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Relationship of Serum Zinc, Insulin, and C-Peptide in Patients with Type 2 Diabetes Treated at a Tertiary Care Center

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ABSTRACT

Pancreatic beta cells have been shown to use zinc in the production and secretion of insulin and C-peptide. It is important to investigate the potential for a link between these factors, especially in respect to diabetes mellitus. Taking blood levels of Insulin, C-peptide, and zinc in people with type 2 diabetes, we hoped to analyze this connection in the Indian population. One hundred patients with type 2 diabetes were surveyed cross-sectionally in a tertiary hospital in North India (MMIMSR). Zinc levels were evaluated spectrophotometrically, whereas C-peptide and insulin levels in the serum were determined using chemiluminescence immunoassay. Serum C-peptide and insulin levels were both reported to be elevated (2.59 1.45 ng/ml and 11.24 10.57 IU/ml), whereas serum zinc levels were within reference ranges (97.82 11.27 g/dl). However, zinc levels were shown to have extremely significant inverse relationships with both serum C-peptide (r=0.649, p0.001) and insulin (r= -0.423, p0.001). Our findings suggest that zinc may have a role in the etiology and treatment of type 2 DM because of its high correlation with blood insulin and c-peptide levels in these individuals

Keywords Diabetes Mellitus,Zinc, Insulin,C-Peptide.

INTRODUCTION

Type 2 DM, which was until recently considered to be a disease of the rich countries, is now emerging in epidemic proportions in the developing countries [1]. 72.1 million people, which is about 1/6th of all the adults with diabetes in the world, live in India. The number is expected to rise to 134.3 million in 2045 [2].Significantly raised levels of insulin have been reported in type 2 diabetics in some studies [3,4]. Recent studies have also shown that C-peptide, a co product of insulin secretion, is a biologically active peptide with a host of physiological roles [5,6,7]. Raised C-peptide levels have been reported in diabetics in some studies [8,9]. Zinc, one of the most important trace elements in our body, is required for multiple steps in insulin synthesis, release and action [10,11]. Estimation of serum zinc levels in diabetic patients has yielded varying results with some authors like Basaki et al and Al- Maroof et al reporting decreased levels while Zargar et al reporting normal levels and Fujimoto et al reporting raised levels [12-15]. With the above background, the current study was planned. Serum insulin, c-peptide zinc levels were estimated in North Indian type 2 diabetic patients. The objective was to see if there was a relationship between the serum levels of these parameters which could then be explored for therapeutic benefit.

MATERIALS AND METHODS

The study was conducted in the Departments of Biochemistry and General Medicine, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, Haryana on hundred type 2 diabetic patients admitted in the Department of General Medicine /attending the Medical OPD. Prediagnosed diabetic patients, >30 yrs of age, were chosen for the study. Subjects with Type 1 DM, patients receiving insulin, supplements or medications containing zinc (multi vitamins etc), pregnant and lactating women and patients with thyroid dysfunction were excluded from the study. Patients were selected randomly.

DM was defined according to the American Diabetic Association (ADA) guidelines [16], which are as under:

- HbA1C >6.5%. OR
- FPG >126 mg/dL (>7.0 mmol/L). (Fasting = no caloric intake for at least 8 h.)*OR
- 2-h plasma glucose>200mg/dL (>11.1mmol/L). OR
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >200 mg/dl (>11.1mmol/L).

Clinical data

Demographic and clinical data were collected with the help of a self designed, pretested questionnaire.

Sample collection and transport

For accurate comparison to established normal values, fasting morning serum samples were obtained after an overnight fast of atleast 8 hrs. The blood was collected aseptically, preferably from anticubital vein in a plain red-top venipuncture tube containing clot activator (*BD™ Vacutainer™*) (without anticoagulants or gel barrier). Care was taken to ensure that no hemolysis took place during or after sample collection. The samples were allowed to clot for 30-60 minutes at room temperature. This was followed by centrifugation using *Remi™ R-23* research centrifuge. Serum was then separated and aliquots were made. Serum C-peptide, insulin, fasting blood glucose and zinc levels were then measured. Samples that required storage for upto 5 days were kept under refrigeration at 2-8°C. Samples that required storage for longer periods (upto 30 days) were stored at -40°C in deep freezer (*Remi™* Ultra Low Freezer).

