Comparative Analysis of Serum Trace Elements and Nephrotoxic Elements between Diabetic Nephropathy and Non-Diabetic Kidney Disease Patients

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ABSTRACT:

Background: Diabetic nephropathy (DN) is a major complication of diabetes mellitus and a significant contributor to end-stage renal disease (ESRD). This study investigates the relationship between serum trace elements (Zn, Cu, Mg, Fe) and nephrotoxic elements (As, Pb, Cd, Hg) with renal function in diabetic patients with nephropathy, diabetic patients without nephropathy and non-diabetic chronic kidney disease patients, in addition to controls.

Methods: A cross-sectional study was conducted at King Abdulaziz Air Base Hospital, Dhahran, from December 2021 to May 2022, involving 123 participants divided into four groups: diabetic nephropathy, diabetes without nephropathy, chronic kidney disease (CKD), and healthy controls. Serum levels of trace and nephrotoxic elements were measured using inductively coupled plasma mass spectrometry. Renal function tests and glycated hemoglobin (HbA1c) levels were also measured. Pearson's and Spearman's correlation analyses were performed to assess the relationships between these element levels and renal function.

Results: The mean (SD) serum levels of Zn 85 (25) µg/dL and Cu 110 (35) µg/dL were significantly higher in the diabetic nephropathy group, while Fe 17.3 (6.9) µmol/L, As 0.33 (0.74) µg/dL, and Pb 7.7 (2.3) µg/dL were significantly higher in the diabetes without nephropathy group. Only serum magnesium levels were significantly higher in the chronic kidney disease group. Interestingly, mercury was highest in the control group at 1.14 (0.81) µg/dL. Serum creatinine showed direct correlations with trace elements Zn (r = 0.406) and Cu (r = 0.358), and with nephrotoxic elements As (r = 0.328), Pb (r = 0.384), and Hg (r = 0.287). HbA1c was positively correlated with Zn (r = 0.307), As (r = 0.309), and Pb (r = 0.365), while it was inversely correlated with Mg (r = -0.308) and Fe (r = -0.359).

Conclusion: In this study, trace elements Zn and Cu, as well as toxic elements As and Pb, were positively correlated with serum creatinine and negatively impacted renal function. Glycated hemoglobin was positively correlated with Zn, As, and Pb, while it was inversely correlated with Mg and Fe. Both trace and toxic elements are associated with renal function and HbA1c, highlighting the need for further research.

Background

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus (DM) and a leading cause of end-stage renal disease (ESRD) [1]. In Saudi Arabia, the prevalence of DM is approaching 18.3%, one of the highest rates in the world [2]. Of those with DM, 25-40% will develop diabetic nephropathy, significantly contributing to the burden of chronic kidney disease (CKD) and ESRD in the country.

The pathophysiological mechanisms of DN are complex, involving hyperglycemia, oxidative stress, inflammation, and altered mineral metabolism [3]. Increasing interest has been directed toward understanding the role of trace and nephrotoxic elements in the pathogenesis of DN [4]. The kidneys are particularly vulnerable to damage from high glucose levels, which can trigger oxidative stress and inflammation [5]. Oxidative stress, defined by an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense mechanisms, plays a pivotal role in the development and progression of DN [6]. ROS can damage cellular components, including lipids, proteins, and DNA, leading to cellular dysfunction and apoptosis [7].

Trace elements such as zinc (Zn), copper (Cu), magnesium (Mg), and iron (Fe) are essential micronutrients involved in various enzymatic and biochemical processes, including the regulation of oxidative stress and inflammation [8]. In addition to their diverse metabolic and physiological roles, Zn, Cu, and Fe serve as cofactors for antioxidative enzymes such as superoxide dismutase (SOD), cytochrome oxidase, and catalase, respectively [9–12]. Magnesium is directly involved in enzymes that catalyze the glycolysis pathway and insulin secretion [13]. Dysregulation in the levels of these trace elements has been linked to oxidative stress, contributing to the development of diabetic nephropathy (DN) [14, 15].

