ORIGINAL ARTICLE

The Urinary Levels of Some Essential and Toxic Metals in Type 2 Diabetic Patients and Non-diabetic Healthy Control Human Subjects

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ABSTRACT

Objective: *To compare the levels of some selected essential and toxic metals in type 2 diabetic (T2D) patients and non-diabetic (ND) healthy controls. Furthermore, the study aims to evaluate the possible interrelationship of metals in urine of T2D patients and ND healthy controls.*

Methods: *The study was conducted at the Diabetes and Cardio-Metabolic Disorder Laboratory, Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan, which comprised T2D patients and an equal number of ND healthy controls. Two biofluids,* ie*, blood and urine samples of diabetes and control subjects were considered. Magnesium (Mg), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), manganese (Mn), iron (Fe) and zinc (Zn) levels were measured in the urine samples of both groups. Metal concentrations were assessed by atomic absorption spectrophotometry, whereas biochemical parameters in the serum of the study subjects were estimated using commercial kits on Microlab-300. Graph Pad Prism 5 was used for statistical analyses.*

Results: *There were 49 T2D patients with an average age of 53.5 ± 10.4 years and 49 ND healthy controls with a mean age of 45.2 ± 9.2 years. Fasting serum glucose and triglycerides were significantly (*p *< 0.05) higher in diabetics relative to their ND control subjects. The levels of Mg, Zn and Pb were found to be significantly higher (*p *< 0.05). Fe decreased more than 30% in urine samples of T2D patients compared with the ND control group. Cu, Cr, Mn and Ni manifested no change. A significant positive correlation of Pb with Zn (r = 0.3284,* p *= 0.02) and Mn (r = 0.3648,* p *= 0.01) was found in T2D patients.*

Conclusion: *An imbalance in the levels of selected metals was observed in urines of type 2 diabetes when compared with controls. Furthermore, statistical results revealed positive correlations between toxic metal Pb, with essential trace elements,* ie*, Mn and Zn. Thus, it could be concluded that toxic metals, especially Pb may have a role in renal dysfunction with successive loss of essential metals such as Zn and Mn through urination.*

Keywords: Essential metals, toxic metals, type 2 diabetes, urine.

INTRODUCTION

Diabetes mellitus (DM) is one of the lifestyle-related diseases that is the most widespread worldwide. Diabetes mellitus is usually classified as type 1 diabetes and type 2 diabetes (T2D). In the case of the former case, the hyperglycaemia results from a severe insulin deficiency caused by the destruction of pancreatic beta cells (1). Over the time, it can be regulated by using different

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insulin modalities (2, 3). In the latter case, glucose elevation develops because of insulin resistance (IR) and insufficient insulin secretion. Insulin is secreted from pancreatic beta cells in response to increased blood glucose levels. Insulin binds to its receptor on the surface of target tissues and stimulates glucose uptake by the cells. This result in blood glucose level being decreased. A decreased response to insulin may result in impaired glucose tolerance or T2D, which is characterised by elevated blood glucose level and IR (4). Several factors such as heredity, age, smoking, obesity, diet, sex, sedentary life style, socioeconomic status and hypertension, *etc*, are involved in the disease development. Among all these parameters, balanced diet and active lifestyle play vital roles in T2D prevention (5, 6).

The quality and quantity of fat and carbohydrates-based diet intake have significant impact on the risk of T2D (7). Imbalance in biologically active form of many metals such as iron (Fe), magnesium (Mg), zinc (Zn), chromium (Cr), manganese (Mn), lead (Pb), nickel (Ni), and copper (Cu), participate in the alteration of glucose metabolism of T2D patients (8). These metals are involved in the structural and catalytic activities of many metalloenzymes. Magnesium is needed as a cofactor for more than 300 enzymes, particularly involved in glucose homeostasis. The reduced plasma level of Mg has been reported in poorly controlled T2D patients (9, 10). Prolonged hyperglycaemia is also due to increased and decreased urinary and blood plasma levels of Zn. The decreased plasma level of Zn adversely affects the ability of pancreatic islet cells to produce and secrete insulin (11). Zinc plays a key role in the storage and secretion of insulin. Previous studies show that people with T2D have suboptimal Zn status due to its low absorption and increased urinary excretion (8). Copper is needed for the catalytic activity of superoxide dismutase that takes part in the protection of cells from superoxide radicals (8).

