ZINC LEVELS IN TYPE 2 DIABETES MELITUS : A PRELIMINARY STUDY OF DIABETIC NEPHROPATHY PROGRESSIVITY

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Summary Zinc is one of the trace elements which involved in carbohydrate metabolism. Some studies found there are zinc levels alterations in Type 2 Diabetes Mellitus (T2DM) individuals, in which zinc level are decreased further if diabetic nephropathy occur. Diabetic Nephropathy is a complication of DM, which characterized by the presence of microalbuminuria. In this cross-sectional study, serum zinc levels and MA were measured and then compared between three groups (control group, T2DM with hypertension, and T2DM without hypertension). Zinc serum were measured using ELISA method, while microalbuminuria used *Immunoturbidimetry* method. 42 samples were collected, consist of 15 subjects in control group, 11 in T2DM with hypertension, and 16 in T2DM without hypertension. The urine albumin / creatinine ratio was higher in T2DM group compared to the controls (p=0.001), but the serum zinc levels between control group and T2DM (p=0.317) show no significance. There was significant differences in albumin/creatinine ratio (p=0.002) but not in serum zinc (p=0.582) between T2DM with hypertension compared to other two groups, and there was no association between serum zinc levels in T2DM with albuminuria and T2DM with normoalbuminuria (p=0.758). We concluded that zinc levels in T2DM with albuminuria are higher than T2DM with normoalbuminuria, although there was no significant correlation. This is an ongoing preliminary study.

Key Words zinc, type 2 diabetes mellitus, albuminuria.

Type 2 Diabetes Mellitus (T2DM) is a metabolic disease, marked by high blood glucose level (hyperglycemia) and decrease of insulin production or insulin action which is produced by pancreas gland.(1) There is an increasing tendency of T2DM prevalence in this world according to World Health Organization (WHO) which in 2012, it caused 1.5 million of death.(2)

T2DM is a condition caused by impaired insulin secretion by pancreatic β-cell, and impaired insulin function which is a result of insulin resistance. Several risk factors that caused insulin resistance are sedentary lifestyle and unhealthy dietary habit.(3)

Hyperglycemia in T2DM activates six major pathways (increased polyol pathway, increasedadvanced glycation end products/AGEs, activation of PKC isoform, increased hexosamin flow, and increased reactive oxygen species/ROS) which caused complication. either acute or chronic complication. Chronic complication of diabetes was further classified into micro and macrovascular complication. Diabetic Kidney Disease (DKD) is one of these chronic complication of T2DM, which characterized by persistent albuminuria, persistent decrease in glomerular filtration rate (GFR), increase in arterial blood pressure and increase in cardiovascular morbidity and mortality. (4)Structural kidney changes which confirmed by kidney biopsy differentiate DKD and nephropathy diabetic (ND) which was caused by diabetes. Screening for albuminuria in T2DM patients could help early detection and treatment of DKD, which indicates the escalation of cardiovascular morbidity and mortality in diabetes patients.(5)

The occurrence of albuminuria in DKD was assumed due to an increase in glomerular permeability in DM patients, which is caused by hyperglycemia. Glucose could bind several proteins in a non-enzymatic manner, which binds protein in glomerular filtration barrier and caused permeability changes thus stimulates mesangial matrix growth.Albuminuria is a sign of glomerular changes, and can be classified to three stages : normoalbuminuria (<30 mg albumin/g creatinin), microalbuminuria (30-300 mg albumin/g creatinin), and macroalbuminuria (>300 mg albumin/g creatinin). (5)

Previous studies found correlation in trace elements levels and T2DM, which several trace elements have corresponding influence with T2DM.[6] It was found that blood lowering glucose action of insulin was amplified by some trace elements such as chromium, magnesium, manganese, molybdenum, selenium and zinc.[7]

Zinc is one of the trace elements in the body which support many functions. Some references mentioned zinc plays a role in insulin action therefore affect carbohydrate metabolism. Moreover, zinc has the antioxidant and anti-inflammation effect, and involved in lymphocyte B and T function. [8; 9]

Chronic hyperglycemia in T2DM associated with lipid peroxidation and oxidative damage in cells, which caused antioxidant imbalance. Several studies shown declining of zinc serum in T2DM patients, accompanied by increasing urine zinc excretion which worsened zinc serum status. Previous studies found decline in serum zinc and increase urine zinc excretion[8; 10; 9]

Albuminuria indicates kidney complication of DM, and more severe inflammatory process. [11] Inflammatory process in DKD/ND implies increasing of oxidative stress. This study compare zinc levels in T2DM with control group, and then make further comparison between T2DM with hypertension (HT) and without HT. Zinc levels will be compared between albuminuria and normoalbuminuria group.

