Research Article

Serum Levels of Selenium, Copper and Zinc in Children with Type I Diabetes Mellitus

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Abstract
Background: In recent years, the oxidative stress-induced free radicals have been implicated in the pathogenesis of TID. It has been also reported that elements like selenium (Se), zinc (Zn), and copper (Cu) involved in lipid peroxidation may play a role in the pathogenesis and exacerbation of this disease. **Objective:** The aim of the present study was to measure the serum levels of selenium, copper and zinc in children with TID and compare them with that of apparently healthy children and to correlate their levels with glycated hemoglobin (HbA1c%). **Subjects and Methods:** Our study was carried on 100 children who were classified into 2 groups; Group I: included 60 diabetic children with mean age 7.8±2.1 years old, 25 male (41.7%) and 35 female (58.3%), a mean disease duration of 5.21±2.59 years. Group II: included 40 healthy controlled children with mean age 6.4±1.9 years old, 20 male (50%) and 20female (50%). **Results:** The Se and Zn levels of children with TID were significantly lower than tlwse of controls. Glycated hemoglobin (HbA1c) levels were found to be negatively correlated \Vith Se and Zn levels. On the other hand, Cu levels of children with TIDM were significantly higher than those of controls and higher in uncontrolled diabetic children tlmn in the controlled diabetic children and there was a significant positive correlation between glycated hemoglobin (HbAlc%) with Cu levels. The negative correlation between Se, Zn and HbA1c levels may show poor metabolic control of the disease and effect of oxidative injury. Those elements should be closely monitored during the course of TID and supplementation of these elements may be beneficial both for controlling diabetes and preventing long teen oxidative injury related to diabetic complications.
Keywords: Selenium, Zinc, Copper, HbA1c, Type 1 Diabetes mellitus

Introduction
Type one diabetes mellitus (TIDM) is a chronic heterogenous group of disorder which affect body metabolism occurs due to defects of insulin metabolism, it emerges from autoimmune destruction of insulin-producing pancreas islet cells in which environmental <md genetic factors may have a role in the pathogenesis of this complex disease (Maritim et al., 2003).

Diabetes pathogenesis is considered to be multi-factorial, and oxidant damage is one of the factors in the etiology of diabetes mellitus in children (Ozenc et al., 2015).

There is a strong relation between some essential metal elements and TlDM (Ozeuc et al., 2015).

Selenimn, an essential trace element with antioxidant properties, is a component of complex defense system against oxidative stress (selenitml-dependent glutathione peroxidases and selenoproteins) (Rayman et al., 2012).

Copper, an important part of many essential enzymes involved in a number of vital biological reactions. It participates in oxidation-reduction reactions in energy metabolism. Indeed Abnormal metabolism of Copper may have a role in the pathogenesis of chronic inflammatory

disorders including TlDM (Lin et al., 2014).

Zinc, an essential element, is usef11l in synthesis, storage and secretion of insulin. Zinc, is a component of many enzymes. The function of zinc in the body metabolism is based on its enzymatic affinity, way of a zinc enzyme complex or zinc metalloenzyme, It plays an important role in t11e maintenance of several tissue functions (Ortega eta!., 2fJ12).

Subjects and Methods

This was a prospective cross sectional study was carried out at pediatric endocrinology outpatients clinic, Minia university children and maternity Hospital during the period from October 2016 till April 2017.

.This study included 100 children grouped as following:

Group I (case group):-

It included 60 children diagnosed as having TID according to ADA, (2016) criteria (ADA., 2016), their age ranged from 5- 15 years with a mean of 7.8±2.1 years, 25(41.7%) of them were males while 35 (58.3%) were females.

They were furtherly divided into 2 subgroups according to HbAlc level:

Group I A: 25 controlled diabetic children 12(48%) of them males and 13(52%) of them females, with HbAlC :S 7.5.

Group I B: 35 uncontrolled diabetic children 13(37%) of them male and 22(62.8%) of them females, with HbAlC > 7.5 (JSPAD, 2014).

- Group II (control group):

It included 40 apparently healt11y control children age and sex matched with case group with a mean of 6.4±1.9 years, 20 (50%) of them were males and 20 (50%) were females.

