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Research Article

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Immunomodulatory Trace Elements Zinc and Copper Concentrations in Diabetic Individuals within Port Harcourt Metropolis, Rivers State, Nigeria

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Abstract Trace elements such as Zinc and Copper modulate immune functions that affect the formation of insulin in the pancreatic beta cells. This study aimed to determine Zinc, Copper, Albumin and Total protein in diabetic individuals within Port Harcourt metropolis, Nigeria. Plasma Zinc, Copper, Albumin and Total protein concentrations of 28 diabetic individuals aged between 30-75 years were compared to 24 healthy non diabetic individuals aged between 30-75 years. Atomic absorption spectrophotometer was used to determine Zinc and Copper concentrations while bromocresol green method was used to determine Albumin. Total protein concentration was determined using biuret method. The results showed that the mean ± standard deviation of Zinc, Copper, Albumin and Total protein for diabetic individuals are 0.58 ± 0.14 mg/L (Zinc), 0.028 ± 0.16mg/L (Copper); 42.64 ± 4.10g/L (Albumin) and 66.57 ± 11.52 g/L (Total protein) respectively while the values for non – diabetic individuals are 0.80 ± 0.30 mg/L (Zinc), 0.05 ± 0.01 mg/L (Copper), 38.5 ± 5.56 g/l (Albumin,) 58.83 ± 9.42 g/l (Total protein) respectively. The concentrations of Zinc and Copper were significantly lower in diabetic individuals when compared with control ($p<0.05$). This study shows that diabetic condition affects the concentrations of trace elements such as Zinc and Copper.

Keyword: Trace elements, Zinc, Copper, Diabetes, Total protein, Albumin

Introduction

"Immonomodulatory trace elements are those elements that are capable of modifying one or more immune function" [1]. Immunomodulatory trace elements such as zinc and copper are required for proper body function.

Zinc is an essential regulatory ion that functions in protein synthesis, catabolism and energy metabolism [2]. Zinc functions in the storage and secretion of insulin [3]. Zinc is an essential regulatory ion that functions in protein synthesis, catabolism and energy metabolism [2]. Zinc is found in almost every tissue in the body, and acts as a powerful antioxidant in preventing cancer from carcinogenic substance [4]. Zinc modulates immune functions that affect diabetes pathophysiology and also aid in the formation of insulin in the pancreatic beta cells [3]. Zinc helps the immune system function properly; it is needed for cell growth, wound healing and breakdown of carbohydrate for energy. Low zinc level increases the effect of stress on the body. Zinc deficiency leads to thinning of hair, weak immunity, persistent diarrhea, anemia, poor wound healing and depression [5].

Excess intake of zinc can cause zinc toxicity which can lead to disturbance in copper metabolism, vomiting. It may affect cardiac function and impair pancreatic enzymes like amylase and lipase [5].

Copper play an important role in maintenance of the body metabolism, growth and repair of the body. It helps the body carry out enzyme reactions and to maintain the condition of connective tissues by building collagen. Copper

makes protein available by releasing iron in the blood there by influencing protein metabolism. Copper has anti inflammatory ability that relieves pain and stiffness associated with arthritis, joint pains and repair of connective tissues. Copper and other trace elements such as Zinc are required to balance thyroid activity. Copper aids in the neutralisation of free radicals which can cause damage to cells.

Total Protein reflects the total amount of protein in the blood. Low total protein may indicate malabsorption, malnutrition, kidney disorder and inflammatory conditions while elevated levels may indicate inflammation and infection in the system.

Albumin is a globular protein that is not glycosylated. It constitutes 50 percent of human plasma proteins. Albumin is produce in the liver and serves as a carrier for molecules such as hormones, bilirubin, fatty acids, cations and thyroxine. Low Albumin causes liver disease, protein losing enteropathy, malabsorption, malnutrition and malignancy while high levels of Albumin are caused by dehydration [6].

