Türk Biyokimya Dergisi [Turkish Journal of Biochemistry - Turk J Biochem] 2006; 31 (3); 107–114.

Research Article [Araştırma Makalesi]

Yayın tarihi 18 Eylül, 2006 © TurkJBiochem.com

[Published online 18 September, 2006]

Influence of Age, Gender and Duration of Diabetes on Serum and Urine Levels of Zinc, Magnesium, Selenium and Chromium in Type 2 Diabetics in Calabar, Nigeria

[Yaş, Cinsiyet ve Diyabet Süresinin Calabar Nijerya Tip 2 Diyabetlilerinde Serum ve İdrar Çinko, Magnezyum, Selenyum ve Krom Seviyelerine Etkisi]

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ABSTRACT

The study aimed to demonstrate the possible effect of some variables such as age, gender and duration of diabetes on the serum and urine levels of zinc, magnesium, selenium and chromium in type 2 diabetic subjects and a control group of non-diabetic individuals. Serum and urine zinc (Zn), magnesium (Mg), selenium (Se), chromium (Cr) and creatinine; fasting plasma glucose (FPG) and serum urea levels were determined in 60 diabetic subjects of both genders aged between 40-75 years and 40 age matched non diabetic subjects from Calabar, Nigeria using colorimetric methods and atomic absorption spectrophotometry (AAS). Elderly diabetics (55-75) have significantly ($p<0.05$) lower serum Se and Cr and higher urinary trace elements than the younger age group (40-54). Female diabetics have significantly ($p<0.05$) higher serum Zn and Se and lower urinary Mg and Zn than diabetic males. Serum and urine trace elements concentrations varied significantly ($p<0.05$) with the duration of diabetes. Aging and increasing duration of diabetes alters the metabolism of Zinc, Magnesium, Selenium and Chromium by decreasing their serum concentration and increasing their urinary excretion. The gender related differences in trace element levels in diabetics, might be attributed to hormonal imbalance associated with the diabetic state.

Key Words: Type 2 diabetics, Age, Gender, Zinc, Magnesium, Selenium, Chromium

ÖZET

Bu çalışmada yaş, cinsiyet ve diyabet süresi gibi değişkenlerin tip 2 diyabetli hastaların serum ve idrar çinko (Zn), magnezyum (Mg), selenyum (Se) ve krom (Cr) seviyelerine olası etkisi incelenmesi amaçlanmıştır. Calabar Nijer'li 40-75 yaş arasında olan ve hem kadın hem de erkek 60 diyabetik ile yaş grubu uyumlu 40 normal kişide, serum ve idrar Zn, Mg, Se, Cr, kreatinin ve açlık plazma glukoz (FPG) seviyeleri atomik emilim spektrofotometrisi (AAS) ve kolorimetrik yöntemler kullanılarak belirlenmiştir. Yaşlı diyabetiklerde (55-75 yaş) genç diyabetiklere (40-54 yaş) oranla belirgin olarak ($p<0.05$) daha düşük serum Se ve Cr seviyesi saptanırken idrar eser ögeleri daha yüksek bulunmuştur. Kadın diyabetik grubunda erkeklere oranla belirgin olarak ($p<0.05$) yüksek serum Zn ve Se miktarı ile düşük idrar Mg ve Zn seviyesi saptanmıştır. Yaşlanma ve diyabet süresi Zn, Mg, Se ve Cr metabolizmasını serum derişimlerini düşürüp idrarla atılımlarını arttırarak etkilemiştir. Diyabetiklerde Zn, Mg ve Se seviyelerinde cinsiyete bağlı farklılıklar diyabetik durum ile ilişkili hormonal dengesizliğe bağlı olabilir.

