Evaluation of some trace elements in type 2 diabetes mellitus patients

Dr. Numan Abduallateef [1] , Suhayla Khalied [2]

1. M.B.Ch. B, D.C. Path. Department of chemical pathology, Central public Health Lab.(CPHL)
2. M.Sc. Biochemistry, collage of Agriculture, University of Baghdad

Abstract

Noninsulin dependent diabetes mellitus is supposed to be associated with fluctuations in the plasma levels of several trace elements. There is accumulating evidence that the metabolism of several trace elements is altered in patients with noninsulin dependent diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progression of this disorder. **Aim** The aim of the present study is to compare the levels of essential trace elements including copper (Cu), zinc (Zn), magnesium (Mg), iron (Fe), and calcium (ca) in type 2 diabetic patients , healthy controls . and their possible association with age, sex and glycemic status**. Materials and methods** The comparative study included 100 type 2 diabetic patients and 30 as healthy control. Blood samples of all the subjects were collected after an overnight fasting for the determination of blood glucose, magnesium, zinc, copper, iron and calcium. Fasting blood sugar (FBS) was determined by enzymatic colorimetric assay to confirm the status of the patients and the controls while metals' concentrations were measured with Atomic Absorption Spectrophotometer. **Results** The mean values of serum Mg and Zn levels were significantly reduced in blood samples of diabetic patients as compared to control subjects ($p<0.01$).The serum Cu , Fe and Ca were significantly higher in blood samples of diabetic patients compared to normal subject ($p<0.01$). Sex and age did not affect the trace elements concentration except Cu showed a statistically significant increase with age. Glycemic status effect the trace elements concentrations significantly. **Conclusion** The results confirm that alteration of some essential trace metals are associated with diabetes mellitus.

تقدير مستويات بعض العناصر النزرة لدى مرضى السكريII

د .نعمان عبداللطيف[1] و سهيلة خالد[2]

1 - مختبر علم الامراض الكيميائي - مختبر الصحة المركزي العام – وزارة الصحة.

2 - كلية الزراعة – جامعة بغداد-وزارة التعليم العالي و البحث العلمي.

الخلاصة

هناك ارتباط مفترض بين داء السكري غير المعتمد على الانسولين (داء السكري II) وبين تقلبات مستوى عدة عناصر نزرة في بلازما دم المرضى المصابين بهذا الداء. هناك تراكم لشواهد تدل على إن استقلاب عدة عناصر نزرة يُغيَّر في مرضى داء السكري II وإن لهذه المغذيات دور محتمل و محدد في نشوء المرض و تطوره اللاحق .

الهدف من الدراسة

مقارنة مستوى عدة عناصر نزرة لدى مرضى مصابين بداء السكري II بمستواها لدى اصحاء وتقصي علاقة ذلك بالعمر و الجنس ومستوى السكر في الدم. العناصر النزرة التي شملتها الدراسة هي :

(ca) كالسيوم و(Fe) حديد و(Mg) مغنيسيوم و(Zn) خارصين و(Cu) نحاس

الطرائق و المواد

الدراسة المقارنة تضمنت 100 مصاب بداء السكري II و 30 أصحاء. أُخذت عينات الدم من كل الذين شملتهم الدراسة وهم في حالة صوم ليلي و ذلك لتقدير مستوى كلوكوز الدم(باستخدام الطريقة الانزيمية اللونية) و العناصر المذكورة انفا(باستخدام مقياس طيف الامتصاص الذري)

النتائج

اظهرت نتائج التحليل الإحصائي ما يلي:

ارتفاع معنوي واضح في مستويات النحاس والحديد والكالسيوم لدى مرضى السكري مقارنة مع مجموعة الأصحاء و انخفاض معنوي واضح في مستويات الزنك والمغنيسيوم لدى مرضى السكري مقارنة مع مجموعة الاصحاء. عدم تأثر مستوى هذه العناصر بالعمر او الجنس بينما اظهر النحاس زيادة واضحة مع تقدم العمر.فيما تأثرمستوى هذه العناصر بمستوى السكر بصورة واضحة.