All children witl1 TID treated witl1 insulin and had no other medications or supplemental intake of vitamins and other micronutrients. Insulin treatment consisted of multiple daily injections, frequent fasting, and postprandial capillary glucose monitoring and adjustment of the insulin dose accordingly.

Blood samples were obtained from the children after an overnight fast under complete aseptic technique, by sterile venipuncture and divided into:

1- One ml was collected in plain tubes for fasting blood glucose level.

2- Two ml was collected in tubes containing EDTA as anticoagulant for HbA1c assay.

3- Three ml was collected in plain tubes, left to clot for 30-60 minutes at room temperature and then, tubes were centrifuged at 1500 rpm for 15 minutes and separated serun1 was directly transferred to be kept frozen at – 20^{o}c until Se, Cu and Zn assay to be done.

Statistical analysis of the data of this study was performed using statistical package for the social sciences (SPSS) statistical package version 20.

Data were given as mean±standard deviation. Statistical significance was set at a p value of <0.05.

Results

Table (1): Comparison between diabetic children and control group as regard the studied laboratory data.

	Group I Diabetic children (N 60)	Group II Control children (N 40)	P-value
	Mean±SD	Mean±SD	
Fasting Glucose (mg/dl)	153.4±25.1	86.6±18.09	0.005*
HbAlc (%)	8.7±2.2	5.9±0.5	<0.001*
Selenium (ng/ ml)	37.2±9.8	109.5±16.8	<0.001*
Copper (ug/dl)	201.9±16.4	134.1±27.7	<0.001*
Zinc (ug/dl)	46.1±9.3	87.3±15.7	<0.001*

*** (p) was considered Significant if< 0.05**

Table (I) shows significant lower levels of selenium and zinc in diabetic children than in control group. On the other hand, there were significant higher levels of glucose, HbAlc and copper in diabetic children than in control group.

Table (2): Comparison between controlled and uncontrolled diabetic children as regard the studied laboratory data.

	Controlled (HbAlc <7.5) (N 25)	Uncontrolled (HbAlc ≥7.5) (N 35)	P-value
	Mean±SD	Mean±SD	
Selenium (ng/ml)	46.4±3.0	30.6±7.4	<0.001*
Copper (ug/dl)	185.8±11.2	213.4±6.9	<0.001*
Zinc (ug/dl)	54.9±6.2	39.7±4.8	<0.001*
HbAlc%	6.6±0.4	10.3±1.6	<0.001*

*** (p) was considered Significant if< 0.05**

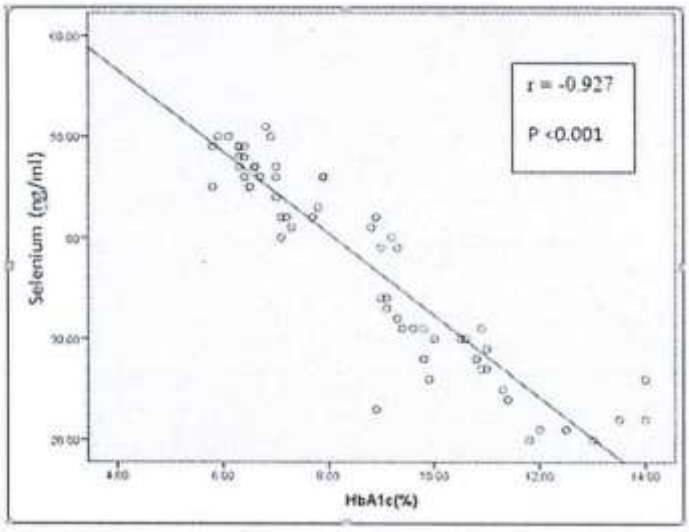
Table (2) shows significant lower levels of selenium, and zinc in uncontrolled diabetic children than in controlled diabetic children. On the other hand, there were significant higher levels of copper and HbA1c in uncontrolled diabetic children than in controlled diabetic children.

