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Serum and Urine Levels of Chromium and Magnesium in Type 2 Diabetics in Calabar, Nigeria

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ABSTRACT

Alterations in trace elements have been reported in a number of disease states. Deficiency of some trace elements has been correlated with the presence of diabetic complications. It is not known whether differences in trace elements status are a consequence of diabetes and hyperglycaemia or alternatively whether their deficiencies contribute to the expression of the disease. The study was aimed at determining the concentrations of essential elements chromium and magnesium in the serum and urine of diabetics and non-diabetics in Calabar, Southern Nigeria. Serum and urine chromium (Cr), magnesium (Mg) and creatinine, fasting plasma glucose (FPG) and serum urea levels were determined in sixty diabetic subjects (29 males and 31 females) and forty age-matched non-diabetic subjects (15 males and 25 females), using colorimetric methods and atomic absorption spectrophotometry. Body mass indices (BMI) of the subjects were also determined. Statistical analysis was done using t-test and correlational analysis. The BMI, FPG, and urine Mg were significantly ($p<0.05$) higher and urine creatinine lower in diabetics than non-diabetics studied. No significant ($p>0.05$) differences were observed in serum urea, creatinine, Mg, Cr, and urine Cr levels of both groups. A significant negative correlation ($p<0.01$, $r = -0.441$) was observed between serum Mg and urine Mg of diabetics. No association was seen between serum Mg and serum Cr levels and urine Cr and urine Mg of the diabetic population of the study. Diabetes enhances urinary magnesium loss but does not seem to affect serum and urine chromium levels. There is need for further study on the mechanism of urinary magnesium loss in diabetes.

INTRODUCTION

Interest in the role of trace elements in medical research has been growing in recent decades. Many trace elements seem to be essential for humans and various metabolic processes are dependent on normal trace elements concentrations. Their deficiencies have been implicated in various mal trace elements including diabetes, anaemia, depression, ageing, low sexual potency and heart disease (Arora and Gores, 1996). Morradian and Morrely (1987) in their review article on the serum

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micronutrient status in diabetes stated that the relationship between nutrition and diabetes was suspected as early as 1674 and that over the last twenty years, numerous studies have found alterations in micronutrient status of patients with diabetes mellitus. In some studies, deficiency of certain minerals or vitamins has been correlated with the presence of diabetic complications. It is now known that dietary modifications can help considerably in improving blood glucose control and therefore reduce many complications caused by this condition (Broadhaust, Schmidt and Reeves, 1997). Chromium, as a component of the Glucose Tolerance Factor (GTF) is essential for normal carbohydrate and lipid metabolism in mammals (Davis *et al*., 1997). It acts by activating insulin receptor kinase activity up to seven fold (Vincent, 1999), thus increasing insulin autoamplification (Davis & Vincent, 1997) and efficiency (Watts, 1999). Magnesium, on the other hand, serves as a cofactor for some enzymes of the glycolytic pathway and enhances the ability of insulin to activate tyrosine kinase (Suarez, 1993). The clinical significance and evaluation of these elements in regard to diabetes remains conflicting (Chatterjee, Mukhejee and De, 1999), probably due to number of subjects, sex and laboratory processing (Zargar *et al*., 1998, Zargar *et al*., 2002).

The serum and urine levels of chromium and magnesium were determined in diabetic and non-diabetic population in this study to ascertain if diabetics were deficient of these elements.

SUBJECTS AND METHODS

Study Design

The study was designed to asses the serum and urine levels of chromium and magnesium among known 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. Informed consent was sought and 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, 45-75 years at the time of the study, known type 2 diabetic patient for the past five years, and non-diabetic according to the 1999 World Health Organization diagnostic criteria for diabetes (WHO, 1999). 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 known type 2 diabetic patients (29 males and 31 females) and forty non-diabetic subjects (15 males and 25 females) were used as control.

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

Sample collection

After an overnight fast, fasting venous blood samples were collected asceptically from the subjects via

venepuncture for fasting plasma glucose, serum chromium and magnesium determination. Assay for serum urea and creatinine were also done to test for renal function. Fasting spot urine samples were also collected into sterile chemically clean universal containers for urine chromium and magnesium determination and also for urine creatinine estimation to correct for urine flow rate of individuals. Urine trace elements concentrations are expressed per gram of creatinine.

Methods

Fasting plasma glucose was estimated using the glucose oxidase method of Barharm and Trinder (1972), serum urea was estimated using the diacetyl monoxime method of Veniamin and Vakirtz-Lemonias (1970), serum and urine creatinine was estimated using the modified Jaffes reaction method of Spencer (1986), while serum and urine chromium and magnesium were estimated using the flame atomic absorption spectrophotometry.