Anahtar Kelimeler: Tip 2 diyabet, Yaş, Cinsiyet, Çinko, Magnezyum, Selenyum, Krom

Kayıt tarihi 25 Ekim 2005; kabul tarihi 06 Mart 2006
[Received 25 October 2005; accepted 06 March 2006]

INTRODUCTION

The relationship between nutrition and diabetes was suspected as early as 1674 and over the last 20 years, numerous studies have found alteration in micronutrient status of patients with diabetes mellitus (1). Deficiencies of trace elements and minerals have been implicated in the development of the various diabetic complications. It is not known whether differences in trace elements status are a consequence of diabetes or alternatively whether they contribute to the expression of the disease (2). Mechanisms that ensure optimal distribution of an element over a range of intakes constitute a system of homeostatic regulation for that element (3). The homeostatic regulation of trace element concentration and distribution in the body are affected by a number of factors including dietary intake, sex, age and presence of disease such as diabetes (4). Insulin resistance is a common finding in the elderly people and as such ageing is associated with an impaired glucose handling (5). Reductions in renal function have also been associated with ageing (6). Decline in physiological functions with age may influence absorption, excretion and metabolism of micronutrients (7,8). Sex related differences have also been observed in the reference values of some trace elements (9), while long standing diabetes may exert its influence via development of various diabetic complications such as nephropathy. All these suggest that there has been a lot of influence on the normal body metabolism of trace elements.

The influence of age, gender and duration of diabetes on serum and urine levels of Zinc, Magnesium, Selenium and Chromium in diabetics were determined in this study.

Material and Methods

Patients and Study Design

Type 2 diabetic patients attending the diabetic clinic of the University of Calabar Teaching Hospital (UCTH) and non-diabetic subjects selected from apparently healthy individuals attending the staff clinic of the hospital were included in the study. Informed consent was obtained from subjects before recruitment into the study. The ethics committee of University of Calabar teaching hospital approved the study protocol. The inclusion criteria for the study were as follows; Age, 40-75 years at the time of the study, known type 2 diabetic patient for the past two years, and non-diabetic according to the 1999 World Health Organization diagnostic criteria for diabetes (10). Exclusion criteria were as follows; pregnancy in diabetic subjects and controls, presence of renal complications and hypertension.

Selection of subjects

Subjects were randomly selected from the population group specified above based on fulfillment of the inclusion criteria. A total of one hundred subjects were recruited for the study. Sixty type 2 diabetic patients (29 males and 31 females) and forty non-diabetic subjects (15 males and 25 females) were used as controls. Both diabetic and non diabetic subjects were grouped as follows:

Gender: Males and Females

Age groups: Younger age group (40-54 years) and Elderly (55-75 years)

Duration of Diabetes: ≤ 4 years, 5-9 years, 10-14 years and ≥ 15 years.

Body weight and height were measured and used to calculate the BMI, which was used as a measure of relative body weight. Blood pressures of subjects were taken at three intervals one month prior to sample collection to rule out the undiagnosed hypertension. A structured questionnaire was used to obtain the data on occupation, physical activity, lifestyle pattern as smoking and alcohol consumption, past and present illness and medication.

Sample collection

After an overnight fast, fasting venous blood samples were collected aseptically from the subjects via venepuncture for fasting plasma glucose, serum zinc, magnesium, selenium and chromium determination. Assay for serum urea and creatinine were also done to test for renal function. Fasting spot urine samples were also collected into the sterile chemically clean universal containers for urine zinc, magnesium, selenium and chromium determination and also for urine creatinine estimation. Urine trace elements concentrations are expressed per gram of creatinine.

Methods

Fasting plasma glucose was determined using the glucose oxidase method of Barharm and Trinder, (1971) (11), serum urea concentration was estimated using the diacetyl monoxime method of Veniamin and Vakirtz-Lemonias, (1970) (12), serum and urine creatinine contents were determined using the modified Jaffes reaction method of Spencer, (1986) (13); and zinc, magnesium, selenium and chromium levels in serum and urine were estimated using the flame atomic absorption spectrophotometry (Buck Scientific model 210 VGP, 1992) with graphite furnace using air/acetylene flame.