Table (3): Correlations between HbAlc with serum selenium, copper and zinc in diabetic children**.**

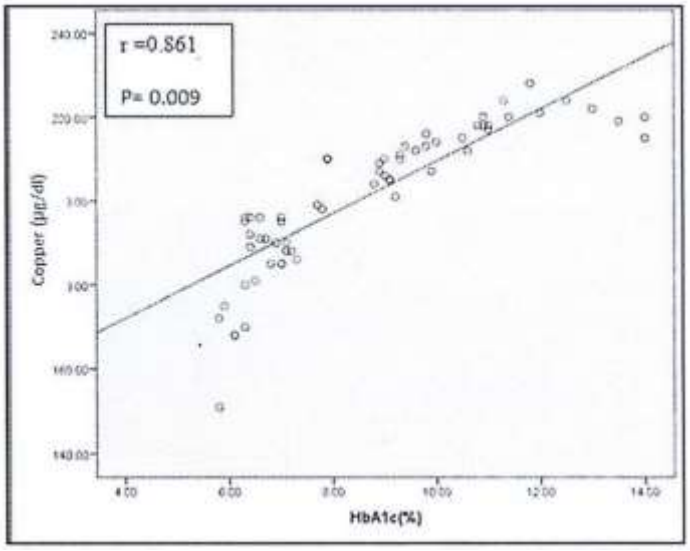
	HbAlc	
	R	**P-value**
Selenium(ng/ml)	**-0.927**	**<0.001***
Copper(1-1g/dl)	**0.861**	**0.009***
Zinc(1-1g/dl)	**-0.900**	**<0.001·**

***(p) was considered Significant if < 0.05**

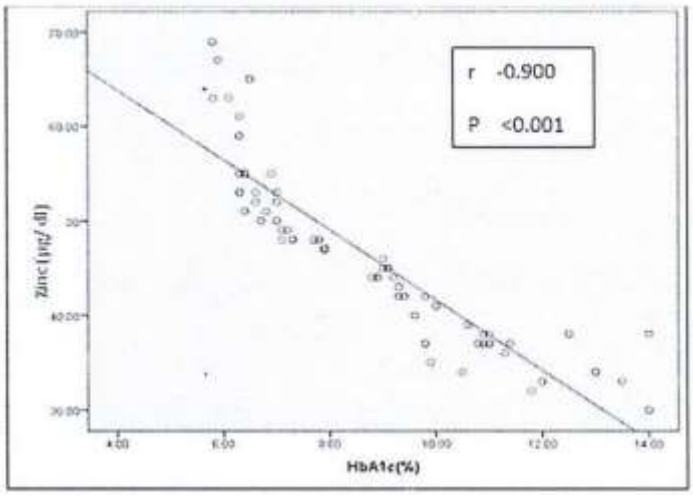
There were significant strong negative correlations between Se, Zn and HbA1c in diabetic children (r=-0.927, p<O.OOl; r=-0.900, p<0.001 respectively) (Fig. I and 3). On the other hand, there was a significant positive correlation between Cu and HbAlc in diabetic children(r=-0.861, p 0.009) (Fig. 2).



(Fig. 1): Correlation between levels of selenium and HbAlc in diabetic children.



(Fig. 2): Correlation between levels of copperand HbAlc in diabetic children.



(Fig. 3): Correlation between levels of zinc and HbAlc in diabetic children.

Table (4): Relationship between duration of diabetes and selenium, copper, zinc and HbA1c%.

	TIDM cases duration <5 years (N 33)	TIDM cases duration <5 years (N 27)	P-value
	Mean±SD	Mean±SD	
Selenium (ng/ml)	37.3±9.5	35.03±10.5	0.383
Copper (ug/dl)	202.5±17.4	198.2±15.2	0.318
Zinc (ug/dl)	45.6±9.4	46.7±9.2	0.635
HbA1c%	8.8±2.2	8.6±2.3	0.723

Table (4) shows insignificant difference in levels of Se, Cu and Zn in diabetic children as regard the duration of the disease.

Discussion

Strict glycemic control is essential for preventing the complications of diabetes. HbAlc% is the stable product of non-enzymatic irreversible glycation of the beta chain of hemoglobin by plasma glucose and is formed at rates that increase with increasing plasma glucose levels. HbA1c % levels provide an estimate of plasma glucose levels during the proceeding l-3 months (ADA, 2014).