Whereas Zn protects sulfhydryl groups of proteins and enzymes by its antioxidant properties from free radical attack in the body, it is also reported that glycated proteins bind transition metals and their glycocholates play a key role in the aetiology of peripheral vascular dysfunction in T2D (12). Chromium is involved in glucose metabolism by enhancing the effects of insulin. Certain low molecular weight Cr (LMWCr) compounds may enhance the response of insulin receptors to insulin. The ability of LMWCr compounds to activate the insulin receptors depends upon its Cr contents. When the insulin level drops due to normalisation of blood glucose level, LMWCr compounds may be released from the cell in order to diminish its effects (13).

Iron is a potential candidate in many cellular reactions that produce reactive oxygen species (ROS). Such reactions contribute to tissue damage and increased oxidative stress, thereby leading to the pathogenesis of T2D. Numerous studies have suggested a link between high body iron stores and diabetes pathogenesis (14, 15). Similarly, Pb is dangerous to most of the human body organs and interferes with metabolism and cellular functions. Some other toxic metals such as cadmium and arsenic are implicated as they disrupt the glucose uptake and alter the related molecular mechanism in glucose regulation (16, 17).

The concentration of these metals in various biofluids is adversely changed with disease state, particularly in serum and urine. Among these biospecimens, urine is good since it is freely obtainable and non-invasively tested (8). Therefore, in the present study, the urine samples of T2D patients were considered to assess various metal levels in their samples relative to the non-diabetic (ND) apparently healthy control subjects. Mutual interactions of toxic and essential metals were also calculated.

SUBJECTS AND METHODS

Study subjects

The study population comprised 98 Pakistani Punjabi adults including 49 ND control subjects and 49 well-characterised T2D patients. The ND and T2D age (mean ± SD) was 45.2 ± 9.2 and 53.5 ± 10.4 years, respectively, with an age range of 35–80 years. A well-defined questionnaire was used to obtain information regarding occupation, physical activity, lifestyle patterns and dietary habits, past and current illness, and medication status. Anthropometric measurements of body height, weight and waist and hip circumference were also measured. T2D patients with nephropathy and malignancy were not included in the present study. After measuring height and weight, body mass index (BMI) (kg/m^2) was calculated by dividing weight (kg) by height in metre square (m^2). An informed consent was obtained from all subjects for their participation in this study. For the collection of urine and blood samples, camps were organised in Faisalabad, Pakistan. All subjects were informed to attend sample collection sites on a specific day in the morning after 12–14 hours overnight fast. During the fasting period, the subjects were only allowed to drink plain water. The study received approval from the institutional ethics committee.

Biological samples (blood and urine)

A venous blood sample was taken in a gel-containing vacutainer without an anticoagulant between 8 and 10 AM, after an overnight fast of 12–14 hours duration. The blood taken in vacutainers was allowed to clot and subsequently centrifuged for 10 minutes at 900 rpm to separate the serum. This was stored at −20ºC prior to downstream analysis. Serum were analysed for biochemical tests such as fasting glucose, cholesterol, triglycerides, total protein, *etc*. These biochemical parameters were estimated by colorimetric/spectrophotometric methods using commercial kits (DiaSys, Germany) on a semi-automatic clinical chemistry analyser (Microlab-300, Merck, Germany).

The urine samples of the same ND control subjects and T2D patients were also collected for the analysis of various metals. Different aliquots were stored at −20ºC until carrying out further analyses.

Urinary metals analysis

Varian double beam flame atomic absorption spectrophotometer (FAAS) (AA240 FS, Australia), with manual background correction, was employed for the determination of transition metals such as Zn, Cr, Cu, Mn, Pb, Ni and Fe and macronutrients such as Mg in the urine samples of T2D and control subjects. The analyses were carried out by using a hollow cathode lamp of respective metal under standard instrumental operational conditions. Acetylene gas was used as fuel.

