Advances in Biochemistry
2020; 8(4): 57-61
http://www.sciencepublishinggroup.com/j/ab
doi: 10.11648/j.ab.20200804.11
ISSN: 2329-0870 (Print); ISSN: 2329-0862 (Online)

Assessment of Serum Zinc and Copper Levels, and Cu / Zn Ratio Among Patients of Côte D'Ivoire with Type 2 Diabetes

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To cite this article:

N'Guessan Assieoussou Jean-Luc, Boyvin Lydie, Bahi Gnogbo Alexis, Moke-Bedji Louise Odile, Yaye Yapi Guillaume, Ankotche Amos, Djaman Allico Joseph. Assessment of Serum Zinc and Copper Levels, and Cu / Zn Ratio Among Patients of Côte D'Ivoire with Type 2 Diabetes. *Advances in Biochemistry*. Vol. 8, No. 4, 2020, pp. 57-61. doi: 10.11648/j.ab.20200804.11

Received: November 5, 2020; **Accepted**: November 20, 2020; **Published**: November 27, 2020

Abstract: Type 2 diabetes or diabetes mellitus is a metabolic disorder characterized by an increase in blood sugar level. It is caused by a lack of insulin secretion, insulin action, or both. There are approximately 501,530 people living with diabetes in Côte d'Ivoire with an estimated prevalence of 5.19% in 2013. Unfortunately impairment of zinc and copper leads to increased oxidative stress, insulin resistance and diabetic complications. The study aimed to assess the zinc and copper status in patients with type 2 diabetes. This study involved 80 type 2 diabetics and 80 non-diabetic as controls. Glycated hemoglobin (HbA1c) was analyzed in whole blood using the Hitachi Roche Cobas C311. The serum assays of zinc and copper then of glycemia were carried out using a flame-air / acetylene atomic absorption spectrophotometer (AAS) of the brand Varian Spectr AA-20 Victoria, Australia and on the Cobas C311 respectively. A significant decrease in zinc content ($P < 0.0001$) and a significant increase in copper content as well as the Cu / Zn ratio ($P < 0.0001$) were observed in type 2 diabetic patients compared to non-diabetic control. The lower serum zinc values and the high Cu / Zn ratio could justify the high level of oxidative stress in patients with type 2 diabetes. Assessment of zinc and copper is essential in the monitoring of complications due to type 2 diabetes.

Keywords: Copper, Côte D'ivoire, Oxidative Stress, Type 2 Diabetics, Zinc

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by an increase in blood glucose level. It results from a defective insulin secretion, insulin action or both [1]. Type 2 diabetes accounted for about 90-95% of all diabetes cases [2]. In 2019, the International Diabetes Federation (IDF) estimated that 463 million people are living with diabetes and this figure is expected to reach 578 million in 2030 and 700 million in 2045 worldwide. In Côte d'Ivoire, the 2013 IDF estimates indicated the number of diabetics to be 501,530, with an estimated prevalence of 5.19% [3].

Diabetes-induced hyperglycemia is considered to be a leading cause of diabetic vascular complications and is associated with oxidative stress, impaired metabolism of trace elements and lipids. These changes can affect homeostasis [4]. Distruption of trace elements and increased oxidative stress in diabetes may contribute to insulin resistance, and then the development of diabetes [5, 6]. Several studies have reported that the metabolism of certain trace elements is altered in diabetes and that these alterations may be a factor contributing to the pathogenesis and progression of this disease [7, 4].

Zinc and copper are trace elements that are not synthesized by the body. They must be provided in sufficient quantity through feeding. They play a central role in the oxidation /

antioxidant mechanism. The imbalance in favor of oxidation leads to higher susceptibility to damage of oxidative tissues, thus leading to the pathogenesis of diabetes or diabetic complications [8].