Antioxidant system disorder and/ or lack of antioxidant elements such as Se, Cu and Zn, changes in pancreatic antioxidant enzyme activities are major factors accused in the destruction of pancreatic B cells and may be related in the development of diabetes (Ozenc et al., 2015).

In our study serum Selenium concentrations were reported to be decreased in children with TID than in the healthy control group (p value < 0.001) (table 1) and our results were in agreement with Ozenc et al., (2015) who reported a significant decrease of serum Se in patients with TID. The lower selenium status measured in diabetes might be explained by effect of the disease, or its associated inflammation, on selenium status. For example, a systemic inflammatory response produces cytokines that inhibit the expression of SEPP1 and will reduce plasma selenium (Rayman., 2012).

Another studies suggested that current daily intake of dietary selenium may be inadequate to protect human health (Rayman., 2012). In addition, experimental and observational prospective studies indicate a diabetogenic effect of selenium at low levels of intake (Vinceti et al., 2009). The supplementation of Se might have significant value for the treatment of diabetes and prevention of complications (Ozenc et al., 2015).

On the other hand, our results were in contrast with Tabar et al., (2012) and Asare et al., (2013) who reported elevated of serum Se in T2D patients. The high levels of selenium concentrations lead to over expression of GPx 1, which removing hydrogen peroxide, a second messenger in the insulin signaling cascade and insulin binding to its receptor lead to insulin resistance.

In addition, Se levels were significantly lower in uncontrolled diabetic children than in the controlled diabetic children (p value <0,001) (table 2) and there were a significant negative correlation between Se and HbAlc levels (r = -0.927, P < 0.001), (table 3) and (figure 1). This was in agreement with Ozenc et al., (2015), who found decreased serum Se levels that negatively correlated with HbAlc levels in diabetic children and explained that by with poor control, more hyperglycemia lead to excess levels of reactive oxygen species that interfere with the expression of SEPPI and will reduce selenium concentrations.

In our study, no significant correlation between disease duration and level of selenium was observed (Table 4). This was in agreement with the result by Forte et al., (2013), who found insignificant differences were observed between the Se concentrations and duration of the disease.

In the present study, there was a significant higher levels in serum copper in children with TIDM in comparison to healthy controls (p value <0.001) (table 1) and our result was in agreement with Lin et al., (2014) and Salmonowicz et al., (2014), who stated that serum copper levels were significantly elevated in patients with TID in comparison to control.

The increase in copper levels in patients with TID may be attributed to hyperglycaemia, which stimulates glycation and causes release of copper ions from copper binding sites of proteins. The release of copper ions into blood further accelerates the oxidative stress (Olaniyan et al., 2012). Copper in its free form is a potent cytotoxic element because of its redox chemistry. It readily participates in Fenton and Heiber Weiss reactions to generate reactive oxygen species (Sarkar et al., 2010).

On the other hand, our result was in disagreement with Basaki et al., (2012), who stated that serum copper levels were reduced in 20 Iranians diabetic patients, but Ekmekcioglu et al., (2001) observed no change in copper levels in diabetic patients and control.

In addition, serum copper levels in our study were significantly higher in uncontrolled diabetic children than in the controlled diabetic children (p value <0,001) (table 2). Moreover, there was a significant positive correlation between Cu and HbAlc (r = 0.861, P < 0.009) (table 3) and (figure 2). This was in agreement with EvliyaoiJlu et al., (2004), who found a strong positive correlation between copper and HbA1c that represents blood glucose regulation. The relation between HbA1c, which shows the glucose regulation, and the increasing copper value supports that Cu is an important marker for oxidative stress, because HbA1c levels rise with the poor control of diabetes mellitus.

In our study, there was no significant correlation between disease duration and level of copper (Table 4).This was in agreement with the result by Olaniyan et al., (2012), who stated that no significant differences observed between the Cu levels and duration of the disease.
An unbalanced level of Zn in the body can reduce the activity of the antioxidant enzymes and to contribute to the tissue damage (DiSilvestro., 2000).