Analytical procedure

For mineral estimation, 5 mL of each urine sample were digested in aqua regia and heated up to dryness. The residues were dissolved in 10 mL of concentrated HNO_3 and heated carefully until the solution turned colourless. It was then transferred into 25 mL flasks, and volumes were made up to the marks by de-ionised water. These sample solutions along with standards were aspirated to FAAS. For Mg estimation, serum samples were diluted (1 mL of urine in 25 mL of aqueous solution) with distilled water prior to their aspiration into an atomic spectrophotometer for analysis. The amount of each element in the sample was determined from its respective calibration curve. The analysis of each sample was made in duplicate, and the averaged value is reported in results.

RESULTS

General physical and biochemical characteristics of study subjects

General characteristics and laboratory findings of the studied human subjects are given in Table 1. A total of 49 ND control (males = 28 and females =21) subjects and 49 T2D (males = 20 and females = 28) patients were considered for selected metals analysis such as Cu, Mg, Cr, Fe, Zn, Ni and Mn using an atomic absorption spectrophotometer. The mean age of the ND control and T2D subjects was 45.1 ± 9.2 and 53.5 ± 10.4 years, respectively. Mean BMI (28 ± 0.7 kg/m^2) of T2D patients was significantly higher (p = 0.005) than the BMI (25.5 ± 0.34 kg/m^2) of the ND control group. There was also a significant difference (p = 0.004) in the mean value of waist hip ratio (WHR) (0.97 ± 0.08) of T2D patients when compared with the WHR (0.92 ± 0.6) of the ND control group. Fasting serum glucose and triglycerides were significantly ($p < 0.05$) higher in diabetics relative to their ND control subjects. On the other hand, fasting serum cholesterol, total proteins and creatinine were not significantly ($p < 0.05$) different between T2D and ND subjects (Table 1).

Urinary metal levels in T2D patients versus non-diabetic (ND) control subjects

In urinary metal analysis, the levels of Mg and Zn were found to be significantly higher ($p < 0.05$) in urine

Table 1: Anthropometric and biochemical characteristics of ND and T2D subjects

Subjects Parameters	Non-diabetic controls (N = 49)	T2D patients (N = 48)	*p*-value
BMI (kg/m^2)	25.5 ± 0.34	28 ± 0.7	0.005
WHR	0.92 ± 0.06	0.97 ± 0.08	0.004
Glucose (mg/dL)	88.7 ± 1.7	199.4 ± 10.7	< 0.0001
Cholesterol (mg/dL)	183.5 ± 6.6	176.3 ± 6.2	NS
Triglycerides (mg/dL)	127.9 ± 9.9	179.3 ± 15.07	0.005
Total proteins (g/dL)	7.7 ± 0.5	7.6 ± 0.13	NS
Creatinine (mg/dL)	0.73 ± 0.05	0.78 ± 0.04	NS

T2D: type 2 diabetes; ND = non-diabetic; BMI = body mass index; WHR = waist hip ratio; NS = non-significant.

samples of T2D compared to the ND control group. The mean values of Mg and Zn were 17.7 ± 1.6 and 0.46 ± 0.03 ppm for T2D patients, while in the case of the ND control group, the levels of Mg and Zn were obtained as 11.48 ± 1.3 and 0.33 ± 0.03 ppm, respectively. The statistical analyses show that the levels of these two metals, Mg and Zn, were 35 and 28% higher in the urine samples of T2D patients relative to their respective ND control subjects. On the other hand, in urine samples, the Fe level (1.16 ppm) in the T2D group was found to be lower ($p = 0.005$) than the value (1.77 ± 0.2 ppm) obtained for Fe in the urine samples of ND control human subjects. The Pb metal was found to be significantly higher ($p = 0.03$) in the urine of diabetics relative to the ND control group. The comparative levels of Pb in T2D patients and ND control subjects were 0.047 ± 0.003 and 0.036 ± 0.003 ppm, respectively. The calculated percent fold increase of Pb in the urine samples of T2D patients was about 29% as compared with the ND control subjects. The comparative urinary levels of Cu, Cr, Mn and Ni were higher in T2D patients than in ND control subjects, but these differences were not statistically significant (Fig. 1).

