



Evaluation of random blood sugar, alkaline phosphatase and zinc levels in type 2 diabetes mellitus subjects

Authors

Dr Prabhakar Singh Bais¹, Dr Amitabh Agarwal²

¹Asst Prof. & Head, Department of Biochemistry -MLB Medical College & Hospital- JHANSI-284128

²Asso.Prof. Department of Physiology, Prasad Institute of Medical Science –Lucknow

Abstract

Objective: The purpose of present study was to evaluate the RBS, ALP, and zinc in the individuals having T2DM and to compare the study parameters with healthy controls of Bundelkhand region. The other aim was to analyze the association between study parameters in healthy control subjects group and T2DM subjects group.

Results: We observed an insignificant in age in the present study when compared between the two groups. Whereas, comparison between two groups, of parameters such as the values of RBS, ALP, and serum zinc we observed a significant difference. In the patient group, positive correlation was observed between ALP versus zinc and a negative correlation was observed between age and RBS; RBS and serum zinc. In the control group, we found positive correlation between ALP and zinc.

Conclusion: In conclusion, we have demonstrated as zinc depletion aggravates insulin resistance and may result in increased loss through urine, it may be advisable to periodically monitor zinc concentrations in T2DM subjects.

Keywords: Type 2 Diabetes mellitus, Alkaline phosphatase, Hyperglycemia, Zinc.

Introduction

There are many etiologic hypotheses for hyperglycemia in Type 2 Diabetes Mellitus (T2DM), such as genetic defect^[1], loss of insulin sensitivity^[2], HDLc defect^[3], oxidative stress^[4], glucose toxicity^[2,4], low concentrations of chromium^[5], zinc^[6], and melatonin^[7]. It is possible that several of these hypotheses, e.g., trace element deficiencies^[5,6], mitochondrial defect^[8], and oxidative stress^[2], may interact as pathogenetic mechanisms for insulin resistance in T2DM. Bioavailability of minerals is especially vulnerable to free radical damage which is reported to be high during hyperglycemia might

change the oxidation state of minerals, making it to excrete through urine^[9]. Loss of minerals may lead to decrease in its bodily content and such loss might affect the concentrations of minerals like zinc.

Extensive studies of all the metabolic abnormalities under taken so far have not been able to provide an insight into all the pathophysiologic alterations in diabetes. New areas of problems keep surfacing as a result of the complex interactions among the various factors. A few published reports of both in vitro and in vivo studies on the interactions of Alkaline Phosphatase (ALP) enzyme activity and glucose

drew attention to their alterations in diabetic states^[10,11]. In one study, ALP activity was increased in the plasma of diabetic rats^[12]. In another study, in contrast to T2DM, starvation is associated with a decrease in ALP activity which is reversed by re feeding^[13]. However, the level of ALP has not been evaluated in case of T2DM subjects in this specific region.

Zinc is a necessary as a co-factor for several enzymes that play a vital role in glucose metabolism^[14]. Studies have demonstrated lower zinc level as a common feature in patients with T2DM^[14-18], although diabetes can induce lower zinc levels, zinc deficiency has also been proposed as a risk factor for T2DM^[14-18]. Studies on models demonstrated that zinc has a negative effect on the signaling process of insulin^[17,18]. Similarly, some studies^[19,20] revealed that zinc administration has a beneficial effect on insulin action and glucose metabolism. In a study^[21,22], found an inverse correlation between zinc intake and risk of T2DM. Moreover, zinc depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with T2DM^[23,24], as well as on the evolution of complications such as retinopathy, thrombosis and hypertension^[1-3]. Interestingly, a study showed lower serum zinc is a strong independent predictor of the development of T2DM^[25]. Several studies have shown lower serum zinc concentrations in T2DM compared to healthy controls [Agrawal et al., 2011; Koenig et al., 2006]. However, there is no clear indication or hypothesis to trace whether zinc deficiency occurs first or development of T2DM.