In our study, serum Zinc levels were significantly lower in children with TID than in the healthy control group (p value <0.001) (table 1) and these results were in agreement with Kazi et al., (2008) and Olaniyan et al., (2012), who reported decrease levels of zinc in T2D patients.

Our result was in agreement with Ozenc et al., (20 IS), who reported that serum zinc levels in the diabetic group were lower than in the control group and explained by hyperzincuria or decreased gastrointestinal absorption of Zn, or both in diabetic patients.

In fact, some authors found beneficial effects in Zn supplemented T2D patients and referred of an effective improvement in their glycemic control (Al-Maroof and Al-Sharbatti., 2006).
On the contrary, other studies found unchanged levels of Zn (Serdar et al., 2009) and (EvliyaoiJlu et al., 2004), while Zargar et al., (2002), reported increased plasma level of Zn in type1 diabetic patients.

In addition, zinc levels were significantly lower in uncontrolled diabetic children than in the controlled diabetic children (p value <0,001) (table 2). Moreover, there was a significant negative correlation between Zn levels and HbA1c% (r= -0.900 p<0.001), (table 3) and (figure 3). This was in agreement with Ozenc et al., (2015), who found decreased serum Zn levels that negatively correlated with HbA1c% levels in diabetic children.

Quilliot et al., (2001) explained the negative correlation between Zn levels with HbA1c% levels in diabetic children by hyperglycemia, as assessed by fasting plasma glucose and by plasma HbAlc, which was responsible for the increased zinc excretion and the decreased superoxide dismutase activity.

Jansen et al., (2009) had hypothesized that the plasma Zn concentration may be related to the duration of the disease, such that the initial elevation of the plasma levels at the onset of the disease (when B-cells destruction occurs) is followed afterwards by a drop when elevated urinary Zn excretion overcomes the release of Zn from B-cells. However in our study, no clear relationship between disease duration and level of Zn was observed (Table 4).

So, zinc supplementation can protect harmful effect of diabetes-induced oxidative stress and it can be hypothesized that serum Zn levels should be closely monitored during the course of TID and supplementation may be given to patients (Ozenc et al., 2015).

Conclusions

Lower Se and Zn levels and higher Cu levels in diabetic children than control group indicate the effect of oxidative stress in pathogenesis of diabetes and metabolism of insulin.

Also the negative correlation between HbA1c and Se, Zn levels and positive correlation with Cu level show the negative effect of poor diabetic control on oxidative system or the effect of oxidative stress on poor control of diabetes.

References

1. Al-Maroof R and Al-Sharbatti S. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type-2 diabetics. Saudi Med J, 2006; 27: 344-50.
2. American Diabetes Association (ADA). Classification and Diagnosis of Diabetes Mellitus. Diabetes Care, 2014; 37(1): 85.
3. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. Diabetes Care, 2016; 30 (1):42-7.
4. Asare GA, Osae S, Nortey ·ENN, Yambire FK, Amedonu E, Doku D and Annan Y. Evaluation of serum metallothionein-1, selenium, zinc, and copper in Ghanaian type 2 diabetes mellitus patients. Int J Diabetes Dev Ctries, 2013; 33:86-95.
5. Basaki M, Saeb M, Nazifi S and Shamsaei HA. Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. Bioi Trace Elem Res, 2012; 148(2): 161-4.
6. Di-Silvestro R. Zinc in relation to diabetes and oxidative disease. J Nutr, 2000; 130:1509-11
7. Ekmekcioglu C, Prohaska C, Pomazal K, Steffan I, Schernthaner G and Marktl W. Concentrations of seven trace elements in different hematological matrices in patients with type 2 diabetes as compared to healthy controls. Bioi Trace Elem Res, 2001; 79:205-9.
8. Evliyaoglu O, Kebap9Ilar L, Uzuncan N, Kthvaslan N, Karaca B, Koca9elebi Rand Yensel N. Correlations of serum Cu+2, Zn+2, Mg+2 andHbA1c in type 2 and type 2 diabetes mellitus. Turk J Endocrinol Metab, 2004; 2:75-9.
9. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C, Oggiano Rand Madeddu R. Blood metals concentrat on in type 1 and type 2 diabetics. Biol Trace Elem Res, 2013; 156(1-3):79-90.
10. Jansen J, Rosenkranz E, Overbeck S, Warmuth S, Mocchegiani E,Giacconi R, Weiskirchen R, Karges W and Rink L. Disturbed zinc homeostasis in diabetic patients by in vitro and in vivo analysis of insulinomimetic activity of zinc. J Nutr Biochem, 2012; 23:1458-66.
11. Kazi T, Mridi H, Kazi N, Jamali M, Arain M, Jalbani Nand Kandhro G. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients.

