Original Article

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Biochemistry Section

Zinc Status in Type 2 Diabetic Patients: Relation to the Progression of Diabetic Nephropathy

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ABSTRACT

Background and Objectives: Zinc deficiency often occurs in patients with diabetes. Therefore, the relationship between zinc status and progression of nephropathy in diabetes has been explored.

Materials and Methods: Total 300 diabetic patients and 100 non-diabetic healthy subjects (age matched) were selected followed by informed consent and divided into five groups as I: non-diabetic normotensive control; II: diabetic normotensive; III: diabetic hypertensive; IV: diabetic normotensive with microalbuminuria; V: diabetic hypertensive with microalbuminuria. The blood samples of all subjects were collected and analyzed for serum zinc, serum creatinine, and estimated-glomerular filtration rate (e-GFR). Urine zinc, creatinine and microalbuminuria concentrations were determined.

Results: The serum zinc levels were low ($p<0.01$) in diabetic patients as compared to non-diabetic control subjects. The lower levels ($p<0.001$) of serum zinc were observed in Group IV and V as compared to group I-III. Significantly low levels of e-GFR ($p<0.05$) and high levels of microalbuminuria ($p<0.001$) were observed in diabetic patients with low serum zinc level as compared to normal serum zinc level. Serum zinc level in diabetic patients was inversely correlated with serum creatinine($r=-0.331$, $p<0.001$), microalbuminuria ($r=-0.587$, $p<0.001$) and positively with e-GFR ($r=0.194$, $p<0.01$).

Conclusion: It is evident from this study that advancing diabetic nephropathy represented by decreasing GFR and increasing microalbuminuria is associated with lower serum zinc levels. It thus indicates the need for determining serum zinc levels and the effectiveness of zinc supplementation in diabetic patients, particularly during the assessment of kidney damage.

Keywords: Diabetes, Nephropathy, Zinc

INTRODUCTION

Diabetic nephropathy is a microvascular complication associated with diabetes causing slow deterioration of kidneys leading to end-stage renal disease [1]. The nephropathy associated with diabetes has been attributed to oxidative stress [2]. Oxidative stress can be caused either by the increased production of reactive oxygen species (ROS) or a deficiency in antioxidant defense. Antioxidant deficiency can result from low intake of vitamins, such as vitamin C and E, or impaired synthesis of enzymes, such as super oxide dismutase, catalase and glutathione peroxidase, due to zinc deficiency [3]. Chronic zinc deprivation generally results in an increased sensitivity to the effects of oxidative stress due to deficiency of these enzymes [4].

It is known that low-dietary zinc intake and low levels of serum zinc are associated with a high prevalence of cardiovascular diseases, diabetes and glucose intolerance [5]. It is not always clear which comes first; the effects of diabetes mellitus and hyperglycemia on serum zinc metabolism or the effects that follow alterations in zinc homeostasis on carbohydrate metabolism. Perhaps more accurately, there are concurrent, semi-independent events that give rise to the effects of one on the other. Consequently, considering the possible modulating effects of zinc on antioxidant functions, a restored zinc status in individuals with type 2 diabetes mellitus might counteract the deleterious effects of oxidative stress and help to prevent the complications associated with diabetes [6].

Although several factors are involved in the genesis of diabetic nephropathy, low zinc status appears to contribute to the diabetes associated renal injury [5]. Measurement of serum zinc levels may be considered medically necessary in diabetic patients and might be used as a marker of nephropathy in concomitants with other nephropathy detection markers. Little is known, however, about the utility of serum zinc level in the assessment of nephropathy in diabetic patients. As data on the subject is scarce particularly in the diabetic patients of Iraq in this complication group, the present study was designed to determine the alterations of serum and urine zinc in terms of vascular damage to identify the progression of nephropathy in diabetes.