MATERIALS AND METHODS

This cross-sectional preliminary study conducted in Wahidin Sudirohusodo hospital, Makassar, Indonesia, from January 2018 to October 2018. Ethical clearance was issued by Ethics of Biomedical Research in Humans, Hasanuddin University Faculty of Medicine. Inclusion criteria were DKD patients who were treated in Internal Medicine policlinic, aged 20-70 years old. Exclusion criteria were subject with chronic liver disease, urinary tract infection, gestational diabetes, T2DM patients which known less than five years, DKD with dialysis, history of fever within last 72 hours, and history of zinc-containing supplement in the last one month. Number of samples wasdetermined using a formula for correlative analysis research.

Before blood and urine samples and other data were collected, subjects were informed about this study and enrolled as they agree to participate in this study by signing the informed consent. Subjects were interviewed regarding their intake which was using three days food recall 24 hours (2 week days and 1 holiday/weekend day). Blood pressure were measured using a standard mercury sphygmomanometer. Creatinin serum was analyzedusing ELISA (*enzyme-linked immunosorbent assay*) method. Body weight was measured using a SECA weight scale (level of accuracy 100 g), body height was measured using stadiometer with maximum capacity 200 cm (level of accuracy 0.1 cm). Albuminuria was screened using spot urine, albumin urinwas analyzed by Immunoturbidimetry methodwhich will be compared with urine creatinine, while zinc serum were analyzed using ELISA method.T2DM patients was classified as normoalbuminuria if albumin/creatinine urine ratio is <30mg/g creatinine, and albuminuria if albumin/creatinine urine ratio is ≥30 mg/g creatinine.

We collected 42 sampleswhich divided to three subgroup, 15 in control group, 16 in T2DM without HT, and 11 in T2DM with hypertension.And the data was further analyzed in T2DM group, and divided between albuminuria and normoalbuminuria. Data was analyzed using version 18 SPSS.

RESULTS

Baseline characteristic for control group and T2DM group was shown in table 1. Using independent t-test, we found difference in systolic blood pressure and albumin/creatinine urine ratio, with albumin/creatinine urine ratio was higher in T2DM patients (p=0.001). Zinc intake was slightly higher in control group (p = 0.017). There were no differences between zinc serum within T2DM group and control group.

Table 2 show albumin/creatinine urine ratio, zinc serum and zinc intake in control group and T2DM group which analyzed using Kruskal Walis test and one way ANOVA. There was significant difference between each T2DM group (with an without HT) and control groupfor albumin/creatinine urine ration (p=0.002) with the highest ratio was within T2DM with HT group (135.0 µg/mg creatinine). Zinc serum (p=0.583) nor zinc intake (p=0.066) show significant differences within each group, but increase in zinc serum was in line with zinc intake.Zinc serum was highest in control group (37.04 µg/dl) and lowest in T2DM with hypertension group (33.37 µg/dl).

We compared the distribution of zinc serum level in T2DM patients with and without albuminuria (table 3). There was no significant difference in zinc serum level (p=0.758) within both group, and higher zinc level was found in T2DM subjects with albuminuria.

DISCUSSION

Zinc level in human is determined by zinc intake, metabolism, and excretion. The major causes of zinc deficiency are inadequate intakeof diets, malabsorption of nutrients, and excessive loss ofzinc and increased demand of zinc. Other condition which affect zinc levels are circadian variations (high level in the morning, andlow in the afternoon), food variations and some drug usage.[12]

There is considerable evidence that several biochemical pathways (enhanced polyol activity, increased formation of advanced glycation end products (AGEs); activation of protein kinase C (PKC) and increased hexosamine pathway flux), which are activated by hyperglycemia, associated with the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress.[13]

Increasing oxidative stress in T2DM plays a role in complications of DM. Under physiological condition, increase of ROS will activate endogenous antioxidant. But in DM, overproduction of ROS exceeds the endogenous antioxidant.ability in addition of accompanying processes. Therefore, ROS accumulation leads to the activation of stress-sensitive intracellular signaling pathways that, in turn, promote cellular damage and contribute to the diabetic complications development and progression[13]

Zinc, one of the trace elements, takes part in the

regulation of chronic inflammatory statusthrough the reduction of inflammatory cytokines and reduces oxidative stress by participating in the synthesis ofantioxidant enzymes and acts as a catalyzer of enzymes, taking part in lipid, carbohydrate, and protein metabolism. Other role of zinc was involved in the synthesis, storage, and release ofinsulin, which suggests the critical role of this microelement in the progression of type-2 diabetes mellitus.[14]

Physiological concentration of zinc inhibits the production of reactive oxygen species. The antioxidant effect of zinc may be mediated through direct action of zinc ion, its structural role in antioxidant proteins, and modulation metallothionein induction.[14]

Previous studies found zinc levels were lower in subjects with T2DM which in line with our result (table 2).[15; 16; 8]Although when the subjects were classified by albuminuria severity, our findings was the opposite of previous study (table 3). We found zinc serum was higher in albuminuria group in T2DM subjects compared to normoalbuminuria. The first findings supports the theory 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. But as the worsening of albuminuria, which is a marker of diabetic nephropathy, we found zinc serum was higher.