Although low serum zinc concentrations in diabetics have also been found in the above mentioned studies, there are no reported data for diabetics living in Bundelkhand region. Therefore, the purpose of present study was to evaluate the RBS, ALP, and zinc in the individuals having T2DM and to compare the study parameters with healthy controls of Bundelkhand region. The other aim was to analyze the association between study parameters in healthy control subjects group and T2DM subjects group.

Materials & Methods

The study was conducted in the Department of Biochemistry, Maharani Laxmi Bai Medical College (MLBMC), Jhansi. Age & sex matched forty human individuals having a normal glycaemic status were taken into healthy control group. Forty T2DM subject, on treatment were included in T2DM group. The diagnosis of T2DM was made according to the norms laid by American Diabetes Association 2018. The diagnosis of T2DM group subjects was done by the consultants of General Medicine department of MLBMC. Exclusion criteria were type 1 diabetes individuals, less than five years of known duration of T2DM, and with complications. Inclusion criteria for healthy controls were non-diabetic, not taking supplementations, and having no other complications.

Sampling Procedure

Random venous blood (5ml) were drawn into gray and plane vials, 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 minutes. Samples were stored in aliquots at -20° C until assayed. Plasma glucose was estimated by using the method Glucose Oxidase and Peroxidase (DPEC – GOD/POD) purchased from Arkray Healthcare Pvt Ltd. The reagents were prepared according to the instructions provided in the kit manual. Serum Alkaline Phosphatase estimated by King & King's method of Span Diagnostics Ltd. Serum Zinc estimation by colometric method of Coral Clinical Systems. Glassware was immersed in 1.6 mol/L nitric acid for 24 H, then rinsed four times with water, and micropipette tips prior to use were rinsed in 0.8 mol/L nitric acid and then rinsed for three times in water and were allowed to dry on a absorbent paper shown not to contaminate with minerals.

Statistical Analysis

Microsoft excel was used to perform statistical analysis. Unpaired 't' test was performed to

compare the means of variables between two groups. Pearson correlation was used to find the association between two variables. $P < 0.05$ was considered significant.

Results

Figure 1 shows age, RBS, ALP, and zinc mean values observed in T2DM subjects and control subjects respectively. We observed an insignificant in age in the present study when compared between the two groups. Whereas,

comparison between two groups, of parameters such as the values of RBS, ALP, and serum zinc we observed a significant difference ($P < 0.001$).

In the patient group (Table 1) positive correlation was observed between ALP versus zinc ($R = 0.2814$, $P = 0.002$) and a negative correlation was observed between age and RBS ($R = 0.2264$, $P = 0.003$); RBS and serum zinc ($R = -0.3132$, $P = 0.034$). In the control group, we found positive correlation between ALP and zinc ($R = 0.1251$, $P = 0.01$).

