Therapeutics, Pharmacology and Clinical Toxicology
Vol XIV, Number 1, March 2010
Pages 53-56

ORIGINAL RESEARCH

RESEARCH ON THE INFLUENCE OF BIGUANIDES TREATMENT ON COPPER CONCENTRATION IN NON-INSULIN-DEPENDENT DIABETES MELLITUS PATIENTS.

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Abstract. We have studied the plasmatic and urinary concentration of copper in a group of adult patients with non-insulin-dependent diabetes mellitus (NIDDM). Our study enrolled 30 patients with NIDDM, naïve for antidiabetic medication, recruited from the Diabetes, Nutritional and Metabolic Diseases Clinic of Faculty of Medicine Constanţa. The patients received treatment with Metformin, 1000 mg/day. We measured the plasmatic and urinary concentration of copper (Cu) and the concentrations of: glucose (Gl), HDL, LDL, cholesterol (Col), triglycerides (Tg), glycosylated hemoglobin (HbA1c), before and after 3 months of treatment. The same measurements were performed on a control group (CG) of healthy adults of both genders. The results were statistically interpreted. The data revealed a significant difference in the plasmatic concentration of copper, the NIDDM group displaying higher values than those in the control group (111,91+/-20,98 vs. 96,33+/- 8,56 μg/dl, p<0,001) prior to the administration of Metformin. The treatment with Metformin for 3 months did not significantly modify the plasmatic concentration of copper (111,91+/-20,98 vs. 101,23+/-21,73 μg/dl) nor the urinary concentration of this cation (53,35+/-23,79 vs. 51,70+/- 22,13 μg/24h). The plasmatic concentration of copper positively correlated with the levels of HbA1c, triglycerides and cholesterol.
Keywords: NIDDM, copper, Metformin, HbA1c

Introduction

Copper is an essential trace element for the human organism, capable of fluctuating between the oxidized Cu^{2+} and the reduced Cu^{+} state, being co-factor for many enzymes, therefore displaying redox activity: for iron oxidation, pigment synthesis, neurotransmitters' synthesis, antioxidant reactions, peptides' amidation, connective tissue formation; it also interferes in the metabolism of iron, in hematopoiesis, porphyrin synthesis and other metabolic processes. The enzymes in which copper is co-factor are: cytochrome oxidase (energy generation), Cu-Zn superoxide dismutase (protection from oxidative stress), ceruloplasmin (iron mobilization), tyrosinase (pigmentation), peptidyl, glycyl amidating monooxidase (neuro and bioactive peptide modification) (Puig S et all 2002).

This same redox activity of Cu renders it a potentially toxic metal when accumulated to high levels or in an uncontrolled manner. Both the deficiencies and the excess of copper are associated with specific

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clinical manifestations. (Bremner I, 1998)

Diabetes mellitus is a chronic metabolic disorder that is associated with the increased free radical production leading to oxidative damage: the oxidative stress.

Copper has pro-oxidant effects; it participates in radical reactions such as conversion of superoxide to hydrogen peroxide and hydroxyl radicals, in Fenton-type reactions, it catalyses the oxidative modification of LDL, in vitro and in the arterial wall. Copper also has antioxidant effects, through CuZnSOD, which catalyses the dismutation of superoxide.

Many of the pathological effects of copper overload are consistent with an oxidative damage to membranes or macromolecules. Ceruloplasmin, the major copper-containing plasma protein, may act as either antioxidant or pro-oxidant, depending on ambient conditions (Ferns GAA,1997).

For all these reasons, in this work, we have studied the plasmatic and urinary concentrations of copper, in a group of NIDDM patients and in a healthy adults group.

Materials and methods

We assessed a group of 30 adult patients with NIDDM: 18 (60%) male, and 12 (40%) female, with a medium age: interval 30-40 y, 2 (6,7%); 40-50y, 13 (43%); 50-60y, 15 (50%), (figure 1) and a control group of 17 healthy adults: 8 (47,1%) male and 9 (52,9%) female, with a medium age: interval 30-40y, 6 (35,3%); 40-50 y, 7 (41,2 %); 50-60y, 4 (23,5%) (figure 2). The diagnosis of NIDDM was established in the Diabetes Clinic of Clinical County Emergency Hospital Constanța, in accordance with the European guide for diabetes (NICE 2008). All the patients received Metformin (Siofor^{R}, Berlin Chemie) 1000 mg. orally/ day.