Statistical analysis

The significance of difference between the groups was assessed by student's t-test analysis. Variations within and among groups were tested using the one-way analysis of variance. P value <0.05 was considered statistically significant.

RESULTS

Table 1 shows some descriptive data for diabetics and non diabetic subjects used in the study. In the diabetic population, 48.33% of the subjects were males while

Table 1: Descriptive Data For Diabetics And Non-diabetics

Data	Diabetics, n (%)	Non-diabetics, n (%)
Gender		
Male	29/60(48.33)	15/40(37.50)
Female	31/60(51.67)	25/40(62.50)
Age groups (years)		
40-54	44/60(73.33)	32/40(80)
55-75	16/60(26.67)	8/40(20)
Duration of Diabetes (yrs)		
≤ 4	41/60(68.33)	Nil
5-9	7/60(11.67)	Nil
10-14	7/60(11.67)	Nil
≥15	5/60(8.33)	Nil
Alcohol drinking		
Drinkers	12/60 (20.00)	13/40 (32.50)
Non drinkers	48/60 (80.00)	27/40 (67.50)
Smoking habit		
Non-Smoking	50/60 (83.33)	25/40 (62.50)
Smoking & Duration (yrs)		
1-5	6/60 (10.00)	8/40 (20.00)
6-10	2/60 (3.33)	4/40 (10.00)
11-20	2/60 (3.33)	2/40 (5.00)
>20	1/60 (1.67)	1/40 (2.50)

51.67% were females. 73.33% belong to the younger age group (40-54 years) while 26.67% belong to the elderly (55-75 years). The number of subjects who have been suffering from diabetes for less than 4 years, between 5-9 years, 10-14 years and greater than 15 years were 68.33%, 11.67%, 11.67% and 8.33% respectively. 20% of subjects consume alcohol while 80% were non drinkers. Subjects who have been smoking for 1-5 years, 6-10 years, 11-20 years and greater than 20 years were 10%, 3.33%, 3.33% and 1.67% respectively, whereas 83.33% of subjects were non smokers. In the non diabetic population, 37.50% of the subjects were males while 62.50% were females. 80% belong to the younger age group (40-54 years) while 20% belong to the elderly (55-75 years). 32.50% of subjects consume alcohol while 67.50% were non drinkers. Subjects who have been smoking for 1-5 years, 6-10 years, 11-20 years and greater than 20 years were 20%, 10%, 5% and 2.5% respectively, whereas 62.50% of subjects were non smokers.

Table 2 shows the mean body mass index (BMI), blood pressure (B.P), fasting plasma glucose, serum urea and serum and urine creatinine (creat), Magnesium (Mg), Zinc (Zn), Chromium (Cr) and Selenium (Se) in Diabetic and Non-diabetic Subjects. The BMI, FPG, urine Mg, Zn and Se were found significantly higher and Serum Zn and urine creatinine lower in diabetics than in non-diabetics ($p<0.05$). All other parameters were not significantly different in both groups ($p>0.05$).

Table 3 shows the relationship of age on the serum and urine trace elements concentrations in diabetic and non diabetic subjects studied. The serum Mg levels in the age group of 40-54 years were found significantly higher than those of the age group of 55-75 years ($p<0.05$) while no significant differences were observed in the serum and urine levels of the other elements in both age groups ($p>0.05$) in the non diabetic population. The serum Se and Cr levels were found significantly lower and urinary Zn, Mg, Se and Cr levels higher in the age group of 55-75 years than those of the age group of 40-54 years ($p<0.05$) in the diabetic population.