Biol Trace Elem Res, 2008; 122:1-18.
12. Lin C, Huang H, Hu C, Chen B, Chong I, Chao Y and Huang Y. Trace elements, oxidative stress and glycemic control in young people with type 1 diabetes mellitus. J Trace Elem Med Biol, 2014; 28(1):18-22.
13. Maritim A, Sanders R and Watkins J. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol, 2003; 17(1):24-38.
14. Olaniyan O, Awonuga M, Ajetunmobi A, Adeleke I, Fagbolade O, Olabiyi K, Oyekanmi B and Osadolor H. Serum copper and zinc levels in Nigerian type 2 diabetic patients. Afr J Diabetes Med, 2012; 20:36-8.
15. Ortega, Rodriguez R, Jimenez, Sobaler L, Rodriguez G and Andres. Poor Zinc Status is Associated with Increased Risk of Insulin Resistance in Spanish Children. British Journal of Nutrition, 2012; 107: 398-404.
16. Ozen9 S, Saldir M, San E., etinkaya S, Ye ilkaya S, Babacan O, Fidanct K and Yesilkaya E. Selenium, zinc, and copper levels and their relation with HbA1c status in children with type 1 diabetes mellitus Int J Diabetes Dev Ctries, 2015; 35(4):514-8.
17. Quilliot D, Dousset B, Guerci B, Dubois F, Drouin P and Ziegler 0. Evidence that diabetes mellitus favors impaired metabolism of zinc, copper, and selenium in chronic pancreatitis. Pancreas. 2001; 22: 299-306.
18. Rayman M and Stranges S. Epidemiology of selenium and type 2 diabetes: can we make sense of it?. Free Radic Biol Med, 2013;65:1557-64.
19. Rayman MP. Selenium and human health. Lancet, 2012; 379:1256-68.
20. Salmonowicz B, Krzystek-Korpacka M and Noczynska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. Adv Clin Exp Med, 2014; 23(2):259-68
21. Sarkar A, Dash S, Barik BK, Muttigi MS, Kedage V, Shetty JK. And Prakash M. Copper and Ceruloplasmin levels in relation to total thiols and GST in type 2 diabetes mellitus patients. Ind J Clin Biochem, 2010; 25:74-6.
22. Serdar M, Bakir F, Ha imi A,cellik T, Akin O, Kenar L, Aykut O and Yildirimkaya M. Trace and toxic element patterns in nonsmoker patients with non insulin dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. Int J Diabetes Dev Ctries, 2009; ·29:35-40.
23. Tabar M. Determination of serum selenium in patients with type II diabetes mellitus. Middle-East J Sci Res, 2012; 12:433-5.
24. Viktorinova A, Toserova E, Krizko M and Durackova Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism, 2009; 58:1477-82.
25. Vinceti M, Maraldi T, Bergomi M and Malagoli C. Risk of chronic low dose selenium overexposure in humans: insights from epidemiology and biochemistry. Rev Environ Health, 2009; 24(3):231-48.
26. Zargar AH, Shah NA, Masoodi SR, LawayBA, Dar FA, Khan AR, Sofi FA and Wani AI. Copper, zinc and magnesium levels in noninsulin dependent diabetes mellitus. Postgrad Med J, 1998; 74: 665-8
27. Zargar AH, Shah NA, Masoodi SR, Laway BA, Dar FA, Khan AR, Sofi FA and Wani AI. Copper, zinc and magnesium levels in type-1 diabetes mellitus. Saudi Med 1, 2002; 23: 539-42.