Quantitative assays

Estimation of serum insulin and C-peptide levels was done by Chemiluminescence immunoassay technique on *Autoplex™ CLIA* (chemiluminescence immunoassay) *workstation* using *Acculite™* C-peptide and Insulin test kits (*Monobind ™ Inc.*(US)).

Estimation of zinc was done by Colorimetric method on *Pace plus* ™ semi auto analyser using *Coral™* zinc kit

Fasting blood glucose was estimated by Trinders method (GOD-POD) on *Pace plus* ™ semi auto analyser using *Erba glucose kit(Erba diagnostics Manheim, Germany*. The serum samples were analysed for glucose levels within 2 hrs of sample collection.

Statistical analysis

Statistical analysis was performed using IBM SPSS and Microsoft Excel. Descriptive analysis was performed, followed by graphical representation of different parameters in terms of mean / median and standard deviation / range. Patients were grouped on basis of age, BMI and duration of DM. Since many parameters like insulin, C-peptide, did not follow a Gaussian distribution, log transformation was performed on these parameters to normalize the distribution, as has been reported in several other studies [17-21]. Pearson's coefficient was used to analyse the different parameters for correlation and student t test was performed to analyse the hypertensive and normotensive patients for association. P value <0.05 was considered significant.

Ethical justification

Ethical approval was obtained from Institutional Ethics Committee (IEC) of the institute on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured, and that every finding will be treated with utmost confidentiality and for the purpose of this research only.

RESULTS AND DISCUSSION

Descriptive statistics:

We found that serum insulin levels were raised in diabetic patients. Mean insulin levels were 11.24±10.57 µIU/ml (reference interval = 0-10 µIU/ml). Median, interquartile range and SEM were 7.1, 3.98-13.35 and 1.06µIU/ml respectively. Similar results were reported by Gayoso-diz et al (2013) and Islam et al (2013) [22, 23]. The raised serum insulin levels can be attributed to the fact that type 2 diabetes mellitus primarily involves insulin resistance (unlike type 1 where decreased insulin secretion is the main defect). To overcome this resistance, β cells of pancreas increase the synthesis and release of insulin, thereby leading to hyperinsulinemia.

In our study, we observed that serum C-peptide levels were also raised in diabetic patients 2.59 ± 1.45 ng/ml (reference interval = 0.78-1.89 ng/ml) Median, interquartile range and SEM were 2.00, 1.6-3.7 and 0.15 ng/ml respectively. Similar findings were reported by Kim et al (2011) and Chowta et al (2010) [8,9]. The elevation of C-peptide levels can be explained by the fact that it is produced in equimolar amounts by β cells of pancreas along with insulin by splitting of the common precursor, proinsulin. In patients with type 2 DM, the compensatory increase in C-peptide levels is attributed to the increased rate of synthesis and release of its precursor, proinsulin in an effort to reverse the hyperglycemia seen in the disease. Proinsulin, after release, splits into insulin and C-peptide.

In our study, we found that serum zinc levels were within reference intervals in diabetic patients. Mean values were 97.82 ± 11.27 µg/dl (reference interval = 80-120 µg/dl). Median, interquartile range and SEM were 96.9, 89.55-108 and 1.13 µg/dl respectively (Fig 1). Zargar et al (1998) reported slightly raised zinc levels of 112 ± 32.13 µg/dl (17.19 ±4.92µmol/L) [14]. In other studies, however, many different types of relationships have been reported between diabetes mellitus and serum zinc levels [24,15] . Decreased levels of serum zinc were reported in type 2 diabetic patients by Parham et al (2008) (76 ±16 µg/dl) [25], Ferdousi et al (2012) (72.70±8.43µg/dl) [26] and Al-Maroof et al (2006) (68.9 +/- 11.9 mg/dl) [13]. On the other hand, Fujimoto et al (1986) reported increased zinc levels have been found in patients with diabetes mellitus previously treated with insulin [15]. Similarly Rusu etal (2005) reported raised zinc levels in type 2 diabetics [27]. These differences could be at least partly due to heterogeneity in patient selection, study design and method of detection of serum zinc levels.