Fig. 2 shows a significant positive correlation of Pb with Zn ($r = 0.3284$, $p = 0.02$) and Mn ($r = 0.3648$, $p = 0.01$) in the urine samples of T2D patients. On the other hand, no correlation was found between Pb and other studied metals such as Mg, Fe, Cu, Cr, Mn and Ni. Similarly no relationship was found among Mg, Mn, Zn, Fe, Cu, Cr, Mn and Ni in the same diabetic urine samples.

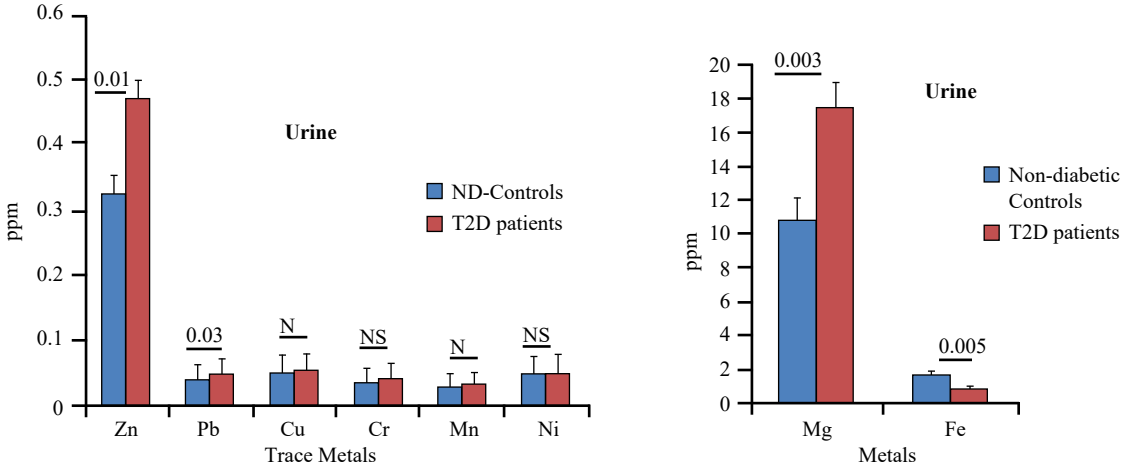

Fig. 1: Comparative urinary metals status of type 2 diabetics and ND control subjects. Zn = zinc; Pb = lead; Cu = copper; Cr = chromium; Mn = manganese; Ni = nickel; Mg = magnesium; Fe = iron; NS = not significant; T2D = type 2 diabetes; ND = non-diabetic.

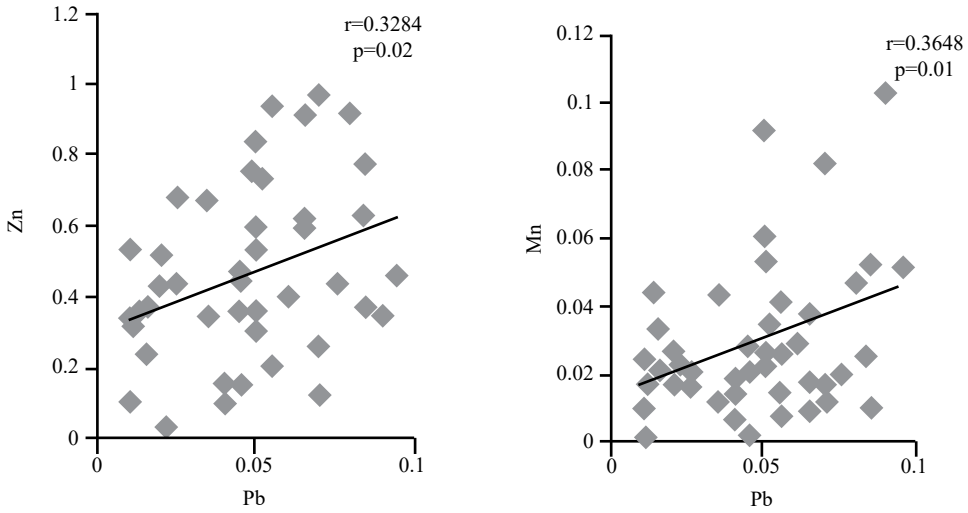

Fig. 2: Correlation analysis of Pb with Zn and Mn in the urine samples of T2D patients. Pb = lead; Zn = zinc; Mn = manganese; T2D = type 2 diabetes.