In addition to trace elements, nephrotoxic elements such as arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) are recognized as environmental and occupational hazards that contribute to kidney damage [16]. Chronic exposure to these elements, even at low concentrations, can induce nephrotoxicity through mechanisms such as oxidative stress, inflammation, and direct cytotoxicity to renal cells [17].

In diabetic patients, altered trace element metabolism and increased urinary losses due to hyperglycemia and glycosuria can exacerbate deficiencies in these elements. Furthermore, the progression of DN impairs the kidneys' ability to regulate trace element homeostasis, leading to further dysregulation [18]. Conversely, the accumulation of nephrotoxic elements in the kidneys can cause renal damage through direct cytotoxic effects and the induction of oxidative stress and inflammation [19].

This study aims to compare the serum levels of trace elements (Zn, Cu, Mg, and Fe) and nephrotoxic elements (As, Pb, Cd, and Hg) in diabetic patients with nephropathy, diabetics without nephropathy, another group with non-diabetic kidney disease, and normal controls. By elucidating the differences in these elements and their correlations with renal function, the study seeks to provide insights into the potential role of trace element dysregulation and nephrotoxic element exposure in the pathogenesis of DN. Beyond conventional therapeutic management of DN, addressing trace element dysregulation and reducing exposure to nephrotoxic elements could offer additional preventive and therapeutic benefits, making this study worthwhile.

Methods

Study Design and Setting

This cross-sectional study was conducted to evaluate the relationship between serum trace elements, nephrotoxic elements, and renal function in diabetic patients with DN, diabetes mellitus DM and in non-diabetic CKD. The study was carried out at King Abdulaziz Air Base Hospital, Dhahran, between December 2021 and May 2022.

Participants were recruited from patients attending the endocrinology and nephrology clinics at King Abdulaziz Air Base Hospital for follow-up, as well as from the general community. A total of 123 participants were enrolled and categorized into four distinct groups: 20 patients with DN (12 male, 8 female), 25 patients with diabetes mellitus (DM) without nephropathy (15 male, 10 female), 22 patients with chronic kidney disease (CKD) without diabetes mellitus (13 male, 9 female), and 56 healthy individuals without DM or kidney disease (28 male, 28 female).

Inclusion criteria were as follows: known type 2 DM patients diagnosed in endocrinology clinics with DN, based on increased urinary albumin excretion (UAE >19 μg/ml and ≤199 μg/min) or the presence of proteinuria (>0.5 g/24 h), with no other renal diseases [20] were included in the DN group. Patients diagnosed with type 2 DM without evidence of DN were included in the DM group. Patients diagnosed with CKD in nephrology clinics without diabetes were included in the CKD group [21]. Healthy individuals without any history of diabetes,

kidney disease, or other chronic illnesses, and with normal laboratory findings, were recruited to participate in the study and included in controls group.

The objectives of the study were briefly explained to all participants, and written informed consent was obtained. Patients with type 1 DM, other chronic diseases, symptoms suggestive of other endocrine or liver diseases, individuals taking mineral or vitamin supplements within the last six months, and pregnant women were excluded from the study.

Socio-demographic data, including age, sex, and duration of diabetes, were collected using a structured questionnaire. Clinical data and laboratory results, including fasting blood glucose (FBS) levels and glycated hemoglobin (HbA1c) (measured using enzymatic methods on the ARCHITECT clinical chemistry analyzer [Abbott, Wiesbaden, Germany]), as well as renal function tests (including serum levels of urea, creatinine, uric acid, sodium, potassium, calcium, chloride, and phosphorus, measured using standard enzymatic methods on the ARCHITECT analyzer), were recorded on the day of recruitment and retrieved from patient records.