Related to zinc involvement in antioxidant activity, Doddigarla et al found serum Zn and SOD levels are significantly inversely correlated with HbA1c levels.[17]On the contrary, Va´vrova et al and Lima et al found increased antioxidant enzymes activity in patient with MS and T2DM. Lima et al state that the increase enzyme activity is a response to counteract oxidative stress in terms of adequate zinc supply.[18; 19]

In a review, Olechnowicz stated that metabolic stress induces a compensatory response, being characterized by increased Zn-mediated mechanisms of antioxidant protection. However, in terms of poor Zn status due to increased Znexcretion or insufficient intake, their mechanisms may notbe activated, resulting in aggravation of metabolicdisturbances.[14]This statement support our findings in T2DM group with albuminuria which zinc serum was higher than those in normoalbuminuria group. Regardless zinc serum was below normal level, it slightly increased in albuminuria group.

In addition to T2DM, several studies found HT as well affect zinc serum although the results were still controversial. Meta-analysis performed by Li et al which published in 2019 found serum Zn level in HT patients was significantly lower than that in controls.[20] While Suryana et al found lower zinc serum level in hypertension subjects in Surabaya, Indonesia which was categorized as deficiency compared to normotensive subjects.[21]On the contrary, Kunutsor et al in his cohort study found higher serum zinc concentration is positively and independently associated with incident hypertension in men.[22] Lower zinc serum in hypertension subjects was assumed due to increase inflammation and oxidative stress.Some HT drug usage also could decrease zinc serum level further.[21; 12]

Our analysis of zinc serum level in T2DM patients with or without HT was in line with Al-Timimi. Although there was no significant difference, zinc serum was lower in T2DM with HT group.

Limitations of this study are drugs usage which was not recorded which can affect zinc level, screening of albuminuria only used spot urine and done one time. The number of samples was still small due to unfinished research.

In conclusion, zinc level in T2DM patients is lower than in healthy people; with lower level was found in T2DM subjects with HT compared to non HT although there was no significant difference. T2DM patients with albuminuria had higher zinc level than those with normoalbuminuriawith no significance. This is a preliminary study which is still in progress.

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Table 1. Baseline characteristic for control group and type 2 diabetes mellitus group.

Variable	T2DM n = 27	Control n = 15	p-value
Age (year-old)	57.85 ± 8.35	46.87 ± 5.8	<0.0001*
BMI (kg/m^2)	23.9	22.37	0.439**
SBP (mmHg)	137.52 ± 19.6	113.2 ± 10.9	<0.0001*
DBP (mmHg)	80	70	0.064**
Serum creatinine	0.875	0.79	0.616**
Albumin/creatinine ratio (µg/mg creatinine)	64	5	0.001**
Zinc intake (mg)	4.78±1.51	5.74±0.95	0.017*
Zinc serum (µg/dL)	34.83	37.04	0.317**
***independent-t test **Mann Whitney test**			

T2DM - type 2 diabetes mellitus; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure.

Table 2. Albumin/creatinine urine ratio and zinc serum level in control group and type 2 diabetes mellitus group.

Group	n	Albumin/creatinine ratio (µg/mg creatinine)		Zinc serum (µg/dL)		Zinc intake (mg)	
		Median	p-value	Median	p-value	Mean	p-value
T2DM + HT	11	135.0	0.002**	33.37	0.583**	4.5 ± 1.49	0.066*
T2DM no HT	16	24.5		35.57		5.04 ± 1.55	
Control	15	5.0		37.04		5.74 ± 0.98	
***one way ANOVA **Kruskal Wallis test**							

T2DM - type 2 diabetes mellitus; HT - hypertension.

Table 3. Zinc serum level distribution in type 2 diabetes mellitus patients.

Variable	n	Zinc serum (µg/dL)		Zinc intake (mg)	
		Median	p-value	Mean	p-value
T2DM albuminuria	15	35.78	0.758*	4.9 ± 1.82	0.549**
T2DM normoalbuminuria	12	32.91		4.59 ± 1.10	
***Mann Whitney test **independent-t test**					

T2DM - type 2 diabetes mellitus.