In our study we found no significant difference in the zinc levels in males and females (98.65 ± 11.15 in females and 97.30 ± 11.41 in males) (p=0.562). Saharia et al (2013) in their study on newly diagnosed type 2 DM cases observed that the mean serum zinc concentration was 80.83 ± 13.1 µg/dL in case of males and 77.56 ± 14.2 µg/dL in case of females. The difference was statistically insignificant (p > 0.05) [28]. Masood et al (2009) also reported similar insignificant gender difference in zinc levels (p=0.10) [24].

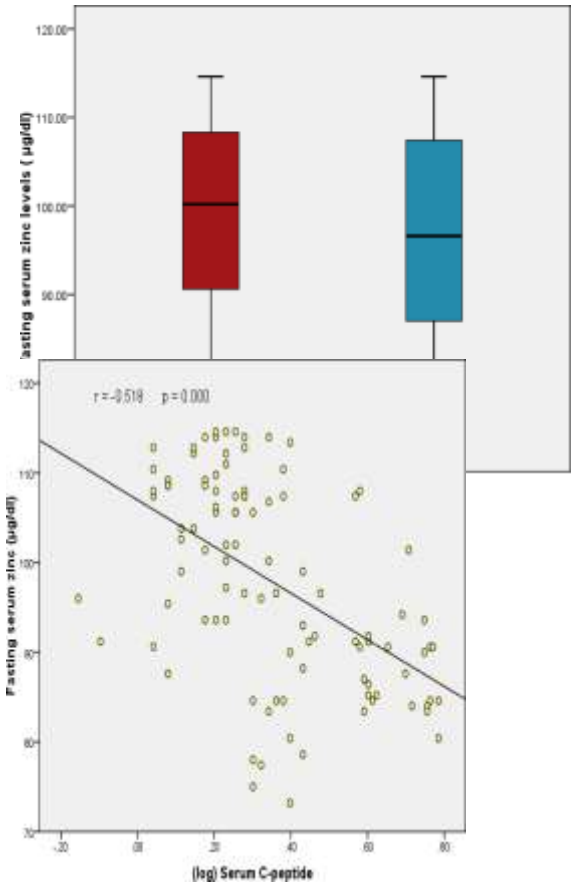

Figure 1: Median and interquartile range of fasting serum zinc levels.

Correlations:

A strong positive correlation was observed between (log) insulin and (log) C-peptide levels. The correlation was statistically highly significant (r=0.649, p=0.000). This strong correlation can in part be attributed to the fact that insulin and C-peptide are released in equimolar amounts from beta cells of pancreas. Because fasting C-peptide levels are 5 -10 % higher than those of insulin owing to a longer half life of C-Peptide, and because unlike insulin, C-peptide does not undergo first pass metabolism in liver and its concentration is not affected by interference from insulin antibodies often present in patients receiving insulin therapy, many authors have proposed the use of C-peptide as a surrogate marker insulin release and β cell function [7,29].

