of protein, we designed this study to assess the concentrations of serum Cr, Zn, Mg and SOD in subjects of T2DM and control and to investigate the effect of Cr, Zn, Mg and SOD versus HbA1c.

2. Material and methods

The study was conducted at Rajiv Gandhi Centre for Diabetes & Endocrinology, a tertiary care in Aligarh, Aligarh Muslim University, Uttarpradesh, India. 50 Age & sex matched and normal glycaemic status subjects were taken into control group. 50 T2DM subject, on treatment were included in patient group. Fasting venous blood (5 ml) were drawn into EDTA and plane vials for estimation of HbA1C, serum Cr, Zn, Mg and SOD content after informed written consent from all the study group subjects with a disposable syringe & needle, under all aseptic conditions. Serum was separated by centrifuging the blood at 3000 rpm for 20 min. Samples were stored in aliquots at −20 °C until assayed. HbA1C was carried by HPLC method on Variant haemoglobin system. For chromium determination serum samples was diluted at ratio 1–4 in perchloric acid + 0.01 mol/l nitric acid and was prepared for atomic absorption spectrometric analysis [19]. All chemical reagents were of analytical grade purchased from Merck and Sigma agents. From the chromium stock solution, the calibration curve was prepared by serial dilution (0.5, 1.0, 1.5, 2.0, 2.5 ng/ml) and for Zinc and magnesium stock solutions (1000 ppm), the calibration 0.5, 2, 4, 6, 8, 10 ppm were freshly prepared by serial dilution of the stock solution. Samples were estimated by atomic absorption spectrophotometer. The light source were 7 mA, 5 mA, 3.5 mA, wavelength of 357.9 nm, 213.9 nm, 285.2 and 0.2 nm, 5 nm, 0.5 nm spectral slit width were used to check the samples, respectively. After obtaining calibration curve, 5 ml of digested serum sample (or aqueous standards) were injected, the absorbance was recorded and the desired elements concentration were determined. Serum SOD activity was measured according to Marklund and Marklund method modified by Nandi and Chatterjee [20–22] by determining the reduction of pyrogallol by superoxide anion. One unit of SOD is defined as the amount of protein that inhibit the rate of pyrogallol reduction by 50%. Results were expressed as units per milliliter and read at 420 nm. All the parameters have been performed in both the group subjects.

2.1. Statistical analysis

Unpaired '*t*' test was performed to compare the means of variables between T2DM patient and control group. Scattered plots were used to know the regression equation of serum chromium, zinc, magnesium and SOD on HbA1c levels. Pearson correlation coefficient was considered to understand the association between two variables. To assess the effect of serum Cr, Zn, Mg and SOD against HbA1C, was done by Odds ratio and Relative risk ratio by taking cut-off values of HbA1c levels ≤6.5 and >6.5 (WHO, 2011), for serum Cr, Zn, Mg and SOD mean value of control subjects was taken into consideration i.e. ≤4.0, >4.0, ≤2.7, >2.7, ≤1.8, >1.8, ≤5.6, >5.6. $p < 0.05$ was considered significant.

3. Results

Insignificant difference ($P = 0.493$) was reported in age (50 ± 4.7 year compared with 50 ± 7.2 year), while body mass Index (26 ± 4.5 kg/m^2 compared with 23 ± 2 kg/m^2) between the T2DM subjects and control subjects showed significant difference (<0.0001). Inverse Pearson correlation coefficient, r (−0.376), (−0.689), (−0.05), (−0.05), (−0.40), (−0.14), (−0.342) and (−0.548) were established when HbA1c of control and T2DM patients were compared with control and T2DM patients of serum Cr, Zn, Mg and SOD variables in that order. This denotes that as the HbA1c is increased, the variables of the present study were in decrease. Out of trend lines of figures (Figs. 2–5), one in each figure displayed a stable decline, with a negative regression ($y = -0.188x + 4.234$, $y = -0.020x + 1.750$, $y = -0.059x + 1.880$ & $y = -0.067x + 3.541$) when HbA1c of T2DM subjects was compared against serum Cr, Zn, Mg and SOD. In another, an incline depicted a positive regression ($y = 0.265x + 4.379$) when HbA1c of T2DM subjects was compared with serum chromium. The overall "*p*"-value (Fig. 1) demonstrated highly significant result at $p < 0.0001$ between the T2DM subjects and controls. In all we can say that enhanced glycation of haemoglobin was associated with a steady refuse in concentration of serum Cr, Zn Mg and SOD of T2DM group compared to control subjects. To know the effect of Cr, Zn, Mg and SOD on the glycation of haemoglobin, odds ratio and relative risk ratio reported were 3.99 (1.23–9.32) & 2.44 (1.10–5.40) for the Cr, whereas for SOD were 3.23 (1.22–8.52) & 2.37 (1.11–5.0) with a "*p*"-value = 0.02. This proves the hypothesis that chromium and extracellular SOD has an effect on glycation of haemoglobin. Serum Zn and Mg did not show statistical significant effect on HbA1c.

4. Discussion

Hyperglycaemia is a major factor in the development of diabetic complications, although the probable mechanism of how it causes is not clearly understood. Nevertheless, oxidative stress is considered the possible cause for the diabetic complications.

This study observed lower levels of serum Cr, Zn, Mg and SOD when T2DM patients were compared with controls. On the contrary, high HbA1c percent was reported when compared between T2DM patients and controls.

This study agrees with the works [23,24] who also demonstrated lower levels of serum chromium in diabetic patients as compared to healthy controls. In contrast, a study conducted by Zima et al. [25] demonstrated no alteration in Cr levels in type

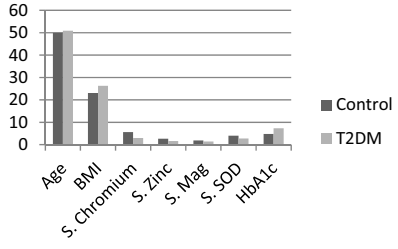

Fig. 1. Bar diagram depicting different values of variables.