Figure 1: Findings of age, RBS, ALP, and Zinc in T2DM and control groups

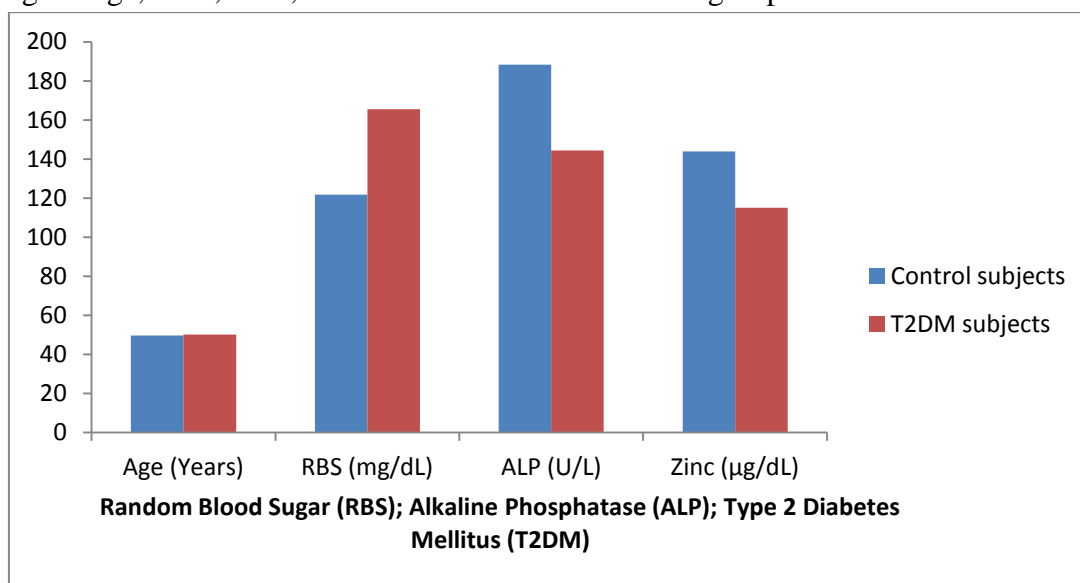


Table 1: Pearson correlation in healthy control group and T2DM group

Variables	Control subjects	T2DM subjects
Age versus RBS	$R = -0.0584$	$R = -0.2264$
Age versus ALP	$R = 0.0248$	$R = -0.0172$
Age versus Zinc	$R = -0.1142$	$R = -0.0960$
RBS versus ALP	$R = 0.3458$	$R = 0.0391$
RBS versus Zinc	$R = 0.2461$	$R = -0.3132$
ALP versus Zinc	$R = 0.1251$	$R = 0.2814$

Discussion

Aims in this study were to compare the plasma RBS, serum ALP, and serum zinc levels of patients with T2DM and healthy controls in Bundelkhand region and also to know the association between the study parameters in respective groups.

The T2DM group subjects showed altered RBS, ALP, and zinc when compared with healthy

control group subjects. Insignificant difference was observed in the age parameter when compared between the two groups.

In the present study pertaining to T2DM group subjects, an inverse correlation between age and RBS was observed, but not in healthy controls. This finding suggests that increased blood sugar in T2DM group may be caused by increase age in the subjects^[2,3]. Literature related to T2DM

reveals that T2DM is one of the aging diseases in the present world and our finding also infers the concerns with age in T2DM subjects^[1,5]. Studies reported that individuals above 40 years of age are more susceptible to develop T2DM^[7,10]. Interesting point is that no correlation was observed in the control group when compared between age and blood sugar, however, we observed insignificant difference in age when compared between T2DM and controls. Keeping in view of this finding, suggests that people who have predisposition to T2DM, are prominently driven towards the initiation of the disease rather than the people who has little predisposition. Moreover, because older adults have the highest prevalence of diabetes, such individuals have traditionally not been included in some studies that involve research on diabetes^[12,13].

For the normal functioning of ALP, zinc is an essential component for the enzyme ALP. It is interesting that a correlation between age and ALP and zinc was positive in the control group and in the T2DM as well. We also observed a negative correlation was observed between age and RBS in T2DM subjects group. At first it seems contradictory, but possible explanation could be that the oxidative stress increases with increased glucose concentration and decrease in zinc is compensatory to the increase in age and the free radical production. Many studies have shown that people with T2DM tend to have higher oxidative stress compared to healthy controls compared with same age group individuals^[2-5]. Though the present did not estimate free radicals but it is clear through the literature that oxidative stress is increased in T2DM patients and also in aged controls^[7-11]. Thus we observed lower ALP levels in T2DM subjects when compared with healthy control subjects.

Zinc is a necessary as a co-factor for several enzymes that play a vital role in glucose metabolism^[14]. Studies have demonstrated lower zinc level as a common feature in patients with T2DM^[15], although diabetes can induce lower zinc levels, zinc deficiency has also been