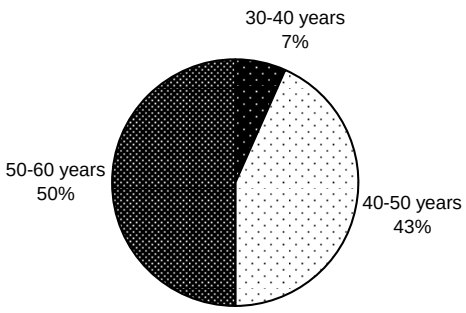

Figure 1. Distribution of the NIDDM patients included in our study group according to the age interval

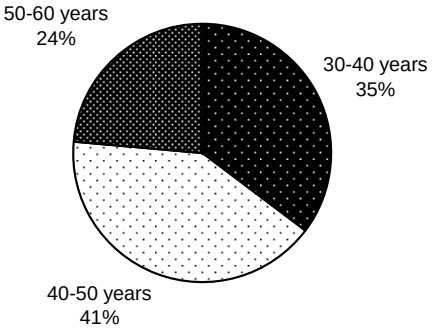

Figure 2. Distribution of the patients included in our control group according to the age interval

The inclusion criteria were: diagnosis of NIDDM, the absence of any other prior antidiabetic medication, the absence of therapy with any products containing copper or other oligoelements.

The exclusion criteria were: renal complications, pregnancy, lactation, hepatic cirrhosis, chronic diarrhea disorder, psychosis. The rules for clinical studies were respected and an informed consent was signed by each patient before recruitment into the study. The patients had the following disorders associated with NIDDM: hypertension (5), peripheral venous disorder (1), chronic bronchitis (1).

Venous blood samples were collected in the morning after an overnight fasting, in special blood collection tubes (vacutainer), for biochemical parameters in red lidded tubes, for HbA1c in mauve lidded tubes with K2 EDTA, for copper determination in green lidded tubes with sodium heparin, and the samples for urine were collected into sterile, chemically cleaned universal containers, from the urine collected over 24 h.

We performed the following determinations: glycemia, HbA1c, cholesterol, HDL, LDL, triglycerides, copper in plasma and in urine/24h. The copper quantitative analysis was done through spectrophotometry, using a special Randox kit, on a Daytona Randox analyzer. We also used Randox serum and urine controls for all analyzed parameters. Heparinized samples were centrifuged at 1500 g for 10 min in order to separate the plasma, and trichloroacetic acid was added in order to precipitate proteins, the supernatant being used for estimation of copper. The same procedure was used for urine samples. The determination of all parameters was performed in a Medical Laboratory from Constanța, with 15189 ISO accreditation.

The results are expressed as means +/- S.D. Differences between groups were examined using the unpaired Student's t-test, and considered statistically

significant at p<0.05; correlations by linear Pearson correlations, the program used being SPSS 12.0.

Results

Comparable means (±SD) for NIDDM and controls, initially and after 3 months of Metformin administration were: plasma copper concentration; 111,91±20,98 and 96,33±8,56 µg/dl, p<0,05, 110,08 ±18,61 and 101,23±21,73 µg/dl. (figure 3).Urinary copper: 53,35±23,79 and 37,51±11,7 µg/24h, p<0,05, 51,7±22,13 and 36±11,66 µg/24h, p< 0,05 (figure 4). Serum glycemia: 245±67,45 and 85,71±13,79 mg/dl, p< 0,05, 192±67,71 and 88±13,92 mg/dl , p<0,05

