Original Article

Correlation between microalbuminuria and urinary copper in type two diabetic patients

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ABSTRACT

Introduction: Diabetes mellitus and its chronic complications may be associated with alterations in the plasma, tissue, and urinary levels of some trace elements like copper. **Materials and Methods:** This cross-sectional study evaluates the 24 hour urinary copper levels in type 2 diabetic patients with microalbuminuria in comparison with patients without albuminuria. **Results:** Forty-two patients with microalbuminuria (case) and 40 patients without microalbuminuria (control) participated in the study. Mean (CI 95%) urinary copper levels were 36.14 (14.54–57.74) and 14.77µcg /L (10.17–19.37) in the case and control groups respectively ($P = 0.003$). There was no significant effect of diabetes duration or HbA1c on urinary copper. **Conclusion:** The present study shows diabetic patients with microalbuminuria have increased urinary copper excretion, however does not exclude the potential toxic effects of this high copper excretion on the progression of diabetic nephropathy.

Key words: Albuminuria, copper, diabetes mellitus, nephropathy, trace elements

Introduction

Copper is an essential trace element that affects much of physiology.[1,2] All cells in the human body require copper for their metabolic needs. Copper serves as an essential cofactor for the activity of cytochrome C oxidase in mitochondria, for Cu, Zn-dependent superoxide dismutase, and other enzymatic systems.[1] These enzymes then critically contribute to a number of key physiological processes including central nervous system functions, connective tissue and blood vessel development, pigmentation, reactive oxygen species detoxification, synaptogenesis, and mitochondrial functions.[3] Copper deficiency decreases the activity of these enzymes, and thus adversely affects the corresponding physiological processes. Excess copper is also deleterious for cell metabolism. Therefore, the level of copper in cells and tissue must be tightly regulated.[1] There is accumulating evidence that the metabolism of several trace elements, e.g., copper is altered in patients with diabetes mellitus[4] and that these nutrients might have specific roles in the pathogenesis and progression of this disorder.[5,6] In some animal studies it has been shown that the treatment with a copper chelating agent reduces insulin resistance and ameliorates glucose intolerance in diabetic db/db mice.[7] but animal studies do not reveal the same results.[8,9]

Chronic complications of glucose metabolism disorders might also be associated with alterations in the levels of some trace elements like copper.[2,5]

Human studies have had paradoxical results with some studies revealing correlation of copper with diabetes mellitus or its complications[4] but other studies revealing no correlation between complications like albuminuria and copper.[10]

It has been also suggested that dysregulation of renal Cu homeostasis may be a key event eliciting development of

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diabetic nephropathy, and selective Cu(II) chelation can protect against pathogenic mechanisms that lead to diabetic nephropathy and might be clinically useful in the treatment of early-stage diabetic kidney disease.[11] On the other hand, overloading of urinary copper to damaged renal tubules may play some roles in the progression of nephropathy in patients with advanced nephropathy.[12]

Regarding to conflicting and limited results about the association of diabetic nephropathy and copper, this study evaluates the correlation of urine copper level and microalbuminuria in type 2 diabetic patients.

Materials and Methods

In this cross-sectional study, Type two diabetic (DMT2) patients who were referred to our diabetes clinic, were enrolled based on the following criteria: history of DMT2 for at least one year, lack of urinary tract infection and lack of heart failure.

After obtaining signed written consent, 24-hours urine samples were obtained from patients for measurement of microalbumin and creatinine. Criteria for microalbuminuria, was albumin more than 30 mg and less than 300 mg in two 24 hours urine samples separated a month.

Patients were categorized in case and control group based on the presence or absence of albuminuria.

Because the natural history of diabetic nephropathy in patients with T2DM is not as clear as in patients with T1DM[13] due to the fact that most of these patients have had untreated diabetes for 10 years (on average) before diagnosis and also the fact that T2DM is largely a disease of an older population, with other co-morbidities that restrict the manifestation of diabetic renal disease. However, microvascular complications of DM are less frequent before five years, but increase significantly after 10 years.[14] So regarding to duration of diabetes, patients were categorized into four groups; first group less than 5 years, second group 5 to less than 7 years, third group 7 to less than 10 years, and fourth group equal or more than 10 years.

Participants were divided into two groups based on HbA1c level, the first group with HbA1c less than 8% and the second group with HbA1c equal or greater than 8%.

The urine copper of patients was measured by atomic absorption spectrophotometry.

Analysis of data was done by SPSS 17. K-S test was used to detect normal distribution of urinary copper level. The independent t-test and ANOVA was used to compare urinary copper levels between groups.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Arak medical University.

Besides obtaining Informed consent, patients participating in the study were assured about the privacy of the data. Participants continued to receive antidiabetic treatment.

Results

Eighty two DMT2 patients participated in the study, 42 patients with microalbuminuria as case group and 40 patients without microalbuminuria as control group. Sixty-nine percent of the participants in the case and 62% of the control group were female. As expected, there was significant difference in the first and second urinary albumin between case and control groups [Table 1].