A strong negative correlation was found between serum zinc and (log) insulin levels. The correlation was highly significant (r= -0.423,p<0.001) (fig 2)

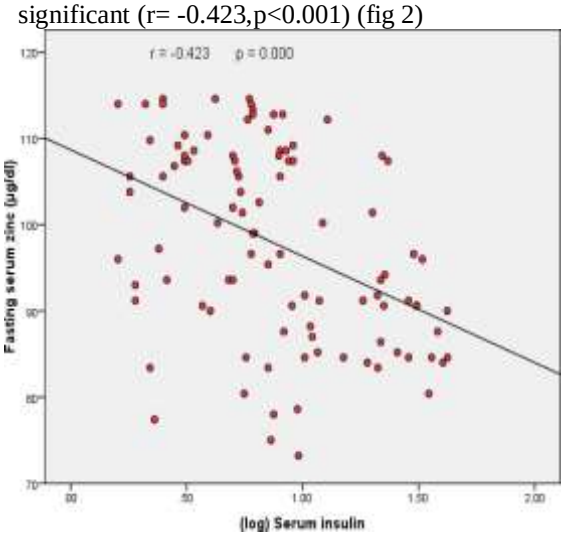

Figure 2: Correlation between fasting serum zinc levels (µg/dl) and (log) serum insulin.

A strong negative correlation was found between serum zinc and (log) C-peptide levels. The correlation was highly significant (r = -0.518, p=<0.001) (fig 3).These negative correlations can probably be explained by the biochemical role played byzinc in insulin release and action. Zinc is known to increase the sensitivity of insulin by several mechanisms. Zinc facilitates hexamerisation and crystal formation of insulin, processes which been suggested that crystal formation increases the degree of conversion of soluble pro-insulin to insoluble insulin [30] Several other modes of action have been described to explain the improved action of insulin by Zinc. It appears that Zinc can have direct insulin-like effects, which may be due to inhibition of the important glycogen-regulating enzyme GSK3. Other mechanisms include stimulation of the postreceptor proteins Akt and PI3-kinase. Zn can also reduce cytokines such as IL-1β as well as NFκB. Zn induces metallothionein synthesis, whereby Zn may have indirect efficacy. [30-32]. All these proposed mechanisms can lead to increased insulin sensitivity (and decreased insulin resistance). In view of the improved sensitivity of insulin, its release by β cells will be decreased. Thus, serum levels of insulin, C-peptide as well as β cell activity will be less if zinc levels are more and vice versa.

Figure 3: Correlation between fasting serum zinc levels (µg/dl) and (log) serum C-peptide.

SUMMARY AND CONCLUSIONS

In our study we found highly significant correlations between serum levels of zinc, C-peptide and insulin in patients of type 2DM. While Serum levels of zinc were within reference intervals, those of insulin and C-peptide were raised in patients of type 2 DM. Given the fact that insulin and c-peptide are fundamental to the origins of

type 2 DM, the strong negative correlation that zinc shares with these parameters points out towards a possible role of zinc in the pathogenesis of the disease. Large scale collaborated studies with a wider patient base are required to through further light on the subject. Further, therapeutic trials evaluating this role a need ofthe hour.

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AUTHOR STATEMENTS

Competing interests

The authors declare no conflict of interest.

Ethical issues

The study was done after obtaining proper ethical approval by the Institutional Ethics Committee of MMIMSR.

REFERENCES

Many people include JC Chan, V. Malik, W. Jia, T. Kadowaki, C. Yajnik, Y. H. Yoon, etc. Asian populations in terms of diabetes incidence, risk factors, and etiology. JAMA. 2009; 301(20): 2129-40.

This is the IDF Diabetes Atlas, Version 8. Diabetes International. The whole paper is available for download at https://www.idf.org/component/attachments/attachments.html?id=560&task=download. Effortlessly updated as of the 21st of November, 2017.

Researchers: Habib SS, M. Aslam, S. F. A. Shah, and A. K. Naveed. High levels of lipoprotein (a) were found in patients with type 2 diabetes mellitus, although low levels of basal insulin were seen in this population. Arq Bras Cardiol. 2009;93(1):25-30 is the referencing format for this publication.