In fact, zinc is an essential trace element which plays a vital role in the maintenance of many biological processes and cellular homeostasis. Zinc deficiency decreases the sensitivity of peripheral tissue cells to insulin and results in decreased membrane fluidity, insulin stability, insulin receptor synthesis, decreased cellular redox potential and increased sensitivity of lipoproteins to oxidative stress [9, 10]. It is also involved in the synthesis, storage, release and conformational integrity of insulin [11]. It also plays a critical structural role for the antioxidant enzyme superoxide dismutase and can stabilize biological membranes to decrease their susceptibility to oxidative damage which can impair cellular functions [12]. As for copper (Cu), it acts as a pro-oxidant, participating in the formation of free radicals, catalyzed by metals. They also act as structural and catalytic components of certain metalloenzymes [4]. Human studies show that diabetic patients can have abnormal serum copper levels [13]. Some authors have shown that in diabetics an increase in copper levels can stimulate glycation and the release of copper ions, which accelerates oxidative stress [14]. Others have shown that copper has insulin-like activity and promotes lipogenesis [15]. Moreover, Cu metabolism can directly or indirectly affect glucose homeostasis; conversely, diabetes can also disrupt Cu metabolism [16].

An imbalance in the Zn / Cu or Cu / Zn ratio may be a better indicator of metabolic disturbance than the Zn or Cu status alone. This ratio in diabetes is used as an important marker for the assessment of vascular complications [17].

In Côte d'Ivoire, very few studies have looked at the link between diabetes and trace elements. Thus, the objective of this study was to evaluate the status of zinc and copper in type 2 diabetics patients living in Côte d'Ivoire

2. Material and Methods

2.1. Ethical Consideration

This study was approved by the National Ethics and Research Committee (CNER) under number N / Ref: 127-18 / MSHP / CNESVS-km.

2.2. Setting, Site and Study Population

This experimental and prospective study took place in the Department of Biochemistry of the Institut Pasteur of Côte d'Ivoire (IPCI) from November 2018 to April 2019. It involved 80 type 2 diabetic patients and 80 healthy non-diabetic controls.

These people were recruited from the diabetes clinic of the University Hospital Center (CHU) of Treichville and at the community-based urban health facility (FSU-COM) of Yopougon toit rouge. The blood samples were sent to IPCI for further analysis.

Type 2 diabetics age between 39 and 74 years were included in this study. On the other hand, type 1 diabetics, pregnants and lactating women, patients with a pathological history such as tuberculosis, toxicological history (alcohol, tobacco, drugs), taking laxatives and food supplements (vitamins; mineral salts) and people who refused to give informed consent were not included in this study.

2.3. Samples Collection

Blood samples were collected from the bend of the elbow by venipuncture from subjects fasting for at least 12 hours in gray tubes containing sodium fluoride and calcium oxalate, in purple tubes containing EDTA and in red tubes without anticoagulant.

The gray and red tubes were centrifuged (horizon centrifuge, supplied by DRUCKER CO USA) at 3000 rpm for 5 minutes to obtain the sera which were stored at -20°C for the assay of biochemical parameters. Blood in EDTA tubes was used for the Hb1AC assay.

2.4. Measuring Anthropometric Data

Weight and height were measured using a mechanical scale and a fixed wall measuring rod respectively. BMI was calculated using the following formula: $BMI = P / T^2$ (P = Weight and T = Height)

2.5. Biochemical Parameters and Trace Elements

The determination of glycemia and glycated hemoglobin was carried out on the COBAS C311 HITACHI spectrophotometer, the principle of which is based on the TRINDER reaction which is an enzymatic and colorimetric method using a chromogen. The intensity of the color developed is directly proportional to the concentration of the assayed substance [18].

Serum zinc and copper concentrations were determined by flame-air / acetylene atomic absorption spectrometry (Varian AA 20). The serum samples were digested with n-butanol (6%) for 30 min at 100°C respecting the ratio of 1/9 (v / v) i.e. 1 mL of serum per 9 mL pure water-n-butanol mixture. The protein precipitation was carried out by diluting 1 mL of serum in 4 mL of a hydrochloric acid solution (2 M) and each sample was stabilized after homogenization according to the method of [19].

The obtained supernatant was aspirated directly into the flame atomic absorption spectrophotometer at the wavelength of 324.8 nm for copper and 213.9 nm for zinc. For the preparation of the calibration range, a standard multi-elements solution concentrated to 1000 ppm was diluted extemporaneously to 1/500 in nitric acid-deionized water (0.03 M).

2.6. Statistical Analysis

Student's t test was used for the comparison of the means of the concentrations. The ANOVA test including Tukey's multiple comparison test for analysis of variance. For both tests, the significance level was set at 5% ($P < 0.05$).