Statistical analysis

The significance of difference between the groups was tested using the t-test analysis. Association between variables was determined using the Pearson's correlational analysis on Microsoft excel and SPSS soft ware 10.0 version (California Inc.). A two sided P value <0.05 and <0.01 was considered statistically significant for the t-test and Pearson correlation analysis respectively.

RESULTS

Table 1 shows the descriptive data of diabetics and non-diabetic subjects in the study. In the diabetic group, 21.67% of the subjects were single, 53.33% were married, 15% were widowed whereas 8.33% were divorced. In terms of occupation, 56.67% were civil servants, 28.33% were businessmen whereas 15% were unemployed. 61.67% went about their daily business by trekking or use of public transport, 8.33% were chauffer driven, 21.67% were self-driven whereas 8.33% used other means of transportation. For social habits such as alcohol intake and smoking, 20% were moderate drinkers, 80% were non-drinkers and 83.33% were non-smokers. 10% had been smoking for 5 years, 3.33% for 6-10 years, 3.33% for 11-20 years and 1.67% for > 20 years. In terms of hypoglycemic agents used by diabetics, 33.33% used biguanides, 50% used sulphonylureas whereas 16.67% made use of insulin. 83.33% had suffered from malaria in the past, 8.33% had typhoid fever whereas 8.33% have had both malaria and typhoid fever.

In the non-diabetic group, 22.5% were single, 50% were married, 15% were widowed whereas 12.5% were divorced. In terms of occupation, 57.5% were civil servants, 30% were businessmen and 12.5% were unemployed. 62.5% of the non diabetics went about their daily activities by trekking or use of public transport, 5% were chauffer driven, 20% were self driven whereas 12.5% fall into other category of transportation. With regards to alcohol intake and smoking, 32.5% were moderate drinkers, 67.5% were non-drinkers and 62.5% were non-smokers. 20% had been smoking for 5 years, 10% for 6-10 years, 5% for 11-20 years and 2.5% for > 20 years. 37.5% had suffered from malaria in the past, 25% had typhoid fever whereas 37.5% have had both malaria and typhoid fever.

Table 2 shows the body mass index (BMI), blood pressure (BP), fasting plasma glucose, serum urea and serum and urine creatinine (creat), chromium (Cr) and magnesium (Mg) in diabetic and non-diabetic subjects. The BMI and fasting plasma glucose levels were significantly ($p<0.05$) higher in diabetics than non-diabetic sub-

Table 1. Descriptive data for diabetics and non-diabetic subjects

Data	*Diabetics, n (%)*	*Non-diabetics, n (%)*
Marital status		
Single	13/60 (21.67)	9/40 (22.50)
Married	32/60 (53.33)	20/40 (50.00)
Widow	9/60 (15.00)	6/40 (15.00)
Divorced	6/60 (8.33)	5/40 (12.50)
Occupation		
Civil Servant	34/60 (56.67)	23/40 (57.50)
Business Men	17/60 (28.33)	12/40 (30.00)
Unemployed	9/60 (15.00)	5/40 (12.50)
Physical Activity		
Trekking/Public Transport	37/60 (61.67)	25/40 (62.50)
Chauffer driven	5/60 (8.33)	2/40 (5.00)
Self driven	13/60 (21.67)	8/40 (20.00)
Others	5/60 (8.33)	5/40 (12.50)
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)
Medication		
Biguanides	20/60 (33.33)	Nil
Sulphonylureas	30/60 (50.00)	Nil
Insulin	10/60 (16.67)	Nil
Past Ailment		
Malaria	50/60 (83.33)	15/40 (37.50)
Typhoid fever	5/60 (8.33)	10/40 (25.00)
Malaria & Typhoid fever	5/60 (8.33)	15/40 (37.50)

Table 2. Mean body mass index (BMI), blood pressure (BP), fasting plasma glucose, serum urea and serum and urine creatinine (creat), chromium (Cr) and magnesium (Mg) in diabetic and non-diabetic subjects