Table 4 shows the relationship of gender with the serum and urine trace elements concentration in Diabetic and Non-diabetic subjects. The serum Zn and Se levels were found significantly higher and urinary Mg and Zn lower in diabetic females than those for the diabetic males

Table 2: Mean Body mass index (BMI), Blood pressure (B.P), Fasting Plasma Glucose, Serum Urea and Serum and Urine Creatinine (creat), Magnesium (Mg), Zinc (Zn), Chromium (Cr) and Selenium (Se) in Diabetic and Non-diabetic Subjects

				Blood	Pressure			Serum						Urine			
Subjects	Subjects	Age yrs	BMI Kg/m^2	Systolic mmHg	Diastolic mmHg	FPG *mmol/L*	Creat µmol/l	Urea mmol/L	Mg mg/L	Zn mg/L	Cr mg/L	Se mg/L	Creat g/L	Mg mg/gcreat	Zn mg/gcreat	Cr µg/gcreat	Se µg/gcreat
Diabetics n = 60	Diabetics n = 60	54.00 ±7.00	25.90 ±4.20	127.00 ±16.00	80.00 ±9.00	8.57 ±4.47	174.00 ±126.50	5.30 ±5.10	13.60 ±4.80	0.60 ±0.28	2.50 ±3.45	0.32 ±0.38	1.66 ±0.52	11.55 ±6.40	0.69 ±0.37	2.53 ±1.20	0.23 ±0.31
Non Diabetics n = 40	Non Diabetics n = 40	50.00 ±10.00	23.74 ±4.20	122.00 ±13.00	75.00 ±14.00	4.14 ±1.00	159.20 ±30.80	4.90 ±1.40	14.10 ±4.80	0.88 ±0.60	2.65 ±1.00	0.28 ±0.24	1.99 ±0.67	5.80 ±4.00	0.41 ±0.18	2.20 ±1.00	0.08 ±0.05
P value	p-value	p>0.05	p<0.05	p>0.05	p>0.05	p<0.05	p>0.05	p>0.05	p>0.05	P<0.05	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05

t-test analysis
p>0.05 is not significant
p<0.05 is Significant

Table 3: Relationship of Age with the serum and urine trace elements concentration in diabetic and non diabetic Subjects.

Age group (years)	Mg mg/L	Serum Zn mg/L	Se Mg/L	Cr µg/L	Mg mg/gcreat	Urine Zn mg/gcreat	Se mg/gcreat	Cr µg/gcreat
Diabetics								
40-54	13.89	0.66	0.38	3.01	14.41	0.64	0.16	2.28
n = 44	±4.90	±0.26	±0.26	±1.25	±3.62	±0.34	±0.07	±1.29
55-75	12.79	0.54	0.17	1.19	11.30	0.88	0.43	3.18
n = 16	±3.55	±0.28	±0.26	±0.09	±4.36	±0.44	±0.45	±1.50
	NS	NS	S	S	S	S	S	S
Non Diabetics	17.55	1.23	0.29	2.63	5.30	0.39	0.09	2.12
40-54	±4.52	±1.26	±0.26	±1.06	±3.56	±0.16	±0.12	±0.95
n = 32								
55-75	13.08	0.81	0.25	2.69	7.81	0.49	0.08	2.75
n = 8	±4.24	±0.24	±0.13	±0.76	±5.34	±0.25	±0.04	±1.09
	S	NS	NS	NS	NS	NS	NS	NS

t-test analysis
$p>0.05$ - non significant
$p<0.05$ - significant
S - significant
NS - non Significant

($p<0.05$), while no significant difference in the serum Mg and Cr, urinary Se and Cr were observed in both diabetic males and females ($p>0.05$). No gender related differences were observed in non-diabetic population

Table 5 shows the relationship of duration of diabetes with the serum and urine trace elements concentration of diabetic subjects. The serum and urine trace elements concentrations varied significantly ($p<0.05$) with the duration of diabetes, with the serum Mg and Zn decreasing and their urinary excretion increasing along with increasing duration of diabetes. Serum Se and Cr showed no specific pattern. No significant variation was observed in urine Cr levels.