3. Results

Epidemiological characteristics: the mean age of type 2 diabetics was 57 ± 1.05 years while that of controls was, 43 ± 1.36 years (Table 1).

The BMI of diabetics was significantly higher (27 Kg / m^2) than that of controls (25 Kg / m^2). Regarding the biochemical profile, the mean concentrations of glycemia and Hb1AC were respectively 1.85 ± 0.08 g / L and 10% in type 2 diabetics against 0.84 ± 0, 01 g / L and 5% in the controls (P <0.05) (Table 1).

The mean zinc values in type 2 diabetics (T2DM) were 8.37 ± 0.29 µmol / L versus 16.76 ± 0.65 µmol / L in non-diabetic controls a significant difference (p <0.05). On the other hand, the mean copper concentrations in diabetics was significantly higher 25.18 ± 0.68 µmol / L than those in controls 17.24 ± 0.61 µmol / L (Table 2). In diabetics, the Cu / Zn ratio was significantly higher 3.51 ± 0.23 µmol / L than in controls 1.19 ± 0.07 µmol / L.

The study showed a significant decrease in zinc concentrations and a significant increase in the Cu / Zn ratio in both groups of diabetics compared to non-diabetic controls group (p <0.05) (Figures 1, 2 and 3).

Table 1. *General characteristic of the study population.*

Parameters	Type 2 diabetics	Controls	P value
Gender (M/F)	33/47	34/46	-
Age (Année)	57 ± 1.05	46 ± 1.36	< 0.0021
BMI (kg/m2)	27	25	0.0884
Glycemia (g/L)	1.85 ± 0.08	0.84 ± 0.01	< 0.0001
Hb1AC (%)	10 ± 0.33	5 ± 0.07	< 0.0001

Normal values: Glycemia (0.75-1.10 g / L), Hb1AC (4.7-6.2%), BMI ≥ 25 Kg / m^2 (Overweight)
BMI ≥ 30 kg / m^2 (Obesity), BMI: 18.5-24.99 kg / m^2 (Normal weight)

Table 2. *Average concentration of trace elements in T2DM patients and controls.*

Parameters	Type 2 diabetics	Controls	P value
Zinc	8.37 ± 0.29*	16.76 ±0.65	< 0.0001
Copper	25.18 ± 0,68*	17.24 ±0.61	< 0.0001
Ratio Cu/Zn	3.51 ± 0.23*	1.19 ±0.07	< 0.0001

Normal values: Zinc (11.5 - 18.5 µmol /L), Copper (11.0 - 22.0 µmol /L), Cu / Zn ratio (1.1 - 1.3)