Sampling

Six milliliters of venous blood were collected after an overnight fasting (approximately 10 hours) from all participants. The blood was drawn into plain vacutainers tubes, allowed to clot and then serum was collected and stored at -70°C until used later for measuring trace elements and nephrotoxic elements analysis.

Measurements of Trace Elements and Nephrotoxic Elements

Serum levels of Zn, Cu, Mg, Fe, As, Pb, Cd, and Hg were determined using inductively coupled plasma mass spectrometry (ICP-MS) technique.Initially, for trace element analysis, serum samples were digested using a microwave digestion method by using a mixture of concentrated nitric acid (69%), concentrated hydrochloric acid (37%), and hydrogen peroxide in addition to the serum sample, all kept for 7 minutes in the microwave and after digestion transferred to acid-washed polypropylene tubes after dilution to be analyzed by ICP-MS analysis as previously described [22].

Sample Size Calculation: The sample size was calculated by using the formula for a two-sample comparison of means as follows:

$n= (Z_{\alpha/2} + Z_{\beta} / d)^2$

Where: n = sample size per group, $Z_{\alpha/2}$ =Z value for the desired confidence level (1.96 for 95% confidence), Z_{β}=Z value for the desired power (0.84 for 80% power), and d = effect size.

The expected effect size estimated based on the expected differences in serum trace element (Zn, Cu, Mg, and Fe) levels and renal function parameters between the groups and based on a previous study [23]. Keeping (α) as 0.05, and 80% power. The calculations indicated a minimum sample size of 20 participants per group to achieve adequate power. This sample size was adjusted to account for potential dropouts and non-compliance, resulting in a target enrollment of 25 participants per diabetic and diabetic nephropathy groups, and a slightly larger control group to enhance the reliability of comparisons.

Ethical Considerations

Ethical approval for the study was obtained from the Deanship of Scientific Research, King Faisal University (Ref. No. EA000627). All participants provided informed consent prior to their inclusion in the study. They were given full information about the study procedures and were assured of their right to withdraw at any time without any consequences.

Statistical Analysis

Data were entered into a computer, cleaned, and double-checked using the Statistical Package for Social Sciences (IBM SPSS, Version 27). The normality of the data was assessed using the Kolmogorov-Smirnov test, and log transformations were applied where necessary to meet normality assumptions. Means (standard deviations) were used to present continuous variables, or medians (interquartile range) if the data were not normally distributed.

One-way Analysis of Variance (ANOVA) or the Kruskal-Wallis test was employed to compare the mean levels of serum trace elements (Zn, Cu, Mg, Fe) and nephrotoxic elements (As, Pb, Cd, Hg) among the DN, DM, CKD, and control groups. Post hoc analyses using Tukey's Honestly Significant Difference (HSD) test were conducted to identify specific group differences. An independent samples t-test was used to compare the duration of diabetes between the DM and DN groups. Pearson's correlation and Spearman's rank correlation coefficients were

calculated to estimate the relationships between serum element levels and renal function markers. The chi-square test was used to compare categorical variables. Statistical significance was set at $p < 0.05$ for all analyses.

Results

The mean(SD) of age in DM group 48.76 (12.72) is significantly higher (p=0.015) than the age in DN 42.50 (25.14), CKD 37.59(18.23), and 33.78(18.76) controls group, Table 1. No significant difference was observed in the mean(SD) of duration of diabetes between the DM 8.5(4.3) and DN 10.2(5.1) group, Table 1.

The mean(SD) of HbA1c was found significantly higher in DN group 7.49(1.99)% compared to other groups DM 7.26(2.25)%, CKD 5.16(0.51)% and controls 5.06(0.39) %, table 1. Likewise, DN group reported the highest value of FBS 172(45)mg/dl compared to DM 158(35) mg/dl, CKD 98(20) mg/dl and controls 90(15) mg/dl (p<0.001), Table 1.