Non-diabetic control subjects and T2D patients were subdivided into ND control males versus ND control females and T2D males versus T2D females for further analysis to investigate gender-related changes in the metal concentrations.

Urinary metal levels of ND control male versus ND female subjects

The statistical analysis of the respective metal levels showed no significant difference in any of the above-mentioned metals between ND control male versus ND control female sub-groups. However, a close look shows that all metals including Zn, Cu, Mg, Pb, Cr, Mn and Ni were found to be higher in the urine samples of ND males, except Fe metals, which was 10-fold higher in the urines of ND females.

Urinary metal levels between T2D male and female patients

Similarly, no significant difference was found between T2D male and female patients with respect to urinary Mg, Pb metals and other trace elements (TEs) including Cu, Zn, Fe, Cr, Mn and Ni. In this case, the urinary levels of Cu, Fe, Cr and Ni were observed non-significantly higher in T2D male versus T2D female patients. On the other hand, Zn, Mg and Mn concentrations were marginally higher in T2D females while comparing with T2D male patients.

DISCUSSION

It has already been established that hyperglycaemia in diabetic patients is linearly linked with the production of advanced glycation end products of several proteins and highly ROS such as H_2O_2, OH and O_2- (18). Metals are central to hold the bioactivity of numerous enzymes and proteins on which life is based. Derangements in the metal (*eg*, Zn, Mg, Cu, Mn, Cr, *etc*) metabolism could lead to the pathogenesis of diabetes. Imbalances in the status of essential metals have been reported as a worsening incidence in the progression of diabetes (19). Several previously published studies manifested the functions of transition and TEs such as Fe, Zn, Mg, Cu, Mn and Cr in insulin action and carbohydrate metabolism. The alterations in these elements attributed to hyperglycaemia and enhanced magnitude of protein glycation in diabetic state. A case-control study revealed alterations in the metabolism of metallic elements such as Zn, Mg, Cu, Mn and Cr in diabetic patients relative to their ND control subjects (20).

In the present study, an increased urinary level of Mg was observed in T2D patients compared with the ND control subjects. These findings are supported by a previously published report (21), in which negative correlation was reported between serum Mg and urinary Mg levels in diabetic patients. This correlation revealed a decrease of serum Mg with concomitant increase of the urinary Mg level. Renal Mg excretion may be regulated by insulin. The mechanism of the urinary loss of Mg may result probably from the reduced capacity of tubular reabsorption of Mg due to osmotic action of glycosuria (9). Previous studies have also revealed an association between hypomagnesaemia and elevated glucose levels. Yet, another study correlated Mg deficiency with fat accumulation in various body tissues, which subsequently leads to obesity-related metabolic disorders (22). This provides a further explanation to high presence (loss) of Mg in the urine of T2D patients as more than 60% of them are obese.

Urinary Fe concentration was less in T2D patients than ND control subjects. This agrees with the previously published results (20), in which concurrent elevation of the serum iron level with decrease urinary excretion of Fe was observed in diabetic patients. Several studies manifested possible association between high iron stores and various metabolic parameters such as blood serum insulin, glucose, dyslipidaemia and obesity (23). Iron is a potential catalyst that produces ROS, which subsequently develop oxidative stress and damages healthy tissues, thereby increasing the risk of T2D (24).

Copper concentrations in various biofluids including urine have been found to be altered in diabetic subjects. The higher urinary level of Cu was observed in T2D patients compared with the ND control subjects in the present study, though the difference was not significant. These results are consistent with the previously published results (25). It has also been manifested that Cu deficiency is an important cause of adiposity development (26).