			Blood Pressure			*Serum*				*Urine*		
Subjects	*Age*	*BMI Kg/m²*	*Systolic mmHg*	*Diastolic mmHg*	*FPG mmol/l*	*Creat µmol/l*	*Urea mmol/l*	*Cr µg/l*	*Mg mg/l*	*Creat g/l*	*Cr µg/g Creat*	*Mg µg/g Creat*
Diabetics n = 60	54.00 ±7.00	25.90 ±4.20	127.00 ±16.00	80.00 ±15.00	8.57 ±4.47	174.00 ±126.50	5.30 ±5.10	2.50 ±3.45	13.60 ±4.80	1.66 ±0.52	2.53 ±1.20	11.55 ±6.40
Non Diabetes 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	2.65 ±1.00	14.10 ±4.80	1.99 ±0.67	2.20 ±1.00	5.80 ±4.00
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

t-test analysis : $p<0.05$ is significant

jects. Urine creatinine was significantly ($p>0.05$) lower in diabetics than non-diabetic subjects. The serum urea and creatinine and urine chromium, though higher in diabetics than in non-diabetics, was not statistically significant ($p>0.05$). The urine magnesium level was significantly ($p<0.05$) higher in diabetics than non-diabetics. The serum chromium and magnesium levels were higher in non-diabetics than in diabetics, but these differences were not statistically significant ($p>0.05$).

Figure 1 shows the correlation graph of serum magnesium and urine magnesium levels of diabetics. A significant ($p<0.01$, $r = -0.441$) negative correlation was observed between these two levels. No correlation was observed between serum chromium and serum magnesium levels and between urine chromium and urine magnesium levels of the diabetic population.

DISCUSSION

Alterations in the status of trace elements have been reported in a number of disease states, trauma and infections. Diseases of the liver and kidney have been known to cause derangements in the homeostatic regulation of trace elements affecting tissue distribution and excretion (Milne, 1999). Excessive accumulation or depletion of trace elements may have significant clinical implications including increased risk for cancer, cardiovascular disease, immune deficiency, anaemia, renal function impairment and bone disease (Behets *et al*., 2001). The actual

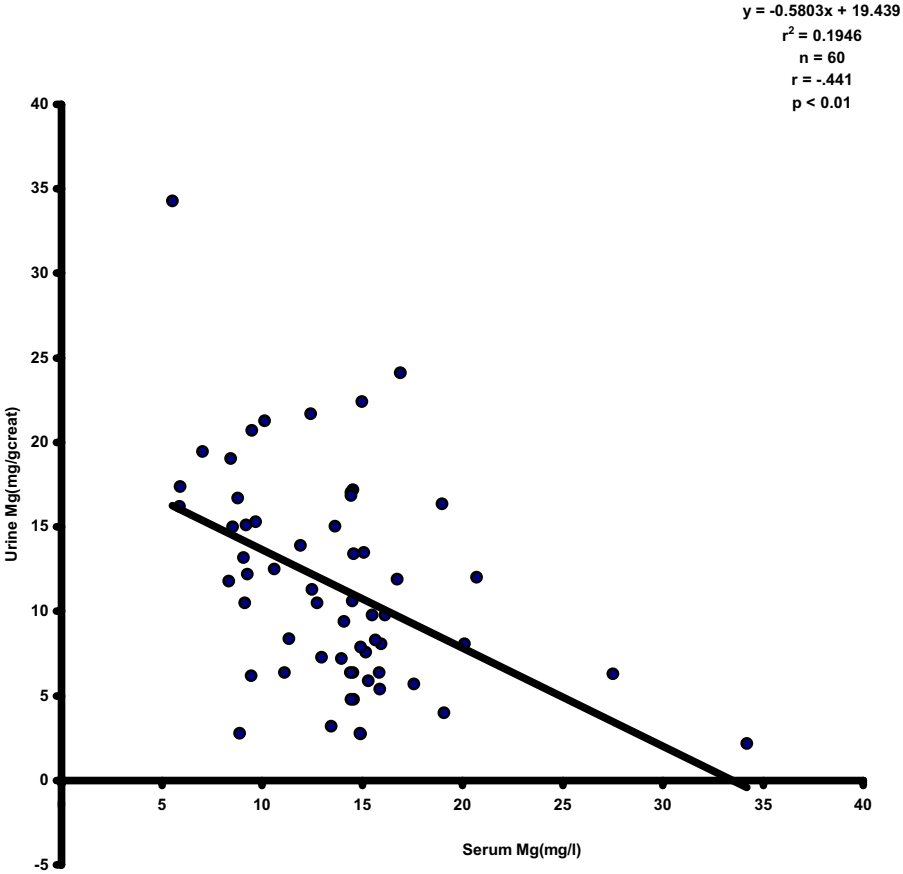

Fig. 1: Correlation graph of Serum Mg and Urine Mg of Diabetes

status of these elements in diabetes and other ailments is still uncertain.

In the present study, lower serum levels of magnesium and chromium were observed in the diabetics compared to the non-diabetic population of the study, though the differences were not significant. This agrees with the works of Ekmecioglu *et al*. (2001) who also demonstrated lower levels of these elements only in the lymphocytes of diabetics and no differences in their levels in other blood components of both groups. However, significantly lower levels of Mg and Cr were reported in serum and hair of diabetics by Nouramonammadi *et al*. (2000).