MATERIALS AND METHODS

This was a cross-sectional study conducted in Duhok Diabetes Center, Kurdistan Region, Iraq, from April to August 2013 in 300 Type 2 DM patients, aged 43.5-71.6 years and 100 apparently healthy subjects with no history of diabetes, aged 45.7-69.2 years. Selection of Patients was random (every 3rd) from previously diagnosed cases who attended the Center for follow-up and management. Controls were selected from staff and sub- staff of Azadi General Teaching Hospital in Duhok city. Patients with chronic liver disease, urinary tract infection, macrovascular disease, gestational diabetes, type 2 DM of less than 5 years duration and those who were newly diagnosed type 2 DM were excluded from the study.

Participants were interviewed and informed about the nature of the study and then, verbal consent was obtained from each subject. The study protocol was approved by Research Ethics Committee of the General Directorate of Health in Duhok Governorate. Patients and controls completed a pre-test questionnaire included anthropometric data and diabetic record.

The groups of patients and control subjects were as follows:

Group I: Non-diabetic, normotensive control subjects

Group II: Diabetic, normotensive patients

Group III: Diabetic, hypertensive patients

Group IV: Diabetic, normotensive patients with microalbuminuria

Group: V: Diabetic, hypertensive patients with microalbuminuria

Blood analysis including serum zinc and creatinine was done. Urine zinc and microalbuminuria concentrations were determined. The glomerular filtration rate (GFR) was estimated by a well-established equation method [6].

Parameters*	Patients n=300	Controls n=100	p-value
Systolic BP (mm Hg)	131.6±16.4	115.6±7.3	<0.001
Diastolic BP (mm Hg)	85.5±10.2	74.0± 6.5	<0.001
Serum creatinine (mg/dl)	0.74±0.33	0.68±0.14	<0.05
Urinary zinc/creatine (ug/g)	2.33±1.18	1.01±0.57	<0.001
Microalbuminuria/creatinine (mg/g)	54.7±68.4	16.2±7.6	<0.001
Serum zinc (ug/dl)	70.0±19.2	86.2±15.2	<0.001
e-GFR (ml/min/1.73m^2)	95.8±26.4	108.2±24.6	<0.001

[Table/Fig-1]: Diabetic patients and controls characteristics **Results are mean±SD*

Group	n	Mean±SD	(95%CI)
Non-diabetic controls			
Group I	100	86.2±15.2*	83.2 - 89.5
Diabetic			
Group II	145	79.2±15.0	74.4-83.9
Group III	41	77.9±17.2	74.9-80.8
Group IV	62	56.8±13.8	53.3-60.3
Group V	52	55.0±14.2	51.0 -58.9

[Table/Fig-2]: Serum zinc levels in diabetic patients and Controls
*Significantly different from diabetic groups (p<0.01)

Group	Group III n=41	Group II n=145	p- value
Systolic BP (mm Hg)	151.0±11.9	122.8±8.9	<0.001
Diastolic BP (mm Hg)	97.3±5.0	79.8±6.2	<0.001
Serum creatinine (mg/dl)	0.74±0.33	0.70± 0.17	NS
Urinary zinc/creatinine (ug/g)	1.92±0.76	1.76±0.66	NS
Microalbuminuria/creatinine (mg/g)	21.0±16.9	20.5±6.0	NS
Serum zinc (ug/dl)	77.9±17.2	79.2± 15.0	NS
e-GFR (ml/min/1.73m^2)	94.1±25.4	97.7±25.2	NS

[Table/Fig-3]: Serum zinc and other parameters in diabetic hypertensive and normotensive patients, *Results are mean+SD, NS: p>0.05

GFR (ml/min/1.73 m^2) = 175x (Serum creatinine$^{-1.154}$) X (Age) $^{-0.203}$ x (0.742 if female).

Serum and urine zinc was analyzed by flame atomic absorption spectrophotometer (Perkin Elmer) using a standardized procedure [7]. The serum and urine creatinine was measured by routine spectrophotometeric method. The microalbuminuria was measured by i-CHROMA™ micro albumin based on fluorescence immunoassay technology [8]. All data was analyzed using the Statistical Package for Social Sciences (SPSS); version 21.0. Independent t-test was used to assess differences in serum and urine analyte among groups. The statistical significance, direction and strength of linear correlation between 2 quantitative variables were measured by using Pearson's correlation coefficient test. Categorical variables were compared by Chi-square test.