Diabetes is a chronic metabolic condition characterized by hyperglycemia, impaired insulin secretion, insulin resistance or both [7]. Patients with high blood sugar experience polyuria (frequent urination), increase thirsty (polydipsia) and hunger (polyphagia). Diabetes that is not properly control or managed can lead to complications, such as cardiovascular disease, nerve damage, kidney damage, eye damage, foot damage, and skin conditions. Trace element concentration such as zinc and copper in diabetes has been shown to modulate immune functions that affect diabetes pathophysiology and also aid in the formation insulin in the pancreatic beta cells.

This study therefore sought to determine immunomodulatory trace element zinc and copper in diabetic individual within Port Harcourt metropolis, Rivers State, Nigeria.

Materials and Methods

Study Area

This study was conducted within Port Harcourt metropolis in Rivers State.

Study Population

The study comprised of a total of 52 subjects aged between 30-75 years. The subject comprises of 28 diabetic individual who were confirmed to be diabetic and 24 non diabetic individual who served as control group. Written and well understood consent was obtained from each participant.

Sample Collection/Preparation

'Blood samples were collected by vein puncture from each subject using vacutainer needle and lithium heparin bottle. Surface of the skin was cleaned with 70% alcohol and allowed to dry. A tourniquet was tied around the arm of subjects above the site for collection. The vacutainer needle was inserted into the vein and 8ml of blood sample was collected into well labeled lithium heparin bottles and flouride oxalate bottles. The blood in the lithium heparin bottle was mixed with the anticoagulant. It was then spun for 5 minutes at 1500 rpm using centrifuge 80 – 1 (by techmel and techmel USA). The plasma samples were separated into plain bottles and stored at 20 °C prior to estimation of zinc and copper concentrations.

Test Methods

Zinc and Copper concentrations were estimated using solar thermo elemental Atomic Absorption Spectrophotometer, model SG 17906.

Albumin was estimated using bromocresol green method [8].

Total protein was estimated using Biuret method [8].

Fasting blood glucose was estimated using oxidase Peroxidase method

Estimation of Zinc and Copper

Zinc and Copper were estimated using solar thermo elemental Atomic Absorption Spectrophotometer model SG 17906

Principle

When small amount of a solution containing trace element (zinc and copper) is been aspirated using the atomic absorption spectrophotometer, the flame coming out of the atomic absorption spectrophotometer changes indicating the type of trace element. The colour is been converted into digital number which appear on the screen of the machine.

Procedure

The blood samples and beakers were labeled individually. 1 ml of each of the plasma was placed in their respective beakers. 6 ml of concentrated analytical grade nitric acid were added to each beaker respectively. 2 ml of concentrated analytical hydrochloric acid ($HClO_4$) were added to each of the beaker. 20 ml of distilled water were added to each of the beaker to digest the plasma. The plasma was placed on a hot plate and was allowed to evaporate to the lowest possible volume (10ml) of the beaker. The plasma was allowed to digest as it was shown by an amber colour clear solution. The plasma was filtered through what man filter paper of 0.2mm. The filtrates were transferred to different volumetric flask respectively. Distilled water was added to each of the voluntary flask to a known mark (20 ml). The plasma was tested using the atomic absorption spectrophotometer. Results were printed out directly from the machine. The controls and test were analysed using same method.

Estimation of Albumin

Albumin was estimated using bromocresol green method [8].

Principle

Bromocresol is an indicator which is yellow at pH 3.4 – 4.2 when it binds with albumin the colour of the indicator changes from yellow to green.

Procedure

The blood samples and tube were labeled from 1 – 28 respectively. The standard and blank was also labeled. 1 ml of bromocresol green (BCG) reagent were pipette into every tube including blank. 10 ul of albumin standard (30 g/l) were added into standard tube. 10 ul of sample was added into sample tube respectively. It was mixed and incubated for 5 minutes at room temperature. It was read at 580 nm using spectrophotometer against a reagent blank. The controls were analysed using the same method.

Estimation of Total Protein

Total protein was estimated using Biuret method [8].

Principle

The cupric in the reagent reacts with the peptide bonds of the protein molecule to give violet or purple coloured complex in an alkaline medium.

Procedure

The blood samples were arranged and the test tubes were labeled blank, standard and test.

1 ml of biuret reagent was added using automated pipette into every tube including blank.