The mechanism responsible for hypomagnesaemia in patients with diabetes mellitus is not completely known. Osmotic diuresis clearly accounts for a portion of the magnesium loss (Chetan *et al*., 2002). It is believed that glycosuria which accompanies the diabetic state impairs renal tubular reabsorption of magnesium from glomerular filterate (Garland, 1992). Renal magnesium handling may be modulated by insulin even in non-diabetic individuals; administration of insulin increases urinary magnesium excretion rate - a rise in magnesium excretion rates in diabetic patients with increasing insulin dosage has been implicated in the hypomagnesaemia seen in diabetics (McNair, 1982). Studies have demonstrated that insulin regulates the intracellular magnesium concentration by stimulating the plasma membrane ATPase pumps and increasing free magnesium entry into the cells (Poallisso, 1998), so the low serum magnesium levels seen in the diabetic population may be a consequence of insulin resistance. Dietary magnesium intake and intestinal hypoabsorption may also be a factor in the low serum magnesium levels of diabetics as diabetics in our locality consume restricted diet as seen from the questionnaire. Hyperglycaemia and high levels of insulin increase chromium excretion (Watts, 1999), so low serum levels of chromium seen in the diabetics has been attributed to insulin resistance, hyperglycaemia and osmotic diuresis resulting from glycosuria, which increase urine chromium excretion (Watts, 1999; Anderson, 1997).

Urine concentrations of Mg were observed to be significantly higher than those of the non-diabetic population. A similar observation was made by Elyazigi *et al*. (1991) on the effects of the diabetic state and related disorders on the urinary excretion of magnesium and zinc in patients, where higher urinary excretion of these elements was demonstrated in the diabetic state. Kisters *et al*. (2000) also reported increased urinary Mg loss in diabetic subjects. Hyperzincuria and hypermagnesuria was also reported by Walter *et al*. (1991) in diabetic subjects when compared with the controls. The mechanism of urinary loss of magnesium may result from depression of the net tubular reabsorption of Mg due to osmotic action of glycosuria and hyperglycaemia rather than any specific effects of insulin on the renal tubules.

No significant difference was seen in the urine chromium concentrations of both groups though the urine chromium concentration of the diabetics was higher than those of the non-diabetics. This contradicts with the findings of Bahijiri (2002), who demonstrated increased urinary excretion of chromium and zinc in elderly diabetics. Insulin administration and hyperglycaemia enhances urinary chromium loss. The reason for the disparity in the two findings is not known and may be attributed to sample size.

This study observed a significant negative correlation between the serum Mg concentration and urinary Mg concentration in diabetic subjects. This may be due to enhanced magnesium utilisation in diabetes. However, no association was seen between serum Cr and serum Mg, and between urine Cr and urine Mg in the diabetic subjects studied. The reason for

this is not known.

The body mass index and fasting plasma glucose were significantly higher in the diabetic population when compared to the non-diabetic population of the study. It has been established that diabetics have higher levels of fasting plasma glucose, glycated haemoglobin and lipoproteins than non-diabetics (Malleucci and Giampietro, 2000; Anetor *et al*., 2002). The higher levels of fasting plasma glucose seen in diabetics are a result of insulin deficiency or insulin resistance associated with diabetes mellitus (Wokoma, 2002).

The findings of this work have shown that diabetic subjects are not magnesium and chromium deficient; rather the diabetic state enhances urinary the loss of these elements.

REFERENCES

Anetor J I, Senjobi A, Ajose OA & Agbedana EO (2002). Decreased serum magnesium and zinc levels, artherogenic implications in type 2 diabetes mellitus in Nigerians. *Nutrition and Health* 16(4): 291-300.

Anderson RA (1997). Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes* 46:1786-1791.

Arora AS & Gores GJ (1996). The role of metals in Ischemia reperfusion injury of the liver. *J Hepatology* 16 (1): 31-38

Bahijiri SM & Mufti AM (2002). Beneficial effects of chromium in people with Type 2 diabetes and urinary chromium response to glucose load as possible indicator of status. *Biological Trace Element Research* 85(2): 97-109.

Barham D & Trinder P (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* 97:142 - 145.

Behets GJ, Dams G, Damment S & Debwe ME (2001). An assessment of the effects of lanthanum on bone in a chronic renal failure (CRF) rat model. *J Amer Soc Nephrology* 12:740-750

Broadhurst CL, Schmidt WS & Reeves JB (1997). Spectroscopy and molecular modeling of Chromium III picolinate and nicotinate complexes used for nutritional supplementation. *Inorganic Biochem* 66:119-130.