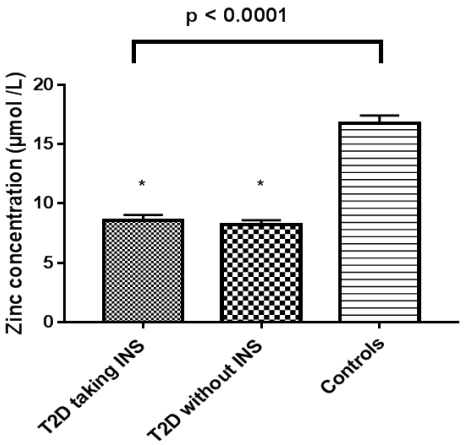

Figure 1. *Zinc Concentration in Diabetics and Controls (INS = Insulin).*

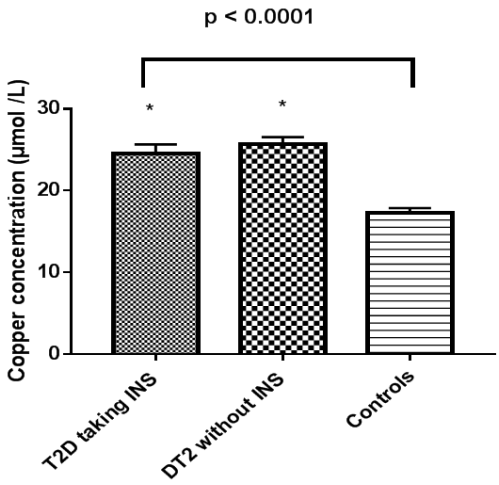

Figure 2. *Copper Concentration in Diabetics and Controls (INS = Insulin).*

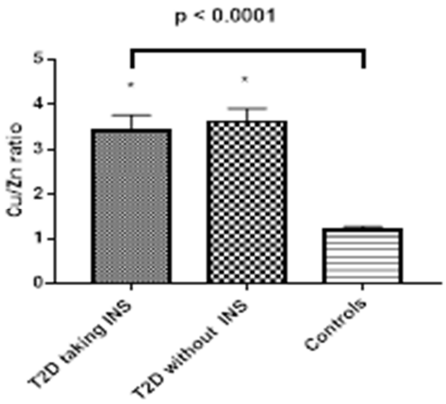

Figure 3. *Cu / Zn ratio in Diabetics and Controls (INS = Insulin).*

4. Discussion

The present study showed a decrease in zinc concentrations in type 2 diabetics compared to controls. These low zinc concentrations are in agreement with several studies, which have also found low zinc levels in type 2 diabetics [20, 21]. These authors indicated that the low serum zinc level in diabetics may be due to diabetes-related hyperzincemia, impaired gastrointestinal absorption and osmotic diuresis [22]. Lower levels of Zn can affect the ability of pancreatic islet cells responsible for insulin production and secretion, as in type 2 diabetes [23]. Zinc is essential for the proper processing, storage, secretion and action of insulin in the beta (β) cells of the pancreas [24]. Indeed six insulin monomers need two Zn 2+ ions to form the hexameric structure on which mature insulin crystals rest [25]. When the zinc concentration decreases, there is a decrease in insulin secretion and peripheral insulin sensitivity. Zinc may be involved in the regulation of the signal transduction mechanism initiated by insulin receptors and the synthesis of insulin receptors. In addition, some authors indicated that the latter is necessary for the maintenance of glucose transporters 4 (GLUT 4) in order to ensure the absorption of glucose in the tissues [26].

The copper concentrations observed in this study were significantly higher in type 2 diabetic patients compared to non-diabetic controls. These results corroborate to those of several authors [27, 21]. It is well known that copper plays a vital role in oxidative stress [28, 4].

Copper in its free form is a potent cytotoxic element due to its redox chemistry. It readily participates in Fenton and Haber-Weiss reactions to generate reactive oxygen species [29]. The increase in Cu ion levels in patients with type 2 diabetes can be attributed to hyperglycemia. This hyperglycemia can stimulate glycation leading to the formation of hydrogen peroxide and the release of copper ions from copper binding sites to proteins [13]. The formation of hydrogen peroxide could cause the superoxide radical to oxidize at higher rates to produce a hydroxide radical responsible for tissue damage [30]. In addition, the increase in hydrogen peroxide could also lead to a decrease in the activity of Cu-Zn SOD, and to the release of copper ions, therefore to an acceleration of oxidative stress [13]. Therefore copper participates in the pathogenesis of diabetic complications.

The Cu / Zn ratio observed in this study were significantly higher in type 2 diabetic patients compared to non-diabetic controls. These results are in agreement with those of Bakacak et al [17]. In fact, hyperglycemia and hyperinsulinemia increase the production of free radicals and decrease the effectiveness of antioxidant defense systems and therefore certain trace elements such as copper and zinc act as antioxidants and prevent membrane peroxidation [4]. The Cu / Zn ratio is an indicator of all the antioxidant defenses to measure oxidative stress. Abnormal Cu and Zn metabolism may affect SOD function and lead to decreased protection of cells against superoxide radicals [31, 7]. Therefore a modification of Cu, Zn and an increase in the Cu / Zn ratio influences the antioxidant defense system and increases the toxic effect of free radicals.

5. Conclusion

The present study shows that a modification of the metabolism of Cu, Zn in particular and of the Cu / Zn ratio is associated with the action of insulin and with oxidative stress in type 2 diabetics. The alteration of these trace elements could represent a major risk factor for the occurrence of diabetic complications.

It is therefore necessary to take into account their associations with chronic hyperglycemia in the management of diabetic patients in order to avoid the occurrence of complications.

Acknowledgements

We thank all the healthy participants and volunteers who agreed to participate in this study. We would also like to thank Dr. Ankocthe Amos, head of the Diabetes Clinic at Treichville University Hospital, who allowed us to recruit patients from his clinic. Finally, we would like to thank the National Research and Ethics Committee for their valuable contributions to this study.

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