20 ul of sample were added into sample tube respectively. 20 ul of protein standard (60g/l) were added into standard tube. It was mixed and incubated for 10 minutes at room temperature and was read at 550 nm using spectrophotometer. The controls were analysed using same method.

Statistical Analysis

Data obtained were analysed using student T – test, p values less than 0.05 were regarded as statistically significant.

Results

This study was conducted to estimate the concentration of zinc, copper, albumin and total protein in 28 diabetic individuals and 24 non- diabetic individuals as control. The results are summarized in table 1-4 as shown below:

Table 1: Concentration of Trace elements (Zinc and Copper) in Diabetic individuals and control with statistical evaluation N=28 for Diabetic individuals and N=24 non Diabetic individuals (control)

Parameters	Diabetic individuals mean ± S.D N=28	Control mean ± S.D N=24	T value	P value
Zinc (mg/L)	0.580 ± 0.140	0.800 ± 0.300	3.4687	0.0011
Copper (mg/L)	0.028 ± 0.016	0.050 ± 0.010	5.8266	0.0001
Albumin (g/l)	42.640 ± 4.100	38.500 ± 5.560	3.0834	0.0033
Total protein (g/l)	66.570 ± 11.520	58.830 ± 9.420	2.6235	0.0115
Copper/zinc	0.049 ± 0.026	0.680 ± 0.019	98.4292	0.0001
Fasting blood sugar (mmol/l)	10.450 ± 2.790	4.750 ± 0.630	9.7842	0.0001

Table 1 shows that all text parameters were significantly reduced in diabetic individuals than non diabetic individuals (P<0.05).

Table 2: Concentration of trace element (zinc and copper) in diabetic individuals with reference to sex

Parameters	Diabetic Females N=18 Mean ± S.D	Diabetic Males N=10 Mean ± S.D	T Value	P Value
Zinc (mg/L)	0.577 ± 0.100	0.597 ± 0.158	0.4116	0.6840
Copper (mg/L)	0.028 ± 0.010	0.029 ± 0.019	0.1839	0.0856
Albumin (g/l)	42.640 ± 3.720	43.600 ± 4.220	0.6241	0.5380
Total protein(g/l)	69.670 ± 8.540	61.000 ± 12.170	2.2098	0.0361
Copper/zinc	0.048 ± 0.015	0.520 ± 0.034	51.1563	0.0001
Fasting blood sugar (mmol/l)	10.620 ± 2.420	10.140 ± 2.880	0.4702	0.6422

Table 2 shows that Total protein was significantly increased in diabetic females when compared to diabetic males (p< 0.05), while all other parameters did not differ between the two groups.

Table 3: Concentration of trace elements (Zinc and copper) in diabetic individuals with reference to sex with n= 18 for diabetic females and N=12 for non diabetics females

Parameters	Diabetic Females N=18 Mean ± S.D	Non Diabetic Females N=12 Mean ± S.D	T Value	P Value
Zinc (mg/L)	0.577 ± 0.100	0.630 ± 0.046	4.7617	0.0001
Copper (mg/L)	0.028 ± 0.010	0.041 ± 0.006	4.0317	0.0004
Albumin (g/l)	42.640 ± 3.720	43.600 ± 4.220	0.6565	0.5169
Total protein(g/l)	69.670 ± 8.540	53.000 ± 6.123	5.8230	0.0001
Copper/zinc	0.048 ± 0.015	0.065± 0.001	3.8972	0.0006
Fasting blood sugar (mmol/l)	10.620 ± 2.420	5.100 ± 0.612	7.6973	0.0001

Table 3 shows that copper concentrations and copper/zinc ratio was significantly reduced in diabetic females when compared to non diabetic females. However, Total protein was increased in diabetic females when compared to non diabetic females.

Table 4: Concentration of trace elements (Zinc and copper) in diabetic individuals with reference to sex with n= 10 for diabetic males and N=12 for non diabetics males.