Mean (CI 95%) urinary copper levels were 36.14 (14.54−57.74) and 14.77 μcg /L (10.17−19.37) in the case and control groups, respectively (P = 0.003) [Table 2]. Regarding to duration of diabetes, we had four subgroups in each group. Mean levels of 24 hours urine copper in four subgroups of case and control groups were compared. There was no significant difference in urinary copper levels between four subgroups in each group ($P > 0.5$) [Table 3]. Also, regarding to HbA1c level, there was not a significant difference of mean urinary copper in patients with HbA1c less and greater than 8%in each group ($P = 0.07$) [Table 4]. There was no significant difference of urinary

Table 1: Mean 24-hour urine albumin level in case and control groups

Times	Mean 24-hour urine albumin (mg±SD) (CI) Group		*P* value
	Control	Case	
First time	12.74 ± 1.1 (10.3 – 15.1)	113 ± 15.4 (82.2 – 143.8)	0.0001
Second time	13.20 ± 1.1 (11 – 15.4)	119.33 ± 17.3 (84.5 – 154.1)	0.0001

Table 2: Mean 24-hour urine copper in case and control groups

Group	Mean urine copper level Mcg/L ±SD (CI)	*P* value
Case	36.14 ± 10.8 (14.54 – 57.74)	0.003
Control	14.77 ± 2.3 (10.17 – 19.37)	

Table 3: Mean 24-hour urine copper level based on duration of type two diabetes

Years of Diabetes	Number of patients (%)		Mean urine copper level±SD (Mcg/L) (CI)	
	Case	Control	Case	Control
<5	16	28	53.33 ± 16.8	14.20 ± 1.7
	38.1	70	(19.73 – 86.93)	(10.8 – 17.6)
5-7	3	4	11.9 ± 2.2	14.62 ± 2.6
	7.1	10	(7.5 – 16.3)	(9.42 - 19.82)
7-10	10	7	23.71 ± 2.5	8.38 ± 0.8
	23.8	17.5	(18.71 – 28.71)	(6.78 – 9.98)
10	13	1	29.20 ± 3.3	9.2
	31	2.5	(22.6 - 35.8)	
total	42	40	36.14 ± 10.8	14.77 ± 2.3
	100	100	(14.54 – 57.74)	(10.17 – 19.37)

Table 4: Mean urine copper level based on HbA1c in case and control groups

	Number of patients		Mean urine copper level ±SD (Mcg/L) (CI)	
HbA1c %	Case (%)	Control (%)	Case	Control
<8	13	22	62.23 ± 19.2	16.96 ± 2.5
	31	55	(23.83 – 100.63)	(11.96 – 21.96)
>8	29	18	24.52 ± 3.1	12.10 ± 1.9
	69	45	(18.32 – 30.72)	(8.3 -1 5.9)
Total	42	40	36.14 ± 10.8	14.77 ± 2.3
	100	100	(14.54 – 57.74)	(10.17 – 19.37)

copper level between two sexes. There was no correlation between age and urinary copper.

Discussion

This study shows significantly higher levels of urinary copper in patients with diabetic microalbuminuria than patients without it. The question is that: does urinary copper excretion leads to damaged kidney and worsens albuminuria or damaged kidney increases urinary copper excretion?

As we could not observe significant effects of HbA1c or diabetes duration on urinary copper, it may reveal that copper excretion is the result, not the cause of diabetic nephropathy. This high urinary excretion of Cu may be due to the excretion of its carrier proteins.[15] Urinary copper excretion may also be due to dissociations from both copper-albumin and ceruloplasmin-copper complexes filtered through the damaged glomerulus.[11,12] It does not exclude that overloading of urinary copper to damaged renal tubules may play some roles in the progression of nephropathy in patients with advanced nephropathy.[12] High urinary copper may be a marker of high renal tissue copper, as evidenced by animal studies, in which, copper chelation improved markers of renal damage.[11] The exact roles of copper in diabetic patients may be really more complicated. For example, metallothioneins (MT) are a group of intracellular metal-binding and cysteine-rich proteins that mainly act as regulator of metal homeostasis such as zinc and copper in tissues. MTs were found to be potent antioxidant and adaptive (or stress) proteins to protect cells and tissues from oxidative stress.[16] Oxidative stress is a well-known mechanism of chronic diabetic complications like nephropathy. Genetically or pharmacologically enhanced MT expression in various organs including heart and kidney provided significant protection from diabetes-induced organ dysfunction such as cardiomyopathy and nephropathy.[16]

Our study has some limitations. First, we did not measure the serum copper and ceruloplasmin. Serum copper concentration may be influenced in diabetic patients[17] but can affect its urinary excretion. Without the results of serum copper as a confounding factor, the interpretation of the results of our study is difficult, although Prabodh in a study from India did not find significant difference of serum copper between diabetic patient with and without nephropathy.[10] Also, some animal and human studies have evaluated serum or urine ceruloplasmin to better clarify the roles of copper in similar settings.[12,15,17] However, multiple other studies have evaluated serum or urinary copper without concurrent evaluation of ceruloplasmin.[5,18,19] Changes of serum ceruloplasmin may complicate interpretation of our findings.

The second limitation is its cross-sectional design, as without a cohort study, precise evaluation of the copper role in diabetic nephropathy is difficult.

In conclusion, the present study shows diabetic patients with microalbuminuria have increased urinary copper excretion, however, does not exclude the potential toxic effects of this high copper excretion on the progression of diabetic nephropathy.

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