The urine Zn level was significantly higher in T2D patients compared with the ND control subjects. Previous clinical studies have reported excessive loss of Zn through urine with decreased values in the blood serum in diabetes as compared with ND control subjects (20, 27). The decreased gastrointestinal absorption with increased urinary excretion of Zn in diabetes may lead to hypozincaemia. It has been suggested that prolonged hyperglycaemia may adversely affect the transport of Zn back into the renal tubules. In addition to this, mutation in the Znt8 transporter gene (*SLC30A8*) contributes to

Zn deficiency. Zn deficiency in diabetes may be associated with increased oxidative stress and tissue damaging (20, 28). Furthermore, Zn is required for the synthesis of insulin hexamer, which is vital to keep the blood sugar level in normal range. It has been reported that mutation in *SLC30A8* gene is associated with T2D (8, 29).

In the present study, the level of Cr in the urine samples of T2D patients was higher than those of respective ND control subjects, but the difference was not significant. It has been extensively studied that Cr takes part in increasing receptors number and binding of insulin to insulin receptors in target organ (30). Trivalent chromium (Cr III) is effective for glucose uptake by the cells. Chromium urinary excretion is expedited in hyperglycaemic condition (30, 31). Anderson *et al*. reported antioxidant effect of the combined supplementation of Cr and Zn in T2D patients (28). Manganese urinary level was higher than their respective ND healthy control subjects. There is no data available in literature which shows comparative urinary excretion of Mn in T2D patients relative to their ND control group. Nevertheless, studies report the possible involvement of Mn deficiency in the pathogenesis of DM (20). However, it has been reported that oral Mn administration improves the insulin sensitivity in diabetic patients (32).

In this study, the mean levels of two toxic metals such as Pb and Ni were studied in T2D patients versus ND control subjects. The results of the present study revealed that the urinary level of Pb was significantly higher in T2D patients as compared with the ND control subjects. Lead is a heavy transition metal that is harmful to most of the human tissues and interferes with the body's physiological functions (33, 34). Lead is ubiquitous environmental toxin that is associated with a wide range of biochemical and physiological dysfunctions (26). A recent study revealed that environmental exposure to Pb is contributing in the development of neuropathy-related complications in T2D patients (35). Several studies have manifested a positive correlation between environmental Pb exposure and reduction in renal function efficiency, and Pb chelating therapies may be effective to improve renal function in ND subjects with chronic kidney disorders (36). Lead also contributes to various metabolic disorders including obesity by substituting vital TEs, *eg*, Zn, Cr and Cu with sequential depletion of anti-oxidative potential of blood in individuals who were frequently exposed to toxic heavy metals including Pb (26).

In the present study, Pb shows positive associations with two essential TEs such as Zn and Mn in the urine specimens of T2D patients, as shown in Fig. 2. Both these TEs (Zn and Mn) act as cofactors for the activity of several enzymes. The increased interactive urinary excretion of TEs such as Zn with toxic Pb metal might adversely affect the levels of these two essential metals in blood sera of T2D patients. As interactions of toxic metals including Pb with essential trace elements have been extensively studied (26). Continuous loss of Zn and Mn over time through urination causes deficiency in TEs levels that may demonstrates the decrease of Zn and Mn in blood of diabetic patients (37).

The urinary Ni level was not different between T2D patients and ND control subjects. There is scant information available in literature; some human and animal studies indicate that Ni deprivation lowers the blood plasma glucose level, which subsequently alters metallic elements (Zn, Fe and Cu) tissue distribution and metabolism (38). An animal study shows that Ni induces oxidative stress in body organs particularly in kidney (39).

In the present study, no association was found between the urinary levels of aforementioned metallic elements with age, gender, clinical and anthropometric characteristics. Kazi *et al*. reported sex- and age-related impact on the bioavailability of TEs (20). The reason for the disparity in the findings is not yet clear and may be attributed to sample size. Simultaneously, the analysis of metals in various biological specimens including urine has been conducted in several previous studies. Among them, blood plasma and urine may be more beneficial, since derangements of metals in blood seems to be reflected in urine of diabetic patients (10). Moreover, urine can be obtained easily and non-invasively compared to with other biofluids such as blood plasma/serum, lacriminal fluid and synovial fluid (8); therefore, urine was used in this study.