proposed as a risk factor for T2DM^[16]. Studies on models demonstrated that zinc has a negative effect on the signaling process of insulin^[19-24]. Similarly, some studies^[17,18] revealed that zinc administration has a beneficial effect on insulin action and glucose metabolism. In a study^[21,22], found an inverse correlation between zinc intake and risk of T2DM. Moreover, zinc depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with T2DM^[23,24], as well as on the evolution of complications such as retinopathy, thrombosis and hypertension^[28-31]. Interestingly, a study showed lower serum zinc is a strong independent predictor of the development of T2DM^[25]. Several studies have shown lower serum zinc concentrations in T2DM compared to healthy controls^[26,27]. However, there is no clear indication or hypothesis to trace whether zinc deficiency occurs first or development of T2DM. We observed significant lower zinc levels in T2DM group subjects when compared to control group subjects and an inverse correlation between serum RBS and serum zinc in T2DM group. The lower level was due to hyperglycemia present in T2DM subjects. Osmotic diuresis is seen in T2DM subjects, cause frequent urination and during this process loss of zinc can occur as suggested by the studies^[28-30]. Similar finding has been shown in studies performed with T2DM subjects^[9].

Conclusion

In conclusion, we have demonstrated that lower zinc levels in T2DM individuals in the Bundelkhand region. As zinc depletion aggravates insulin resistance and may result in increased loss through urine, it may be advisable to periodically monitor zinc concentrations in T2DM subjects. Moreover, further research is imminent to understand the deeper insights into the association of zinc and ALP with T2DM prior to the initiation or after initiation of T2DM.

Conflict of Interest: None Declared

References

1. Selvakumar G, Shathirapathiy G, Jainraj R, Paul PY. Immediate effect of bitter gourd, ash gourd, knol-khol juices on blood sugar levels of patients with Type 2 diabetes mellitus: A pilot study. *Journal of traditional and complementary medicine*. 2017 Oct 1;7(4):526-31.
2. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circulation research* 107(9), 1058-1070.
3. Kontush A, Chapman MJ. Why is HDL functionally deficient in type 2 diabetes?. *Current diabetes reports*. 2008 Feb 1;8(1):51-9.
4. Rains, J. L., & Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine*, 50(5), 567-575
5. Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., & Feng, J. (1997). Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes*, 46(11), 1786-1791.
6. Kinlaw WB, Levine AS, Morley JE, Silvis SE, McClain CJ. Abnormal zinc metabolism in type II diabetes mellitus. *The American journal of medicine*. 1983 Aug 1;75(2):273-7.
7. Peschke E, Stumpf I, Bazwinsky I, Litvak L, Dralle H, Mühlbauer E. Melatonin and type 2 diabetes—a possible link?. *Journal of pineal research*. 2007 May;42(4):350-8.
8. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science*. 2005 Jan 21;307(5708):384-7.
9. Nsonwu AC, Usoro CA, Etukudo MH, Usoro IN. Serum and urine levels of chromium and magnesium in type 2 diabetics in Calabar, Nigeria. *Malaysian Journal of Nutrition*. 2005;11(2):133-42.
10. Chen SC, Tsai SP, Jhao JY, Jiang WK, Tsao CK, Chang LY. Liver fat, hepatic enzymes, alkaline phosphatase and the risk of incident type 2 diabetes: a prospective study of 132,377 adults. *Scientific reports*. 2017 Jul 5;7(1):1-9.
11. De A, Puttannavar R, Rahman F, Adak A, Sahoo R, Prakash BR. Estimation of salivary and serum alkaline phosphatase level as a diagnostic marker in type-2 diabetes mellitus with periodontal health and disease: a clinico-biochemical study. *Journal of Oral and Maxillofacial Pathology: JOMFP*. 2018 Sep;22(3):445.
12. Olmos JM, Perez-Castrillon JL, Garcia MT, Garrido JC, Amado JA, Gonzalez-Macias J. Bone densitometry and biochemical bone remodeling markers in type 1 diabetes mellitus. *Bone and Mineral*. 1994 Jan 1;26(1):1-8.
13. Lumachi F, Camozzi V, Tombolan V, Luisetto G. Bone mineral density, osteocalcin, and bone-specific alkaline phosphatase in patients with insulin-dependent diabetes mellitus. *Annals of the New York Academy of Sciences*. 2009 Sep 1;1173:E64-7.
14. Džúrik R, Steffíková K, Spustová V, Fetkovská N. The role of magnesium deficiency in insulin resistance: an in vitro study. *Journal of hypertension*. 1991 Jan 1;9:S314.
15. Eshak ES, Iso H, Maruyama K, Muraki I, Tamakoshi A. Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: A large population-based prospective cohort study. *Clinical nutrition*. 2018 Apr 1;37(2):667-74.
16. da Silva Bandeira V, Pires LV, Hashimoto LL, de Alencar LL, Almondes KG, Lottenberg SA, Cozzolino SM. Association of reduced zinc status with poor glycemic control in individuals with type 2 diabetes mellitus. *Journal of Trace Elements in Medicine and Biology*. 2017 Dec 1;44:132-6.