الاستنتاج

تشير نتائج هذه الدراسة الى ان التغير في مستوى بعض العناصر النزرة يبدو انه ذو علاقة مع مرض السكري II

Introduction

Type-2 diabetes mellitus results from a defect in insulin secretion and an impairment of insulin action in hepatic and peripheral tissues, especially muscle tissue and adipocytes. A post-receptor defect also is present, causing resistance to the stimulatory effect of insulin on glucose use to occur, and relative insulin deficiency develops. The specific etiological factors are not known, but genetic input is much stronger in type-2 than in type-1. However, type-2 diabetes mellitus is also reported to be caused by obesity[(1)].

Metal ions are known to play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patient[(2)] .

(Cu) is one of the essential trace elements, and has a particular role in cytochrome oxidase function at the terminal end of the mitochondrial electron transport chain. The loss of this activity may contribute to the characteristic swelling and distortion of mitochondria which can be observed in copper deficiency, particularly in metabolically active tissues such as pancreatic acinar cells,entrocytes,and hepatocytes[(3)].

(Mg), another essential trace elements, is a cofactor in both glucose transporting mechanism of cell membranes and various enzymes important in carbohydrate oxidation. It also plays a part in mechanism for energy transfer mediated by phosphate bonds. Previous studies showed that magnesium metabolism might be altered in patients with diabetes mellitus. Some authors found a strong positive association of diabetes with hypomagnesemia, while other demonstrated that increased urinary loss of magnesium caused by osmotic diuresis might contribute to diabetic hypomagnesemia.[(4)]

(Zn) is one of the essential trace elements that is required to maintain the normal physiological function of all form of life[(5)]. It is a component of many enzymes, and plays an important role in the maintenance of several tissue functions, including the synthesis, storage and release of insulin. Zinc deficiency has now been recognized to be associated with many chronic illnesses. Diabetes mellitus(DM) is one of the diseases, which affect zinc homeostasis in different ways[(6)].

Several studies have suggested a possible role of minerals such as iron in insulin resistance or diabetes. (Fe) is a transitional metal and a potential catalyst in many cellular reactions that produce reactive oxygen species. Such reactions contribute to tissue damage and increase oxidative stress, thereby potentially altering the risk of type 2 diabetes[(7),(8)]. It is

increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. In the general population, body iron stores are positively associated with the development of glucose intolerance, type 2 diabetes[9][10].

(Ca) is a versatile intracellular messenger that is used throughout the life cycle of an organism to control diverse biological processes. It has been suggested that diabetes and

cardiovascular disease are linked by a common defect of divalent cation metabolism, including calcium[11].

The aim of the present study is to compare the levels of essential trace elements including copper (Cu), zinc (Zn), magnesium (Mg), iron (Fe), and calcium (ca) in type 2 diabetic patients , healthy controls . and their possible association with age,sex and glycemic status**.**

Material and Methods

Subjects

Collection of data was carried out at the central public health laboratory (C.P.H.L).The diabetic group included 100 NIDDM patients, aged 40-75years,who were chosen from patients with diabetes, attended the medical examinations during the period of the study. An aged matched control group included 30 apparently healthy subjects who were selected the workers from of the same center.

Medical history

All of the recipient have no any other compliant apart from mellituse.

Drug history

50% of the patients was on dianoile 1x3 , 25% have in addition one tab. Of Biguaniol (glucophage). 25% on diatery control only. All of the group have taken sweetam tab on needs of them .