DISCUSSION

Several factors have been described as influencing the serum and urine concentrations of trace elements. This is evidenced by great variability in their serum concentrations from various populations. Some studies have shown that serum antioxidant concentrations are primarily influenced by such variables as sex, age, obesity, tobacco smoking, alcohol consumption and dietary intake (14). Most factors cause a decrease rather than an increase in trace elements concentration. Decreased concentrations are related mainly to decreased nutritional intake, intestinal uptake and altered distribution while increased concentration is reported to result from excessive homeopathic intake, industrial or environmental exposure, smoking and administration of parenteral fluid (15). The possible relationship between such variables as age, gender and duration of diabetes with serum and urine levels of zinc, magnesium, selenium and chromium was examined in this study.

Results from our study demonstrated some degree of variability in the serum and urine trace elements concentrations with age, gender and duration of diabetes. Age appears to exert some significant effects on all subjects of the study. Serum Mg levels decreased with increasing age in the non diabetic population, whereas increasing age decreased serum Se and Cr levels and increased urinary Zn, Mg, Se and Cr loss in the diabetic population. Age related physiological changes, drug therapy, modified dietary requirements and chronic diseases leading to or associated with enhanced consumption or excretion of trace elements might contribute to trace elements deficiency in the elderly (4). A number of surveys have shown Mg, Zn, Se and Cr intakes by old persons to be lower than the corresponding nutrient intakes; this may

Table 4: Relationship of Gender with the Serum and Urine Trace Elements Concentration in Diabetic and Non Diabetic Subjects.

Subjects	**Mg mg/L**	Serum **Zn mg/L**	**Se mg/L**	**Cr µg/L**	**Mg mg/gcreat**	Urine **Zn mg/gcreat**	**Se mg/gcreat**	**Cr µg/gcreat**
Diabetic	12.78	0.49	0.12	2.46	13.27	0.80	0.29	2.62
males	±3.80	±0.29	±0.09	±3.89	±5.19	±0.40	±0.32	±0.82
n = 29								
Diabetic	14.33	0.71	0.41	2.45	9.92	0.59	0.16	2.45
females	±5.60	±0.24	±0.43	±3.15	±6.97	±0.31	±0.28	±1.54
n = 31								
	NS	S	S	NS	S	S	NS	NS
Non Diabetic								
males	12.67	0.85	0.28	2.67	5.40	0.54	0.06	2.00
n = 15	±5.80	±0.13	±0.27	±0.93	±3.87	±0.70	±0.11	±1.14
Non								
Diabetic	14.96	0.89	0.27	2.63	6.06	0.44	0.09	2.36
females	±3.97	±0.76	±0.24	±1.06	±4.18	±0.19	±0.10	±0.91
n = 25								
	NS	NS	NS	NS	NS	NS	NS	NS

t-test analysis
$p < 0.05$ - Significant
$p > 0.05$ - Non Significant
NS - Non significant
S - Significant

be attributed to changes in mineral bioavailability with aging (16). Ageing has been previously associated with low intracellular Mg concentration, probably the consequence of insulin resistant due to ageing (5). Increased urinary loss of trace elements associated with ageing may be attributed to reduction in renal function with ageing. Low magnesium, selenium and zinc status has also been reported in the elderly population (16, 14). A significant decrease in Zn and Se values was also demonstrated in individuals within the age range of 91-110 years when compared with individuals within the age range of 60-90 years by Savarino *et al.*, (2001) (17). Davis *et al.,* (1997) (18) reported a significant age related decrease in mean serum Cr levels in diabetic. Ding *et al.,* (1998) (19) also demonstrated increases in the urinary Cr concentration in both the diabetics and the control group with increasing age. However, lower serum selenium levels were reported in younger women when compared to the elderly (14). Czernichow *et al.*, (2004) (20) also demonstrated a positive correlation between age and serum and red cell magnesium levels in women and a negative correlation of age with urinary Mg in both genders. Naverro-Alarcon *et al.*, (1999) (21) observed no significant correlation between serum and urine selenium levels with patient's age and diabetes. Heterogenity of older adults and their unique rate of ageing may be responsible for the disparity in these results.