RESULTS

The general characteristic of diabetic patients and non-diabetic controls has been shown in [Table/Fig-1]. In diabetic patients, systolic and diastolic blood pressure, urinary zinc/creatinine ratio and microalbuminuria were found to be significantly high (p<0.001) as compared to non-diabetic controls. In contrast, significantly low levels of serum zinc and e-GFR values were observed in diabetic patients as compared to non-diabetic controls (p<0.001).

The mean ±SD of serum zinc levels in non-diabetic controls (Group I) and patient groups (Group II-V), has been shown in [Table/Fig-2]. Significantly (p<0.01) low levels of serum zinc was observed in diabetic, normotensive patients with microalbuminuria (Group IV) and diabetic, hypertensive patients with microalbuminuria (Group V) as compared to non-diabetic controls (Group1), as well as to diabetic, normotensive patients(Group II) and diabetic, hypertensive patients (Group III).

Group	Group V n=52	Group IV n=62	p- value
Systolic BP (mm Hg)	151.1±12.5	122.8±6.7	< 0.001
Diastolic BP (mm Hg)	97.7±8.2	80.8±5.1	<0.001
Serum creatinine (mg/dl)	0.87± 0.62	0.72± 0.82	NS
Urinary zinc/creatinine (ug/g)	3.09±1.47	3.02±1.35	NS
Microalbuminuria (mg/g-creatinine)	107.5±87.3	111.8±81.8	NS
Serum zinc (ug/dl)	55.0 ±14.2	56.8±13.8	NS
e-GFR (ml/min/1.73m^2)	87.1±25.8	99.5±29.1	<0.05

[Table/Fig-4]: Serum zinc and other parameters in diabetic hypertensive and normotensive patients with microalbuminuria,*Results are mean±SD

e-GFR (ml/min/1.73 m2)	Serum zinc levels		
	<70 ug/dl n(%)	>70 ug/dl n(%)	Total
>90(normal or raised)	107(45.7)*	127(54.3)	234
60-89(Mildly decreased)	38 (76.0)*	12(24.0)	50
30-59(Moderately decreased)	14 (93.3)*	1 (6.7)	15
15-29(Severely decreased)	1 (100.0)	-	1
	160	140	300

[Table/Fig-5]: Distribution of diabetic patients with low and normal serum zinc levels, *Chi square test, p<0.01

The [Tables/Fig-3,4] summarize the results in terms of mean±SD. In diabetic, hypertensive patients (Group III) serum zinc levels and other parameters (serum creatinine, urinary zinc/creatinine ratio, microalbuminuria and e- GFR) were seen to be not significantly different from diabetic, normotensive patients (Group II). Similarly, in diabetic, hypertensive patients with microalbuminuria (Group V), serum zinc levels and other parameters were observed to be not significantly differ from diabetic, normotensive patients with microalbuminuria (Group IV), except e-GFR (p,.<0.05). The distribution of patients with low and normal zinc levels has been shown in [Table/Fig-5]. The prevalence of mild and moderate decreased e-GFR was higher in patients with low serum zinc (p<0.01) than in diabetics with normal serum zinc level .Serum zinc levels in diabetic patients were inversely correlated with serum creatinine(r=-0.331, p<0.001), urinary zinc (r=-0.243, p<0.01), microalbuminuria (r=-0.587, p<0.001) and positively with e- GFR (r=0.194, p<0.01) .The correlations between serum zinc levels and systolic and diastolic blood pressure were not statistically significant (r=-0.075and r=-.05), respectively.