Criteria for diagnosis

The criteria for the diagnosis of type two Diabetes Mellitus concludes that any one of the following is diagnostic;

1. Classical symptoms of diabetes,polyurea,polydypsia, and fatigue ,with plasma glucose concentration ≥ 200 mg/dl regardless of the time of the preceding meal .
2. Fasting plasma glucose ≥ 126 mg/dl without caloric intake for at least 8 hr.
3. 2-hr post load plasma glucose concentration ≥ 200 mg/dl during the oral glucose tolerance test

Sample collection

About 10 ml of venous blood was obtained after an over night fast using disposable needle and syringe. Samples were collected between 9.00-11.00 a.m. Each sample was centrifuged at 4000 rpm and the serum was separated and stored at – 20 ˚c until analysis.

Biochemical analysis

Determination of fasting blood sugar (FBS)

Serum glucose was measured by enzymatic colorimetric method using commercial kit (Biocon ® Diagnostic / Germany).

Determination of copper, iron, zinc, magnesium and calcium levels in sera

The serum contents of Cu , Zn , Ca, Mg and Fe were measured by using atomic absorption spectrophotometer. (Perkin-Elmer2380,Norwalk,CT06859-0012,USA)

Statistical analysis:

The statistical analysis system –SAS (2004) was used to data analysis ,results were expressed as Mean ±(SD) ,the T-test (LSD) was used to compare variables among study groups. [12]

Results

One hundred patient with NIDDM (57 male , 43 female) and 30 healthy subject (15 male,15 female) comprised the study group.

Table1 showed the mean fasting blood sugar (FBS),blood Ca, Zn, Mg, Fe and Ca concentration in all subjects. The mean levels of Zn and Mg were significantly lowered ($P<0.001$) in NIDDM as a compare to control group . on the other hand, the mean levels of Cu, Fe and Ca were significantly higher ($P<0.001$) in NIDDM as compared to control group. No difference was found between male and female with respect to Cu, Zn, Mg, Fe and Ca levels as shown in table 2.

Table3 showed that the age had a weak and statistically insignificant effect on serum Zn, Mg, Fe and Ca while Cu shows a statistically significant increase with age.

In table 4 it was found that trace elements significantly influenced by glycemic status except Ca.

Table 1. concentration of trace elements and fasting blood sugar in healthy control and diabetic patients.

Parameters	Means ± SD		T-test(LSD)
	Diabetic patients	Normal healthy control	
Glucose(mg/dl)	261.61 ± 83.93	86.86 ± 17.29	30.593**
Cu (µg/dl)	171.60 ± 22.50	93.36 ± 6.86	8.262 **
Mg (mg/dl)	1.82 ± 0.20	1.98 ± 0.22	0.0863 **
Fe (µg /dl)	174.63 ± 42.66	71.26 ± 17.51	15.832**
Zn (µg /dl)	85.02 ± 14.94	97.86 ± 10.61	5.798 **
Ca (mg/dl)	9.47 ± 0.74	9.03 ± 0. 41	0.280 **

** ($p<0.01$).

Table 2 concentration of trace elements and fasting blood sugar in serum of diabetic patients according to their sex.

Parameters	Means ± SD		T-test(LSD)
	Male	Female	
Glucose(mg/dl)	265.76 ± 87.81	256.10 ± 79.17	33.760 ns
Cu(µg/dl)	172.63 ± 22.32	170.23 ± 22.93	9.054 ns
Mg (mg/dl)	1.80 ± 0.20	1.85 ± 0.21	0.082 ns
Fe (µg /dl)	173.75 ± 44.72	175.79 ± 40.26	17.184 ns
Zn (µg /dl)	84.92 ± 15.11	85.13 ± 14.89	6.019 ns
Ca (mg/dl)	9.57 ± 0.66	9.33 ± 0.82	0.294 ns

ns: non-significant

Table 3. The effect of age in glucose and trace elements level at Diabetic patients

Parameters	Means ± SD		T-test(LSD)
	40-55 years	56-75 years	
Glucose(mg/dl)	249.62 ± 80.60	275.13 ± 86.40	33.151 ns
Cu(µg/dl)	166.98 ± 23.17	176.80 ± 20.75	8.775 *
Mg(mg/dl)	1.81 ± 0.19	1.83 ± 0.22	0.082 ns
Fe (µg /dl)	169.70 ± 45.24	180.18 ± 39.28	16.920 ns
Zn(µg /dl)	85.88 ± 15.64	84.04 ± 14.21	5.959 ns
Ca(mg/dl)	9.56 ± 0.78	9.36 ± 0.68	0.293 ns

* ($p<0.05$).