4 F.C. Goetz, L.R. French, W. Thomas, R.L. Gingerich, and J.P. Clements. Blinded radioimmunoassay assessment of serum insulin in people with impaired glucose tolerance and type 2 diabetes in the Wadena, Minnesota area. Published in Metabolism, 1995;44(10):1371-6.Fifth BL Johansson, SJ Sjoberg, and JW Wahren. Renal function and glucose utilization in type 1 (insulin-dependent) diabetics and the effects of human C-effect peptides. 35, 1, (November 1992): 121-8, Diabetes Research and Clinical Care.

Regarding: 6 Renal function and metabolic control are enhanced by a combination of c-peptide and insulin in people with type 1 diabetes, according to research by Johansson BL, Kernell A, Sjoberg S, and Wahren J. The article appeared in issue 7 of the Journal of Clinical Endocrinology in 1993. Diabetes is a control problem with blood sugar. The reference you need is in Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, eds. Here we have Harrison's Essentials of Internal Medicine, 18th Edition. Page numbers range from 2968 to 2975 in a 2012 book published by Mc Graw Hill Companies in New York.

Many people, including S. Kim, B. Kim, D. Lim, I. Song, J. Jung, K. Lee, and others. Effects of baseline C-peptide levels on atherosclerosis progression in type 2 diabetics. Diabetes Metab. 2011;35(1):41-9. J. \sShenoy Authors: A.K., S.D., P.M. Adhikari, N.K. Chowta, and M.N. D'Souza Diabetic kidney disease and C-peptide levels. Published in Indian Journal of Nephrology, 2010;20(1):25-28.

Obaid M, Pandey R, Singh J, Agrawal BK, and Sodhi K S are the authors. The importance of the rediscovered C-therapeutic peptide. 2013;31(31):1221-8. 11. Journal of Pharmacy and Biological Sciences. Zinc in Pancreatic Islet Biology, Insulin Sensitivity and Diabetes. Maret, W. Reference: Nutrition and Food Science. 2017;22(1):1-8.

Zinc, copper, iron, and chromium levels in young persons with type 2 diabetes mellitus. Basaki, M., M. Saeb, S. Nazifi, and H. A. Shamsaei. Biol Trace Elem Res. 2012;148(2):161–4. Al-Maroof RA, Al-Sharbatti SS. The effect of zinc supplementation on glycemic control in type 2 diabetics. Resources for Authors: This article appears in Volume 27 Issue 3 of the Saudi Medical Journal in 2006.

This includes the work of A.H. Zargar, N.A. Shah, S.R. Masoodi, B.A. Laway, F.A. Dar, A.R. Khan, and A.H. Zargar, among others. Copper and magnesium in non-insulin-dependent diabetes. The Postgraduate Medical Journal printed their article on pages 665-8 in 1998.

Clinical studies examining the relationship between blood trace metal levels and cerebrovascular disease, stomach cancer, and type 2 diabetes. The American Diabetes Association recommends using the citation Hokkoido Igaku Zasshi. 1987; 62(6):913-32. 16. 2017 Diabetes Medical Recommendations. 2017 saw the publication of Diabetes Care 40(S1):S14. You may get a free PDF copy at http://care.diabetesjournals.org/content/diacare/suppl/2016/12/15/40.Supplement 1.DC1/DC 40 S1 final.pdf. As of the 21st of November, 2017. 17. The authors are Okereke IO, T. Kurth, M. N. Pollak, J. M. Gaziano, and F. Grodstein. Fasting plasma insulin, C-peptide, and diabetes: results from the Doctors' Health Study II, 2010. 34(2):200-7. 18. Neuroepidemiology. This is what the research by Katsuki A, Sumida Y, Gabazza EC, Murashima S, Furuta M, and Araki-Sasaki R. Predicting insulin resistance in people with type 2 diabetes via monitoring their homeostasis model over time has been shown to be effective. 19 Shinohara K, Shoji T, Emoto M, Tahara H, Koyama H, Ishimura E, et al. End-stage renal disease patients with insulin resistance: a risk factor for cardiovascular mortality. 2002;13(10):1894-1900 in the Journal of the American Society of Nephrology.