CONCLUSION

Based on the findings obtained in this study, it could be concluded that the normal levels of essential metals are disturbed in T2D patients, which are reflected in their urine samples. The status of essential TEs in various body tissues is influenced by renal excretion. Alteration in the level of one metal may influence the normal levels of other metals. As in the present study, statistical analysis demonstrated a linear relationship between a toxic metal Pb with two essential TEs, *ie*, Zn and Mn in the urine samples of T2D patients. It can also be suggested that toxic metals such as Pb may have a role to induce renal tubular dysfunction in the study diabetic

population. Subsequently, dysfunctional kidneys may become a potential source for the loss of several essential TEs through urine voiding rather than their retention in blood plasma/serum in order to retain the homeostasis of blood and other tissues. Consistent with previous reports, in the present study the high level of Zn was observed in the urine samples of T2D patients. This high urinary excretion of Zn results in decrease levels of Zn in blood plasma/serum, which is the sign of disruptions in the Zn-based antioxidant mechanism. However, the levels of essential trace elements vary in samples studied among individuals. Thus, it may be worth exploring further interactions among various types of metals in T2D patients using large number of urine samples.

Briefly in the present study, in addition to alterations in metal status in the urine samples of T2D patients, statistical analysis demonstrates relationships between more than two metallic elements, which after further large population-based prospective studies may prove to have scientific as well as practical implications in understanding and controlling diabetes or its complications.

AUTHORS' NOTE

The authors declare that they have no conflict of interest about this research publication. ARK conducted and wrote the initial drafts and FRA supervised and critically revised/approved the manuscript.

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REFERENCES

1. Sundsten T, Ortsäter H. Proteomics in diabetes research. Mol Cell Endocrinol 2009; **297:** 93–103.
2. Moller DE. New drug targets for type 2 diabetes and the metabolic syndrome. Nature 2001; **414:** 821–7.
3. Khan AR, Awan FR. Review: mining of protein based biomarkers for type 2 diabetes mellitus. Pak J Pharma Sci 2012; **25:** 889–901.
4. Leahy JL. Pathogenesis of type 2 diabetes mellitus. Arch Med Res 2005; **36:** 197–209.
5. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. New Eng J Med 2001; **345:** 790–7.
6. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. New Eng J Med 2001; **344:** 1343–50.
7. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Sato M et al. Influence of fat and carbohydrate proportions on the metabolic profile in patients with type 2 diabetes: a meta-analysis. Diab Care 2009; **32:** 959–65.
8. Khan AR, Awan FR. Metals in the pathogenesis of type 2 diabetes. J Diab Metab Disord 2014; **13:** 16.
9. Swaminathan R. Magnesium metabolism and its disorders. The Clin Biochem Rev 2003; **24:** 47.
10. Afridi HI, Kazi TG, Kazi N, Jamali MK, Arain MB, Jalbani N et al. Potassium, calcium, magnesium, and sodium levels in biological samples of hypertensive and nonhypertensive diabetes mellitus patients. Biol Trac Elem Res. 2008; **124:** 206–24.
11. Brender JR, Hartman K, Nanga RPR, Popovych N, de la Salud Bea R, Vivekanandan S et al. Role of zinc in human islet amyloid polypeptide aggregation. J Am Chem Soc. 2010; **132:** 8973–3.
12. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C et al. Blood metals concentration in type 1 and type 2 diabetics. Biol Trac Elem Res 2013; **156:** 79–90.
13. Król E, Krejpcio Z. Chromium (III) propionate complex supplementation improves carbohydrate metabolism in insulin-resistance rat model. F Chem Toxicol 2010; **48:** 2791–6.
14. Raj S, Rajan G. Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. Int J Res Med Sci 2013; **1:** 12.
15. Kundu D, Roy A, Mandal T, Bandyopadhyay U, Ghosh E, Ray D. Relation of iron stores to oxidative stress in type 2 diabetes. Nig J Clin Prac 2013; **16:** 100–3.
16. Akinloye O, Ogunleye K, Oguntibeju O. Cadmium, lead, arsenic and selenium levels in patients with type 2 diabetes mellitus. Afr J Biotech 2010; **9:** 5189–95.
17. Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, Guallar E. Arsenic exposure and prevalence of type 2 diabetes in US adults. JAMA 2008; **300:** 814–22.
18. Nishikawa T, Araki E. Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications. Anti Red Signal 2007; **9:** 343–53.
19. Viktorínová A, Tošerová E, Križko M, Ďuračková Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metab. 2009; **58:** 1477–82.
20. Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N et al. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trac Elem Res 2008; **122:** 1–18.
21. Chinyere NA, Opara UCA, Henrieta E, Nathaniel U. Serum and urine levels of chromium and magnesium in type 2 diabetics in Calabar, Nigeria. Mal J Nutr 2005; **11:** 133–142.
22. Song CH, Song IK, Ju SY, Ock SM. Serum magnesium level is negatively associated with fasting serum glucose level in Korean adults. Biol Trac Elem Res 2011; **143:** 612–8.
23. Rajpathak S, Ma J, Manson J, Willett WC, Hu FB. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. Diab Care 2006; **29:** 1370–6.
24. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002; **51:** 2348–54.
25. Ito S, Fujita H, Narita T, Yaginuma T, Kawarada Y, Kawagoe M et al. Urinary copper excretion in type 2 diabetic patients with nephropathy. Nephron. 2001; **88:** 307–12.
26. Padilla MA, Elobeid M, Ruden DM, Allison DB. An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02. Int J Environ Res Pub Health. 2010; **7:** 3332–47.
27. Nasli-Esfahani E, Faridbod F, Larijani B, Ganjali MR, Norouzi P. Trace element analysis of hair, nail, serum and urine of diabetes mellitus patients by inductively coupled plasma atomic emission spectroscopy. Iran J Diab Lipid Disord 2011; **10:** 1–9.
28. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J Am Coll Nutr 2001; **20:** 212–8.
29. Xiang J, Li XY, Xu M, Hong J, Huang Y, Tan JR et al. Zinc transporter-8 gene (SLC30A8) is associated with type 2 diabetes in Chinese. J Clin Endocrinol Metab 2008; **93:** 4107–2.
30. Cefalu WT, Hu FB. Role of chromium in human health and in diabetes. Diab Care 2004; **27:** 2741–51.
31. Diwan A, Pradhan A, Lingojwar D, Krishna K, Singh P, Almelkar S. Serum zinc, chromium and magnesium levels in type 2 diabetes. Int J Diab Develop Ctries. 2006; **26:** 122–3.