Table 4. The effect of level of glucose in trace elements level at Diabetic patients

Parameters	Means ± SD		T-test(LSD)
	Glucose >270	Glucose <270	
Cu(µg/dl)	161.05 ± 18.38	185.58 ± 19.79	7.618 **
Mg(mg/dl)	1.86 ± 0.22	1.76 ± 0.16	0.081 **
Fe(µg /dl)	167.44 ± 44.43	184.16 ± 38.65	15.859 *
Zn(µg /dl)	91.21 ± 13.34	76.81 ± 12.95	5.282 **
Ca(mg/dl)	9.50 ± 0.76	9.42 ± 0.71	0.298 ns

Discussion

There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progress of this disease[13]. Cooper et al showed that Cu metabolism in diabetics is abnormal[14]. Many studies have reported higher copper level in diabetics especially in complicated cases [15]. Our study showed that copper concentrations were significantly higher in NIDDM patients than control group which is consistent with finding of Schlienger et al; Mosaad et al;Zhao et al[16),(2),(17)] . Copper levels have been found to be elevated in IDDM subjects, while urinary excretion of copper has been found to be affected by diabetes mellitus[18)(19)]. In the present study Cu showed a statistically significant increase with age. These finding consistent with finding of Aquilar et al which found that in diabetic patients the age had a statistically significant association with serum copper for each year increase in age which is different, the copper content was higher in the liver of diabetic and lower in a dipose tissue.[20]. Zinc plays a clear role in the glucose metabolism . The results in this study indicate a significant decrease in serum zinc levels in NIDDM patients compared to the normal healthy control. Which consistent with finding of Refaat et al; Zhao et al; Mosaad et al.[6),(2),(17).] Diabetes can affect the balance of zinc in several ways, although it is most likely that hyperglycemia, rather than any primary lesion related to diabetes, causes the increased urinary loss and subsequent decreases in total body zinc[21]. In addition, a review of the literature has shown that in addition to the zincuria, there is evidence that Type 1 and Type 2 diabetics can cause zinc malabsorption. Some researchers have indicated that diabetics may lose zinc by excreting more zinc into the intestine during the digestive processes[22]. Zinc plays a key role in the synthesis, storage and secretion of insulin, and it accounts for the conformational integrity of insulin in its hexameric crystalline form. The addition of zinc to the insulin structure will increase the insulin's ability to bind to its receptor. A decrease in zinc affects the ability of the islet cells to produce and secrete insulin, which could compound the problems of Type 2 diabetics in particular. In addition to the findings that zinc levels are often low in diabetics, it is also felt that zinc (in concert with other micronutrients) may participate as an integral component of antioxidant enzymes . Many of the complication of diabetes may relate to an increase in intracellular oxidant and free radicals associated with decrease in intracellular zinc and zinc dependent antioxidant enzyme[23]. Although this study shows a decrease in zinc with the age yet this was not significant and this consistent with results obtained by many studies, which showed that the elderly are at particular risk of zinc deficiency due to their low energy intake and poor dietary zinc consumption[24]. Diabetes mellitus has been suggested to be the most common