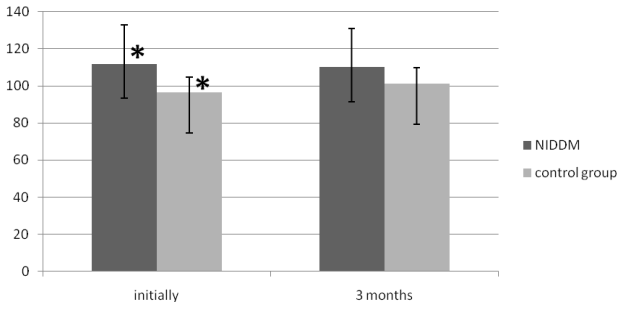

Figure 3. Mean plasma Cu concentration in µg/dl ± SD
** marks a significant difference, p = 0,006*

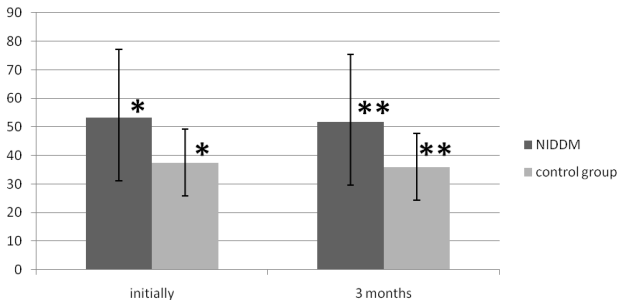

Figure 4. Mean urinary Cu concentration in µg/24 h ± SD
** marks significant difference p= 0,001,*
*** marks significant difference p=0,014*

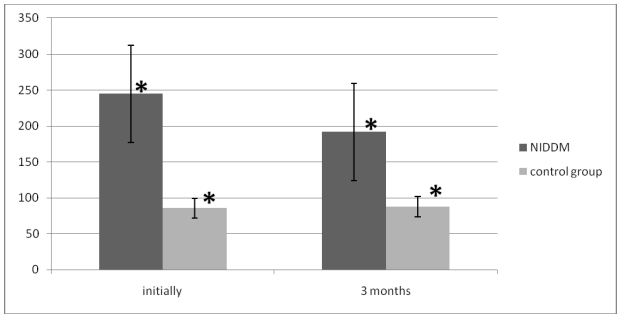

Figure 5. Mean serum glycemia in mg/dl ± SD
** marks significant difference, p<0,05*

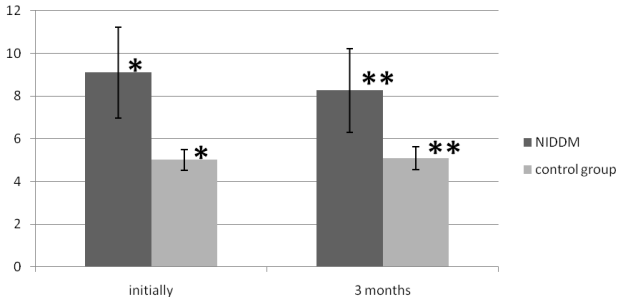

Figure 6. Mean HbA1c ± SD

*marks p< 0,001,
**marks p<0,05

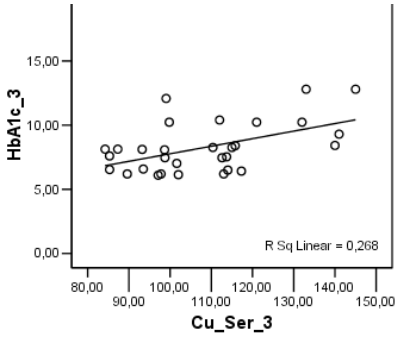

Figure 7. Positive correlation between Cu and HbA1c in NIDDM patients at 3 months

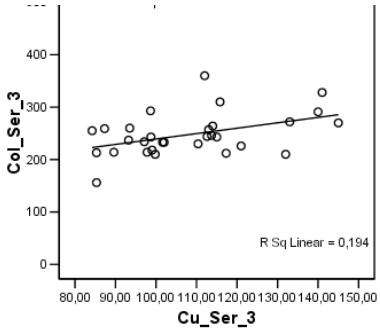

Figure 8. Positive correlation between Cu and cholesterol in NIDDM patients at 3 months