Gender related differences in serum Zn and Se levels were observed in the diabetic population of the study with the females having higher serum levels of Zn and Se than the males. Urinary Mg and Zn levels were found to be higher in the diabetic males than the females. The lower serum levels of Zn and Se observed in the diabetic males may be attributed to increased urinary excretion of these elements in the males. The lower serum Zn levels in the males may also be associated with additional seminal loss of Zn in male diabetics. Milne, (1999) (9) reported higher urinary Mg levels in males than in females. Sex related differences were also reported by Ruiz *et al.*, (1998) (22) who observed significant differences in plasma copper levels between diabetic females and non-diabetic females. Davies *et al.,* (1997) (23) observed significantly lower mean serum Cr levels in males than in females. Ding *et al.*, (1998) (19) also observed higher

Table 5: Relationship of Duration of Diabetes with the Serum and UrineTrace Elements Concentration in Diabetic Subjects.

Duration years	**Serum Mg mg/L**	**Zn mg/L**	Se **mg/L**	**Cr µg/L**	**Mg mg/gcreat**	**Urine Zn mg/gcreat**	**Se mg/gcreat**	**Cr µg/gcreat**
≤ 4	14.90	0.70	0.44	3.30	10.03	0.57	0.14	2.47
n = 41	±4.90	±0.25	±0.40	±3.92	±6.50	±0.29	±0.24	±1.45
5-9	13.80	0.60	0.10	0.94	11.20	0.67	0.09	2.75
n = 7	±2.40	±0.14	±0.04	±0.58	±3.34	±0.21	±0.06	±0.55
10-14	9.30	0.44	0.44	0.08	18.97	1.06	0.69	2.80
n = 7	±2.70	±0.15	±0.33	±0.07	±2.93	±0.27	±0.26	±0.53
≥15	8.20	0.23	0.04	0.34	16.56	1.25	0.64	2.82
n = 5	±1.30	±0.18	±0.01	±0.08	±2.29	±0.36	±0.31	±0.52
p- value	P<0.05	p<0.05	p<0.05	P<0.05	p<0.05	p<0.05	p<0.05	p>0.05

Analysis of variance (ANOVA)
p > 0.05 - non significant
p < 0.05 - significant

urinary Cr levels in diabetic females than in males of same age group, however, such differences in Cr levels were not observed in this study. However, lower serum Zn and Se concentrations have been demonstrated in women when compared to the men (14). Akbaraly *et al*., (2005) (24) reported that plasma Se concentrations were not significantly different between men and women and were not correlated with age in either sex. This sex related differences in the serum and urine trace elements concentrations were not observed in the non-diabetic population, hence the sex difference seen in the diabetic population may be attributed to the diabetic state.

Duration of diabetes seems to exert a significant effect on the serum and urine trace elements concentration of the diabetic population studied. Serum Mg and Zn levels decreased and their urinary excretion increased with increasing duration of diabetes. The decreased serum levels may be attributed to the increased urinary loss of these trace elements. Reductions in renal functions with increasing duration of diabetes have been implicated in urinary trace elements loss (25). However, no correlation has been found between the plasma and urinary Zn levels on the one hand and the duration of diabetes and patients weight on the other hand as demonstrated by Mertz, (1998) (26).

In conclusion, our findings indicate that gender and ageing do not seem to have any significant effect on serum and urine magnesium, zinc, selenium and chromium concentrations in non diabetics. However, in the diabetics, ageing and increasing duration of diabetes enhances urinary loss of these elements, while lower serum zinc and selenium and higher urinary magnesium and zinc excretion was associated with the male gender. These age and gender related differences might be attributed to the hormonal imbalance associated with the diabetic state.

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