metabolic disorder associated with magnesium deficiency[25]. Resnick et al reported intracellular and extracellular magnesium depletion in NIDDM patients[26]. Magnesium levels in our study show significant decrease in NIDDM as compare to control group. This finding consistent with Mosaad et al [2]. The mechanism responsible for magnesium deficiency in patients with diabetes is not completely known. Osmotic diuresis clearly accounts for a portion of the magnesium loss. It is believed that glycosuria which accompanies the diabetic state, impairs renal tubular reabsorption of magnesium from glomerular filtrate[27]. Dietary magnesium intake may also be a factor in deficiency, as the individuals do not consume the fully-recommended daily allowance for magnesium[28] . Recent evidences suggest that insulin can increase free magnesium entry into the cell[29]. Furthermore, in the state of insulin resistance, insulin-induced entry of magnesium is also impaired[30].

Epidemiological studies have reported an association between high iron stores and type 2 diabetes[8]. In the present study, serum iron significantly increase in NIDDM as compart to control group and this finding consistent with finding of Fernandes et al which found that in general population increased body iron stores are possibly associated with occurrence of glucose intolerance,type2 diabetes e.g iron overload[9]. A link has been established between increased dietary iron intake, increase intestinal absorption particularly eating red meat and increased body iron stores, and the development of diabetes . Iron is a strong prooxidant that catalyzed the formation of hydroxyl radicals, and the increase in oxidative stress may be associated with the risk of diabetes . another potential mechanism is direct iron deposition in the pancreatic β-cell that can impair insulin secretion.[31],[8]

Calcium levels in our study declare significant increase in NIDDM as compare to control group. This finding consistent with Joseph et al[32] . Accumulating data from animal models of diabetes and from studying patients with diabetes reveal that intracellular calcium levels are increased in most tissues. The activities of the membrane, adenosine triphosphatase (ATPase) associated cation pumps, which determine intracellular calcium level (i.e., calcium-ATPase and [sodium + potassium]-ATPase), are also altered. The nature of the alteration is often tissue specific and may depend on the level of blood glucose or insulin, or both.[32]

Trace elements under study were not influenced by sex and age but its influenced by glycemic status significantly.

References

1. Olatunbosun (2004). Diagnosis and classification of diabetes mellitus. Diabetes Care 27(Suppl 1): S5-S10.

2. Abou-Seif MA.&Youssef A. (2004).Evaluation of some biochemical changes in diabetic patients.Clinica Chimica Acta 346:161-170

3. Aggett PJ. (1985). Physiology and metabolism of essential trace elements- an outline. In: Taylor A, ed. Clinics in endocrinology and metabolism. Philadelphia: Saunders,; pp 513-43.

4. Muhammad S., Mushtaq A., Khawaja M. and Sajjad H., (2002). Beneficial effects of magnesium supplementation in diabetes mellitus, Pakistan J Med. Research, Vol. 41 No.2

5. Saradesai VM. Inorganic elements (minerals), Chapter6: Introduction to clinical nutrition. 1st . USA**:** Marcel Dekker, Inc; 1998. p. 98-100.

6. AL-Maroof RA. and Al-Sharbatti S.**,**(2006).Serum zinc levels in diabetic patient and effect of zinc supplementation on glycemic control. Saudi Med J ; vol.27(3): 344-350.

7. Opara EC(2004): Role of oxidative stress in the etiology of type 2 diabetes and the effect of antioxidant supplementation on glycemic control. J Investig Med 52:19 –23.

8. Swapnil R.,Joann M. and Walter C.,(2006).Iron Intake and the Risk of Type 2 Diabetes in Women, DIABETES CARE, VOL. 29, No. 6.

9. Fernandez-Real JM, Lopez B A and Ricart W.(2002). Cross talk between iron metabolism and diabetes. Diabetes Care 51; 2345-48

10. Barbieri M, Ragno E, Benvenutti E, Zito GA, Corsi A and Ferrucci L(2001): New aspects of the insulin resistance syndrome: impact on haematological parameters**.** Diabetologia 44:1232–1237.