Urea, creatinine, Na, Cl and P levels were significantly higher in CKD group, while BUN/Cr ratio and uric acids were found significantly higher in DN group ($p < 0.001$), Table 2. Potassium and Ca both were significantly higher in DM group compared to other groups ($p < 0.001$), Table 2.

Table 3 shows the comparison of trace and toxic elements across the four groups, and it shows that serum Zn and Cu were highest in DN group, $p = 0.032$, $p = 0.048$, respectively. Serum Fe ($p = 0.045$), As ($p = 0.041$), and Pb(p<0.001) were highest in DM group, Mg ($p = 0.014$) was the highest among CKD group. Mercury was found to be the highest in control group. Cadmium was comparable between all four groups.

Correlation analysis between renal function tests and trace and toxic elements

Urea is positively correlated with trace elements, Cu ($r = 0.307$, $p = 0.029$), and Mg($r = 0.409$, $p = 0.015$) and positively correlated with toxic elements As (rho =0.289 , $p = 0.045$), Pb (rho =0.356 , $p = 0.024$), and Hg($r = 0.309$, p =0.037). Also, urea is negatively correlated only with Fe ($r = -0.359$; $p = 0.036$).

Serum creatinine is directly correlated with Zn ($r = 0.406$, $p = 0.019$), Cu ($r=0.358$, $p = 0.027$) in trace elements and As (rho = 0.328, $p = 0.034$), Pb(rho = 0.384, p = 0.019) and Hg ($r = 0.287$, p = 0.048) in toxic elements. BUN is positively correlated only with Pb (rho = 0.316, p=0.038) and uric acid levels is positively correlated with Mg($r = 0.507$, p= 0.018).

HbA1c is positively correlated with Zn ($r = 0.307$, $p = 0.025$), As($r = 0.309$, $p = 0.048$), Pb (rho =0.365 , p= 0.027), while it is inversely correlated with Mg($r = -0.308$, p =0.049), and Fe ($r = -0.359$, p=0.028) Table 4.

Discussion

The main finding in this study is that the levels of serum Zn is significantly high in DN group compared to other groups. Additionally, Zn is positively correlated with creatinine and HbA1c. It is well known that, hypozincemia is a common finding in DM, and it is thought to be due to polyuria [24]. In contrary to our findings, many studies reported low levels of serum Zn in patients with DN compared with DM patients. And this decrement in Zn levels can lead to oxidative stress that ends with damage to the renal cells precipitating diabetic nephropathy. While zinc is generally considered beneficial for glucose metabolism and insulin function, its accumulation in renal tissue may contribute to oxidative stress and inflammation in the context of chronic hyperglycemia [25]. However, the reason of higher Zn in DN group is not clear, yet, it can be attributed to the dietary habits of those patients. We do not investigate for dietary sources of these elements in this study, otherwise the findings will be more informative. This finding should be treated with caution since the Zn in the DN group is within the lower limit of the international normal Zn level.

In this study, serum copper levels were found to be significantly higher in the DN group compared to the DM and other groups. Our findings align with those of Zaid and colleagues from Iraq [26], Ezzat and coworkers from Egypt [27] and Xu et al. from China [28]. In contrast, Makhlough et al. reported significantly lower levels [23], while Prabodh and colleagues found no significant difference [29]. This discrepancy in the literature may be due to variations in nutritional sources across these studies[30]. Additionally, serum copper levels are influenced by the rate of glomerular filtration [31], which may reflect the extent of renal damage. It has been reported that in patients with DN, high serum copper is filtered through kidneys and directly damages renal cells and accelerates progression of DN and CKD [32]. Copper accumulation *per se* can induce a form of cell death known as cuproptosis, which has been proposed as one of the mechanisms contributing to DN pathogenesis [33]. Furthermore, copper can act as both a pro- and antioxidant, depending on its concentration [34]. In this study, serum copper levels showed positive correlations with urea and creatinine, indicating an association with

deteriorating renal function. This relationship has been previously reported, as elevated serum copper levels have been found in patients with chronic kidney disease [35].