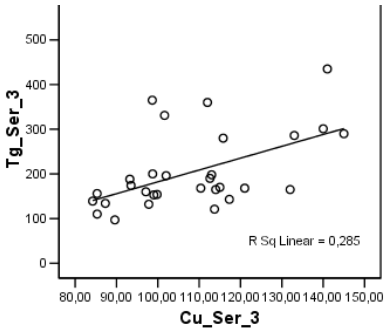

Figure 9. Positive correlation between Cu and triglycerides in NIDDM patients at 3 months

		Mean±SD 0 months	Mean±SD 3 months	p at 0 months between groups	p in NIDDM after 3 months
Cholesterol	NIDDM	258,67 ± 46,59	247,83 ± 40,30		
	CG	227,94 ± 37,59	226,53 ± 37,38	p<0,05	p=0,008
HDL	NIDDM	38,85 ± 10,45	38,57 ± 9,48		
	CG	53,62 ± 10,11	51,78 ± 9,59	p<0,001	p=0,681
LDL	NIDDM	163,79 ± 36,48	165,57 ±30,25		
	CG	147,59 ± 34,02	151,12 ±32,86	p=0,141	p=0,646
Triglycerides	NIDDM	254,43 ±133,49	204,30± 85,47		
	CG	133,59 ± 96,23	118,12 ± 43,61	p=0,002	p=0,001

Table I. Mean and standard deviation values for serum lipids in NIDDM patients and control group; p values

(figure 5). HbA1c: 9,1±2,13 and 5,01± 0,48%, p < 0,001, 8,26±1,95 and 5,08±0,54%, p< 0,05 (figure 6). The values for cholesterol, HDL, LDL and triglycerides are represented in table I. There were positive correlations between: copper and HbA1c (figure 7), Cu and cholesterol (figure 8), Cu and triglycerides. (figure 9).

Discussion and conclusions

NIDDM is a disease in which there is an increase in radical oxygen species (ROS) products, especially due to glycation or lipoxidation processes, auto-oxidation of glucose and oxidizing of glucose and decreased antioxidant defense system. This increased production of free radicals is one of the principal pathophysiological causes of the lesions of vascular endothelium, eye, kidney. The presence of copper in high concentrations can facilitate the production of free radicals through Fenton reaction (Tanaka A et al 2009). The decrease of serum copper is associated with a decrease in the production of ROS in animals with experimental diabetes.

Our data are in concordance with others from literature (Cooper GSJ et al 2009), and show that in the serum of NIDDM patients there is an increased level of copper, compared to healthy subjects (in our case 111,91+/-20,98 vs. 96,33+/- 8,56 μg/dl; p<0,001). The excess of copper is involved in the pathogenesis of some complications of NIDDM. Thus, the copper removal with a copper II selective chelator agent ameliorates left ventricular hypertrophy (Cooper GJ et al 2009, Baker SJL et al 2009). The therapy with Metformin did not significantly influence the serum and urinary copper levels (in serum 111,91+/-20,98 vs. 101,23+/-21,73 μg/dl, in urine 53,35+/-23,79 vs. 51,70+/-22,13 μg/24h) even though it decreased the levels of glycemia (245,2+/-67,45 vs 192,7+/-67,41mg/dl;p<0,005) after 3 months of therapy. This shows that at least for the patients with recently installed NIDDM and without complications, there is no correlation between the decrease in glycemia and the decrease in the plasmatic concentration of copper during the therapy with Metformin. Upon analysis of the copper and HbA1c concentrations, our results show a positive correlation between the two parameters (r=0.517; p<0.003). These data are in concordance with those of Viktorinová et al, which found a positive correlation between serum copper and HbA1c (r=0.709, p<0.001) (Viktorinová et al 2009). There also is a positive correlation between copper and cholesterol (r=0.440,p<0.015), found by (Magalova T et al 1994), and between copper and triglycerides (r=0.534, p<0.002).

Copper excess plays a role in NIDDM pathogenesis.

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