Parameters	Diabetic Males N=10 Mean ± S.D	Non Diabetic Males N=12 Mean ± S.D	T Value	P Value
Zinc (mg/L)	0.597±0.158	0.971±0.323	3.3346	0.0033
Copper (mg/L)	0.029 ± 0.019	0.062 ± 0.005	5.8062	0.0001
Albumin (g/l)	43.600 ± 4.220	43.000 ± 3.740	0.3536	0.7274
Total protein(g/l)	61.000 ± 12.120	64.670 ± 6.680	0.9003	0.3787
Copper/zinc	0.052 ± 0.034	0.071± 0.020	1.6310	0.1185
Fasting blood sugar (mmol/l)	10.140 ± 2.880	4.400 ± 0.187	6.9211	0.0001

Table 4 shows that Zinc and copper were significantly reduced in diabetic males when compared to non diabetic males (p<0.05).

Discussion

This result in table 1 showed that zinc concentration in diabetic individuals is significantly lower than in control. Zinc metal found in every tissue of the body helps to exacerbate the effects of stress on the body [9]. Decrease in zinc concentration in diabetic individual can be as a result of zinc in the blood plasma cells binding to proteins and albumin which helps in transport of various materials across membranes. The cause of decreased plasma zinc level in diabetes may be as a result of an increase in urinary loss as also observed in the work of some researchers [10]. Hyperglacaemia has been postulated to interfere with the active transport of zinc back into the tubular cells [11]. Other possible cause may be disturbed metabolisms of zinc metalloenzymes and an abnormal binding of zinc to tissue proteins which causes hyperzincuria [10].

This study in table 1 also showed that Albumin and Total protein concentrations were increased in diabetic individuals when compared to non diabetic individuals. Albumin regulates the pulling of water into the circulatory system by controlling oncotic pressure of the blood [12]. High Albumin is caused by dehydration. Albumin also contributes in stabilising the endothelial layers. Maintenance of capillary permeability occurs when Albumin reduce oxidative damage and modulate inflammation [13]. Study by some researchers [14], shows that zinc binds to albumin and alpha macroglobulin. This could account for the high albumin concentrations observed in diabetic individuals in this study. Elevated Total protein in diabetic individuals as observed in this study may indicate inflammation or infection. Total protein reflects the total amount of protein in the blood and gives an indication of total immunoglobulin concentrations in the system.

Copper concentrations were also lower in diabetic individuals than non diabetic individuals as shown in table 1. This could be as a result of higher concentration of zinc in diabetic individuals as observe in this study. Zinc is an antagonist to Copper. Metabolism of copper is negatively influence by zinc [15].

Interestingly in table 2, there was no significant difference in Zinc and Copper concentrations when diabetic male zinc and copper concentrations were compared to the values gotten in diabetic females. This may indicate that the concentrations of Zinc and Copper in the plasma are not influenced by difference in sex. This is contrary to the view that difference in sex hormones have a great impact on energy metabolism, body composition, vascular function and inflammatory responses. However, table 2 showed that copper/zinc ratio was significant different in male and female diabetic individuals which could be abnormal metabolism of copper and zinc that affect the function of superoxide dismutase (SOD) and result in decrease protection of cells from superoxide radicals [16]. Table 2 also showed that Total protein was significantly increased in diabetic females when compared to diabetic males. Elevated levels of Total protein could indicate inflammation and infection in diabetic females.

Table 3 showed that Total protein was increased in diabetic females when compared to non diabetic females. This also confirms inflammation and infection in diabetic females as also observed in table 2.

Copper concentrations are lower in diabetic females when compared to non diabetic females in table 3. This result is in agreement with the work of some researchers [17] who observed that plasma level of copper in type 2 diabetic females was significantly lower compared with the non-diabetic females. Lower Copper concentrations observed in diabetic females could be attributed to nephropathy which causes diabetic individuals to excrete copper in their urine.

However table 4 shows that in males, both zinc and copper concentrations was significantly lower in diabetic males when compared to non diabetic males.

Conclusively, this study indicates that zinc and copper concentrations in diabetic in individuals is lower compare to non diabetic individuals. This study also showed that zinc and copper concentration is not influenced by sex. It is advised that diabetic individuals should increase the intake of food containing zinc and copper for the proper function of the immune system, cell growth, wound healing and breakdown of carbohydrate.

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