32. Ekmekcioglu C, Prohaska C, Pomazal K, Steffan I, Schernthaner G, Marktl W. Concentrations of seven trace elements in different hematological matrices in patients with type 2 diabetes as compared to healthy controls. Biol Trac Elem Res 2001; **79:** 205–19.
33. Kazi TG, Jalbani N, Kazi N, Arain MB, Jamali MK, Afridi HI et al. Estimation of toxic metals in scalp hair samples of chronic kidney patients. Biol Trac Elem Res. 2009; **127:** 16–27.
34. Kazi TG, Jalbani N, Kazi N, Jamali MK, Arain MB, Afridi HI et al. Evaluation of toxic metals in blood and urine samples of chronic renal failure patients, before and after dialysis. Renal Fail 2008; **30:** 737–45.
35. Lin J, Lin-Tan D, Yu C, Li Y, Huang Y, Li K. Environmental exposure to lead and progressive diabetic nephropathy in patients with type II diabetes. Kid Int 2006; **69:** 2049–56.
36. Yu CC, Lin JL, Lin-Tan DT. Environmental exposure to lead and progression of chronic renal diseases: a four-year prospective longitudinal study. J Am Soc Nephrol 2004; **15:** 1016–22.
37. Bamgbose O, Opeolu B, Bamgbose J. Levels of cadmium, lead and zinc in urine of randomly selected smokers and non-smokers residents of Abeokuta City, Nigeria. Research J Appl Sci 2007; **2:** 192–7.
38. Serdar MA, Bakir F, Haşimi A, Çelik T, Akin O, Kenar L et al. Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. Int J Diab Develop Ctries. 2009; **29:** 35.
39. Kubrak OI, Husak VV, Rovenko BM, Poigner H, Mazepa MA, Kriews M et al. Tissue specificity in nickel uptake and induction of oxidative stress in kidney and spleen of goldfish, exposed to waterborne nickel. Aqua Toxicol 2012; **118:** 88–96.