Another key finding in this study is the significantly lower levels of serum Mg in the DN group compared to other groups. This is consistent with the findings of Zaid et al. [26], and Ezzat et al. [27]. Adequate intra- and extracellular Mg concentrations, enhance the signaling activity of the tyrosine kinase enzyme in response to insulin hormone [36]. Low Mg levels may either be a consequence or pre-requisite for diabetes complications, including DN [37, 38]. Previous studies have pointed to the accelerated deterioration of renal function in type 2 DM patients with low magnesium levels [39]. Additionally, Mg deficiency is associated with insulin resistance, which exacerbate and further increased risk of nephropathy [40]. Interestingly, in this study, Mg demonstrated protective effects, showing negative correlations with HbA1c and a borderline correlation with creatinine. A very recent clinical trial reported that Mg supplementation in DN patients significantly improved microalbuminuria [41]. This beneficial effect is not limited to the hypomagnesemia patients only but include also normomagnesemic patients [42]. These findings support growing evidence suggesting that magnesium plays a crucial role in glucose metabolism and renal protection [43].

In this study, serum Fe levels in the DN group were higher than in controls, but slightly lower than in the DM group. Experimental studies have shown that iron overload precipitates DM and aggravates the development of diabetic nephropathy. A growing body of evidence has revealed that Fe is heavily deposited in the kidneys of DN patients [44, 45]. While the exact mechanism by which Fe damages renal cells is not fully understood, it is known that free Fe is highly reactive and generates free radicals through the Fenton reaction [46]. Recently, ferroptosis, a distinct type of cell death induced by iron overload, has been implicated in renal cell damage [44]. On the contrary, in animal models of diabetic nephropathy the administration of Fe chelation agents has shown dramatic improvements in terms of decreased proteinuria, and creatinine levels [47]. Furthermore, improvements in glycemic control, as well as enhanced insulin secretion and sensitivity, have also been reported [47]. In this study, Fe showed negative correlations with urea and HbA1c, suggesting a potential protective effect.

In this study, the highest levels of As and Pb were detected in the DM group. However, the levels in the DN group were also still high. Both As and Pb are known nephrotoxic elements and have been linked to renal function deterioration in people with and without diabetes [48, 49]. The severity of renal damage caused by As and Pb is thought to have dose-dependent effect [50]. Previous studies have reported that simultaneous exposure to multiple nephrotoxic elements may have a synergistic effect, accelerating renal damage compared to exposure to a single element [51]. In this study, both As and Pb were positively correlated with creatinine levels and indicate nephrotoxicity. Although the exact mechanisms by which As and Pb damage renal cells are not fully understood, experimental animal models have shown that exposure to Pb increases free radical production, reduces nitric oxide availability, impairs DNA repair mechanisms, and elevates blood pressure, all of which contribute to tubulointerstitial damage and kidney disease [52, 53]. On the other hand, Arsenic induces oxidative stress, inflammation, and epigenetic changes that may contribute to renal damage [54]. Interestingly, the administration of antioxidants and (As and Pb) chelating agents has been shown to markedly alleviate Pb and As renal damage [55, 56]. Beyond their impact on renal function, we also observed a negative correlation between Pb and As levels and HbA1c. The association between dysglycemia and exposure to As and Pb has been reported previously [57, 58]. Experimental animal studies have shown that As directly damages pancreatic β-cells through oxidative stress and reduces intracellular calcium levels necessary for insulin secretion [59].

In this study, Hg levels were notably higher in the DN group compared to the DM and CKD groups. Additionally, a positive correlation was observed between Hg and creatinine levels, suggesting a potential link between Hg exposure and the development or progression of nephropathy in diabetic patients. Mercury is known to induce oxidative stress and mitochondrial dysfunction, which are key mechanisms in the pathogenesis of diabetic nephropathy [60].