20.Example: "Homeostasis Model Assessment of Insulin Resistance Levels as a Proposed Cutoff for Waist Circumference in Japanese Men" by Kamezaki, Sonoda, Nakata, Kashiyama, Muraoka, Okazaki, et al. Exactingly, 21 This was written by R. Muniyappa, S. Lee, H. Chen, and M. J. Quon. Worldwide Medical Journal 2012;51(16):2119-24. Current benefits, limitations, and applications of insulin sensitivity and resistance assessment in live beings. 2008; 294: E15-E26. s22 in the American Journal of Physiology-Endocrinology and Metabolism. P. Gayoso-Diz; A. Otero-Gonzalez; M. X. Rodriguez-Alvarez; F. Gude; C. Cadarso-Suarez; F. Garca; et al. Percentile distributions of the homeostasis model assessment of insulin resistance (HOMA-IR) in a population-representative sample of adults, stratified by gender and age. Here's where findings from EPIRCE come in handy. 2011; 94(1):146-55 Diabetes Research and Clinical Practice.

23. Islam M.R., I.Arslan, J.Attia, M.McEvoy, P.McElduff, A.Basher, and others. Studying the correlation between prediabetes and diabetes using a cross-sectional design in Bangladesh. PLoS ONE. 2013 Oct 8;8(10):e61776.

Twenty-four. References: Masood, N.; Baloch, G.H.; Ghori,

R.A.; Memon, I.A.; Memon, M.A.; and Masood, S.M. Type 2 diabetics, zinc, and magnesium in the blood. Journal of the College of Physicians and Surgeons of Pakistan, Vol. 19, No. 8, Pgs. 483-6, 2009.
25. Those authors are Parham M, Amini M, Aminorroaya A, and Heidarian E. Effect of zinc supplementation on microalbuminuria in people with type 2 diabetes; a randomized, double-blind, placebo-controlled crossover trial. Journal of Diabetes and its Complications 2008;5(2):102-9 S. Ferdousi & A. R. Mia. Newly diagnosed instances of type 2 diabetes and copper and zinc levels. 2012;21(3):475-80. Journal of Medicine in Mymensingh.
Twenty-seven different people contributed to the work: Rusu ML, Marutoiu C, Rusu LD, Marutoiu OF, Hotoleanu C, Poanta L, et al. Diabetic patients' magnesium, zinc, and copper levels are being evaluated. 28 Goswami R.K., Saharia G.K. 8(2005-2), Acta Universitatis Cibiniensis, Seria F, Chemia, pp.61-63. Serum zinc and glycated hemoglobin levels were evaluated in patients with type 2 diabetes mellitus at an Assam referral hospital. Published in J. Lab.
DB Sacks
29. Diabetes is a control problem with blood sugar. Clinical Chemistry and Molecular Diagnosis, 5th Edition: The Tietz Textbook, by Burtis CA, Ashwood ER, and Bruns DE. Originally published by Elsevier: New Delhi, 2012. Pages 1415-1431.
A zinc transporter was developed by K. Lemairea, M. A. Ravier, A. Schraenen, J. W. M. Creemers, R. Van de Plase, M. Granvik, and M. Despite its importance for insulin crystallization, ZnT8 expression is not required for normal glucose homeostasis in mice. PNAS. 2009; 106(35): 14872-7. \s31. We appreciate Drs. Wiernsperger and Rapin's help with this. Recent developments in our understanding of trace elements' impact on glycemic disorders. Diabetic Metabolic Syndrome: A Journal of Clinical and Evaluative Endocrinology and Metabolism. 2010; 2(70):32. Beer, N. L.; Jacobs, S. B.; Grarup, N.; Burtt, N. P.; Burtt, N. P.; Flannick, J.; Thorleifsson, G. Mutations in SLC30A8 that prevent the protein from transporting glucose are protective against developing type 2 diabetes. 46(4):356-73 (2014, Nature Genetics).