11.Berridge MJ, Lipp P, Bootman MD: The versatility and universality of calcium signaling. Nat Rev Mol Cell Biol1 :11 -21,2000

12. SAS. 2004. SAS / STAT Users Guide for Personal Computers. Release 6.12. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).

13. Aguilar MV, Saavedra P, Arrieta FJ, Mateos CJ, Gonzαlez MJ, Meseguer I, et al (2007). Plasma mineral content in type-2 diabetic patients and their association with the metabolic syndromeAnn Nutr Metab; 51:402-6.

14. Cooper GJS, Phillips ARJ, Choong SY, Leonard BL, Crossman DJ, Brunton DH, Saafi EL, Dissanayake AM (2005). Regeneration of Heart in Diabetes by selective Copper Chelation. Diabetes 53: 2501 – 2508.

15. Walter RM, Uriu – hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL (1991). Copper, Zinc, mangenese, and magnesium status and complications of diabetes mellitus. Diabetes Care 14(11): 1050 – 1056

16. Schlienger JL, Grunenberger F, Maier EA, Simon C, Chabrier G, Leroy MI. (1988).Disorders of plasma trace elements in diabetes, relation to blood glucose equilibrium. Presse Med;17:1076-9.

17. Zhao C, Wang H,Zhang J, Feng L. (2008).Correlation of trace elements glucose and body compositions in type 2 diabetcis. Wei Sheng Yan Jiu. 37(5):600-1,605.

18. Walter RM Jr, Urik-Hare JY, Oliu KL, et al. (1991) Copper, zinc, manganese and magnesium status and complications of diabetes mellitus. Diabetes Care;44:1050-6.

19. Hannan M, Raines DA. (1991) Urinary excretion of chromium, copper and manganese in diabetes mellitus and associated disorders. Diabetes Care;18:129-34.

20. Aquilar MV, Laborda JM, Martinez-para MC,Gonzalez MJ, Meseguer I, Bernao A, Mateos CJ(1998).Effect of diabetes on the tissular zn/cu Ratio. J-Trace-Elem-BioI,; 12(3): 155-8.

21. Chausmer AB.,(1998). Zinc, insulin and diabetes, J Am Coll Nutr 17(2).

22. Salgueiro MJ., Krebs N., Zubillaga MB., Weill R., Postaire E., Lysionek AE., Caro RA., Paoli TD., Hager A. and Boccio J. Zinc and Diabetes Mellitus, Biol Tr Elem Res 2001 Vol. 81:215-229.

23. Anderson RA ., Roussel AM., Zouari N., Mahjoub S., Matheau JM. and Kerkeni A. (2001). Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus, J Am Coll Nutr; 20(3):212-8.

24. Wood RJ, Suter PM, Russell RM(1995). Mineral requirements of elderly people. Am J Clin Nutr; 62: 493-505

25. Rude, R. K., Postgrad. Med., 1992, **92**, 217–224

26. Resnick LM, Altru BT, Gupta RK, Laragh JH, Alderman MH, Altm BM. (1993).Intracellular and extracellular magnesium depletion in type-2 (non-insulin dependent) diabetes mellitus. Diabetologia;36:767-70.

27. Garland, H. O., Magnes. Res., 1992, **5**, 193–202.

28. Barbagallo, M., Gupta, R. K. and Resnick, L. M., Diabetologia, 1993, **36**, 146–149

29. Paolisso G., ibid, 1998, **31**, 910–915.

30. Eibl, N. L., Schnack, C. J., Kopp, H. P. and Nowak, H. R., Diab. Care, 1995, **18**, 188–192.

31. Sundara S.,Muhammed G.,Vivian A.,and Sudhir V, (2007)The Role of Iron in Diabetes and Its complications. Diabetes Care. Vol.30,No.7.

32. Levy J.,Gavin JR.,Sowers R.(1995).Diabetes mellitus:a disease of abnormal cellular calcium metabolism? Am J med;99(2)