Cadmium (Cd) levels were comparable across all groups and were barely detectable. However, the nephrotoxic effects of cadmium are well-documented [61]. Very low cadmium levels in this study are likely linked to the environmental and occupational exposures in the study population.

To the best of our knowledge, this is the first study to document the association between trace and nephrotoxic elements in diabetic patients with diabetic nephropathy in Saudi Arabia. The measurements of trace and toxic

elements is not a routine test in clinical practice and seldomly being investigated. This study underscores the potential value of monitoring serum levels of both trace and nephrotoxic elements in diabetic patients. Deteriorating glycemic control may serve as an early warning sign of nephrotoxicity, suggesting the need for timely intervention to prevent or mitigate the development of diabetic nephropathy. However, this study has several limitations that warrant consideration upon interpretation of the findings. First, the cross-sectional design hinders our ability to establish causal relationships between element levels and renal function. Also, in this study we did not account for dietary intake or environmental exposure sources of the measured elements. Additionally, the sample size, particularly in the DN and CKD groups, were relatively small.

To conclude, in this study serum Zn, Cu, As, Pb, and Hg were positively correlated with creatinine levels. While, serum Zn, As, and Pb levels were positively correlated with HbA1c, serum magnesium, and iron levels were negatively correlated with HbA1c. Deteriorated glycemic control may require adjustment for the levels of Mg, Fe, Zn, As, and Pb levels. In addition to their routine work-up, clinician may follow-up the diabetic patients by measuring the concentrations of trace and toxic elements as well. Perhaps, early corrections of these elements may delay or prevent the development of DN. Yet, further research with longitudinal design following-up the DM patients before and after developing DN with consideration for dietary sources for trace elements and investigating the sources for toxic elements exposure is needed.

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Authors Contributions

Ahmed Mohamedain: Conceptualization, visualization, supervision and writing-original manuscript. Fatima Ali Shaabi: Data collection, investigations, laboratory work and results compilation. Ahmed Abdul qader and Nawaf Alkhashram : Research Administration and laboratory work supervision. Dr Sayed Ali, guided and performed the statistical analysis and contributed to the writing of the draft. Lyla Shabi: contributed to the statistical analysis. Hamdan Z. Hamdan, revision of the methodology and critical reviewing and editing of the final manuscript. All authors reviewed and approved the final manuscript. Deepthy Kunnathully Dinesh: data collection and laboratory work. Maujid Masood Malik, supervised the laboratory work.

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Table 1. Socio-demographic and Clinical Characteristics of Study Participants

Variable	DM Group N= 25	DN Group N= 20	CKD Group N= 22	Controls Group N= 56	*p*-value
Mean (SD), Age, years	48.76 (12.72)	42.50(25.14)	37.59(18.23)	33.78(18.76)	0.015
Sex (Male/Female)	15/10	12/8	13/9	28/28	0.325
Duration of Diabetes Mean(SD) years	8.5(4.3)	10.2(5.1)	N/A	N/A	0.210
FBS, (mg/dL)	158(35)	172(45)	98(20)	90(15)	< 0.001
HbA1c, (%)	7.26(2.25)	7.49(1.99)	5.16(0.51)	5.06(0.39)	< 0.001

DM: Diabetes mellitus group; DN: Diabetic Nephropathy; CKD: Chronic kidney disease