Bonsnes RW & Taussky HH (1945). The colorimetric determination of creatinine by the Jaffe's reaction. *J Biol Chem* 15: 591.

Chatterjee J, Mukherjee BB & De K (1999). Trace metal levels of X-ray technicians: Blood and hair. *Biological Trace Elements Research* 46: 211 - 217.

Chetan P, Hans R, Sialy & Devi DB (2002). Magnesium deficiency and diabetes mellitus. *Current Science* 83(12):1456-1464.

Davis S, Mclaren HJ, Hunnisett A & Howard MI (1997a). Age related decreases in chromium levels in 51,665 hair, sweat and serum samples from 40,872 patients, Implications for the prevention of cardiovascular disease and type 2 diabetes mellitus. *Metabolism* 46 (5): 469-73.

Davis CM & Vincent J B (1997b). Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry* 36: 4382-4385.

Ekmekcioglu C, Prohaska C, Pomazal K, Steffan I, Schern T & Marktl W (2001). Concentration of seven trace elements in different hematological

matrices in patients with type 2 diabetes as compared to healthy controls. *Biological Trace Element Research* 79(3): 205-219.

EL-Yazigi A, Hannan N & Raines DA (1991). Urinary excretion of chromium and copper and manganese in diabetes mellitus and associated disorders. *Diabetes Research* 18: 129-134.

Garland HO (1992). Magnesium deficiency in diabetes. *Magnes Res* 5: 193-202.

Kisters K, Schildheuer M & Koneke J (2000). Magnesium deficiency and increased fractional magnesium excretion in insulin dependent diabetes mellitus, Magnesium loading test and blood pressure. *Trace Elements and Electrolytes* 17:67-70.

Malleucci E & Giampietro O (2000). Oxidative stress in families of type I diabetic patients. *Acta Clin Chem* 299:109-117.

McNair P (1982). Renal hypomagnesaemia in human diabetes mellitus, its relation to glucose homeostasis. *Eur J Clin Inves* 12: 81-85.

Milne DB (1999). Trace elements. In: *Tietz Textbook of Clinical Chemistry.* Burtis CA, Ashwood ER (Eds.). 3rd edition. W.B Saunders Company, Philadelphia.

Moradian L & Morely S (1987). The serum micro nutrient status in patients diagnosed of diabetes. *Amer J Clin Nutr* 45:12-13.

Natelson S, Scott ML, & Beffa C (1951). Colorimetric estimation of urea using diacetylmonoxime method. *Amer Med J Clin Patho*l 21: 275.

Nouramomammadi I, Shalmani KI, Shaabani M & Gohari L (2000). Zinc, copper, chromium, manganese and magnesium levels in serum and hair of insulin - dependent diabetics *J Trace Elements and Metabolism* 2: 88 - 100.

Paolisso G (1998). Magnesium. *Diabetologia* 31: 910-915.

Spence K (1986). Analytical reviews in clinical biochemistry; the estimation of creatinine. *Ann Clin Biochem* 23: 1-25.

Suarez Z (1993). Decreased insulin sensitivity in skeletal muscle of hypomagnesemic rats. *Diabetologia* 36:82.

Veniamin MP, Vakirtz-Lemonias C (1970). Chemical basis of carbamidodiacetyl micro method for estimation of urea, citrulline and carbamyl derivatives. *Clin Chem* 16: 3-6.

Vincent JB (1999). Mechanism of chromium action low molecular weight Chromium binding substance. *J Amer Coll Nutr* 1: 6-12.

Walter RM, Uriu-Hare JY & Olin KL (1991). Copper, zinc, manganese and magnesium status and complication of diabetes mellitus Diabetes care 14: 1050 - 1056

Watts DL (1999). Trace elements and glucose disorders. *TEI Newsletters* 11: 2.

Wokoma FS (2002). Diabetes and hypertension in Africa - an overview. *Diabetes International* 12 (12):36-40

WHO (1999). Report of a WHO consultation on diagnosis and classification of diabetes mellitus part 1 (1-30). World Health Organization, Geneva.

Zargar AH, Shah NA, Mashoodi SR, Lauway BA, Dar FA, Khan AR & Wani AI (1998): Copper, zinc and magnesium levels in non-insulin dependent diabetes mellitus. *Post Guard Med J* 74 (877): 665-668.

Zargar AH, Bashir MI, Masoodi SR, Laway BA, Wani AI, Kahan A & Dar FA (2002). Copper, zinc and magnesium levels in type 1 diabetes mellitus. *Saudi Med J* 23 (5): 539-542.