DISCUSSION

In this cross-sectional study conducted in a diabetes center, we found that the mean serum zinc level was significantly lower in diabetics as compared to non- diabetic controls. Diabetic patients with microalbuminuria and low values of e-GFR had a lower mean serum zinc level than other diabetic nephropathy groups. We also found a significant inverse relationship between serum zinc and microalbuminuria; and a significant positive relationship between serum zinc and e-GFR. These findings gave supports in the observation that diabetes affects zinc status and significant decrease was observed in the serum level of zinc at the onset of diabetic complications such as hypertension and nephropathy [9]. One of the most important clinical features of diabetes is its harmful effects on the kidneys small blood vessels (microangiopathy) which may progress to diabetic nephropathy (DNP) [10]. Diabetic nephropathy occurs in approximately one- third of individuals with type-1 and type-2 DM, and is associated with high morbidity and mortality [11]. It is characterized by persistent albuminuria (excretion >300 mg/day,

proteinuria or macroalbuminuria), a relentless decline in GFR, raised arterial blood pressure and rapid progression of other complications like retinopathy, neuropathy, diabetic foot and blood pressure, once proteinuria developed irreversible deterioration in renal functions and renal failure occurs [12]. During progression to DNP and in MAU stages, morphological and structural changes occur in the glomerular and basement membrane of the kidneys, hyperfiltration, thickening of glomerular basement membrane (GBM), mesangial expansion and podocyteloosing. All of these changes will cause leakage of albumin along with other enzymes and trace metals to the urine leading to marked increase of these markers [13]. Therefore, hypozincaemia observed in diabetics can be attributed to hyperzincuria. The results of this study are consistent with finding reported by others [14,15]. In diabetic patients; urinary zinc/creatinine ratio were found to be significantly high as compared to healthy subjects, and the mean serum zinc level was lower in the diabetic patients with microalbuminuria. The decrease serum level of zinc supposed to be the consequence of increased urinary albumin excretion by microvascular damage. A relationship between serum zinc and albuminuria has been observed in several studies for type1 and type2 diabetes [16]. In the present study, there was a trend towards an inverse zinc-microalbuminuria association. As compared with diabetic –normotensive (group II), diabetic- normotensive patients with microalbuminuria (group IV) had significantly lower mean serum zinc concentration and increased urinary zinc excretion. Similarly, diabetic-hypertensive patients with microalbuminuria (group V) have been significantly associated with the consequence reduction in serum zinc level. These findings are supported by the fact that zinc in serum may not affected by increasing blood pressure. It has been reported that as hypertension is a potent risk factor for microalbuminuria [17], it seems that the increase albumin excretion is dependent of change in blood pressure. However, the present study showed that the mean serum zinc level was not significantly differing between diabetic normotensive (group II) and hypertensive patients (group III). It would be useful to undertaken further study to discover more about the mechanism and the effect that blood pressure has on zinc status. It is evident from the present study that kidney damage based on e-GFR affected zinc status; a high percentage of diabetic patients with mild- moderate kidney damage represented by decreased e-GFR had low serum zinc levels. This observation may reflect the rate of progression of the underlying disease. Consequently, considering the possible modulating effects of zinc on antioxidant functions, a restored zinc status in individuals with type 2 diabetes mellitus might counteract the deleterious effects of oxidative stress and helps to prevent complications associated with diabetes.

A limitation of this study may be that we did not measure C - reactive protein (CRP) as an inflammatory factor, which may affect microalbuminuria, and this may change zinc status in diabetic patients, particularly those with microalbuminuria.

CONCLUSION

This study indicated that low- serum zinc was present in one-third of patients with diabetic nephropathy, particularly among patients with microalbuminuria. Unlike hypertension, coexistence of microalbuminuria with diabetes mellitus reflected a strong statistically significant inverse correlation with serum zinc. Advancing nephropathy represented by decreasing GFR and increasing microalbuminuria was associated with decreasing serum zinc levels. It thus indicates the need for determining serum zinc levels and the effectiveness of zinc supplementation in diabetic patients, particularly during the assessment of kidney damage. More elaborate studies including larger sample size are needed to verify the role of serum zinc in screening for early detection nephropathy.

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