17. Samadi A, Isikhan SY, Tinkov AA, Lay I, Doşa MD, Skalny AV, Skalnaya MG, Chirumbolo S, Bjørklund G. Zinc, copper, and oxysterol levels in patients with type 1 and type 2 diabetes mellitus. *Clinical Nutrition*. 2020 Jun 1;39(6):1849-56.
18. Asbaghi O, Sadeghian M, Fouladvand F, Panahande B, Nasiri M, Khodadost M, Shokri A, Pirouzi A, Sadeghi O. Effects of zinc supplementation on lipid profile in patients with type 2 diabetes mellitus: A systematic review and meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*. 2020 Mar 28.
19. Skalnaya MG, Skalny AV, Tinkov AA. Serum copper, zinc, and iron levels, and markers of carbohydrate metabolism in postmenopausal women with prediabetes and type 2 diabetes mellitus. *Journal of Trace Elements in Medicine and Biology*. 2017 Sep 1;43:46-51.
20. Salmerón J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes care*. 1997 Apr 1;20(4):545-50.
21. Meyer KA, Kushi LH, Jacobs Jr DR, Slavin J, Sellers TA, Folsom AR. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. *The American journal of clinical nutrition*. 2000 Apr 1;71(4):921-30.
22. Elsheikh M, Elhefnawy KA, Emad G, Ismail M, Borai M. Zinc alpha 2 glycoprotein as an early biomarker of diabetic nephropathy in patients with type 2 diabetes mellitus. *Brazilian Journal of Nephrology*. 2019 Dec;41(4):509-17.
23. Norouzi S, Adulcikas J, Sohal SS, Myers S. Zinc transporters and insulin resistance: therapeutic implications for type 2 diabetes and metabolic disease. *Journal of Biomedical Science*. 2017 Dec 1;24(1):87.
24. Ruz M, Carrasco F, Rojas P, Basfi-Fer K, Hernández MC, Pérez A. Nutritional effects of zinc on metabolic syndrome and type 2 diabetes: mechanisms and main findings in human studies. *Biological Trace Element Research*. 2019 Mar 15;188(1):177-88.
25. Keane WF, Brenner BM, De Zeeuw D, Grunfeld JP, McGill J, Mitch WE, Ribeiro AB, Shahinfar S, Simpson RL, Snapinn SM, Toto R. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. *Kidney international*. 2003 Apr 1;63(4):1499-507
26. Agrawal P, Arora S, Singh B, Manamalli A, Dolia PB. Association of macrovascular complications of type 2 diabetes mellitus with serum magnesium levels. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2011 Jan 1;5(1):41-4.
27. Koenig W, Khuseyinova N, Baumert J, Meisinger C, Löwel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. *Journal of the American College of Cardiology*. 2006 Oct 3;48(7):1369-77.
28. Walter RM, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. *Diabetes care*. 1991 Nov 1;14(11):1050-6.
29. Turner RC, Cull CA, Frighi V, Holman RR, UK Prospective Diabetes Study (UKPDS) Group. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). *Jama*. 1999 Jun 2;281(21):2005-12.

30. Navarro JF, Mora C, Maci3a M, Garc3a J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. *American Journal of Kidney Diseases*. 2003 Jul 1;42(1):53-61.
31. Viktor3nov3 A, Tošerov3 E, Križko M, Ńuračkov3 Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. *Metabolism*. 2009 Oct 1;58(10):1477-82.