Table 2. Renal functions and electrolytes result in the Study Groups

Variables	DM Group N= 25	DN Group N= 20	CKD Group N= 22	Control Group N= 56	*p*-value
Urea, (mg/dL)	28(10)	45(15)	50(20)	20(5)	< 0.001
Creatinine, (mg/dL)	1.3(0.4)	1.5(0.5)	1.8 (0.6)	0.9(0.2)	< 0.001
BUN/Cr Ratio	21.5(3.2)	30(5)	28(7)	22 (3)	< 0.001
Uric Acid,(mg/dL)	5.1(1.2)	6.2(1.3)	4.8 (1.0)	5.0 (1.1)	< 0.001
Na, (mmol/L)	136.9(3.2)	136.1(3.8)	137.0 (2.4)	136.8 (2.4)	< 0.001
K, (mmol/L)	4.2(0.3)	4.1(0.5)	4.0 (0.5)	4.1 (0.4)	< 0.001
Ca, (mg/dL)	2.38(0.14)	2.34(0.15)	2.32 (0.15)	2.36 (0.14	< 0.001
Cl, (mmol/L)	104.2(3.4)	102.8(2.6)	105.7 (2.2)	105.2 (2.4)	< 0.001
P, (mg/dL)	1.16(0.27)	1.20(0.34)	1.24 (0.30)	1.16 (0.28)	< 0.001

DM: Diabetes mellitus group; DN: Diabetic Nephropathy; CKD: Chronic kidney disease
Data were expressed as mean(SD)

Table 3. Comparison of trace elements and toxic elements in the studied groups

Variables	DM Group N= 25	DN Group N= 20	CKD Group N= 22	Control Group N= 56	*p*-value
Zn (µg /dL)	75 (20)	85 (25)	70 (15)	80 (20)	0.032*
Cu (µg /dL)	100 (30)	110 (35)	95 (25)	105 (30)	0.048*
Mg (mg/dL)	1.8 (0.2)	1.7 (0.3)	1.9 (0.2)	2.0 (0.2)	0.014*
Fe (µmol/L)	17.3 (6.9)	15.8 (5.3)	14.9 (5.0)	14.1 (5.7)	0.045*
As (µg/dL)	0.33 (0.74)	0.17 (0.22)	0.07 (0.14)	0.02 (0.09)	0.041*
Pb (µg/dL)	7.7 (2.3)	4.1 (1.7)	3.5 (1.9)	1.1 (1.4)	< 0.001*
Cd (µg/dL)	0.1 (0.05)	0.1 (0.04)	0.1 (0.04)	0.1 (0.03)	0.932
Hg (µg/dL)	0.54 (0.50)	1.04 (0.38)	0.80 (0.35)	1.14 (0.81)	0.023*

DM: Diabetes mellitus group; DN: Diabetic Nephropathy; CKD: Chronic kidney disease

Table 4. Correlation between Renal Function Tests,HbA1c and trace (Zn, Cu, Mg, Fe) and Nephrotoxic Elements (As, Pb, Cd, Hg) (n = 20)

Variables	Zn	Cu	Mg	Fe	As	Pb	Cd	Hg
	r *p*	*r* *p*	*r* *p*	*r* *p*	*rho* *p*	*rho* *p*	*r* *p*	*r* *p*
Urea	0.226, 0.053	0.307, 0.029*	0.409, 0.015*	-0.359, 0.036*	0.289, 0.045*	0.356, 0.024*	0.059, 0.657	0.309, 0.037*
Creatinine	0.406, 0.019*	0.358, 0.027*	-0.309, 0.057	-0.259, 0.079	0.328, 0.034*	0.384, 0.019*	0.029, 0.789	0.287, 0.048*
BUN	0.106, 0.152	0.158, 0.107	-0.259, 0.072	-0.209, 0.089	0.256, 0.066	0.316, 0.038*	0.043, 0.709	0.208, 0.109
Uric Acid	-0.053, 0.209	-0.105, 0.157	0.507, 0.018*	-0.106, 0.124	0.159, 0.207	0.189, 0.158	0.106, 0.308	0.229, 0.088
HbA1c	0.307, 0.025*	0.256, 0.059	-0.308, 0.049*	-0.359, 0.028*	0.309, 0.048*	0.365, 0.027*	0.076, 0.509	0.259, 0.065

*p-value < 0.05 is statistically significant; r: Person's correlation; rho: Spearman's correlation.