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Serum levels of some Metallic Elements among students of a tertiary institution with different ABO Blood Groups in Nigeria

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Abstract

The clinical importance of ABO blood group system extends beyond immunohaematology and has been associated with diseases, criminal tendencies, personal characteristics and intelligence. Serum copper level was reported to be higher in some blood group type than the others in experimental animals. This study evaluates the serum levels of copper, zinc, magnesium and calcium levels in apparently healthy students in relation to their ABO blood group types. The possible health implications are discussed. ABO blood group and serum levels of copper, zinc, magnesium and calcium were determined using commercially available anti-sera and colorimetry test kits. The mean values were compared between blood group types using ANOVA and Student's-t-test. Of the 200 apparently healthy students (100 males and 100 females) evaluated, 80 belong to blood group O, 56 blood group A, 52 group B and 12 blood group AB. Serum copper levels in the study participants according to their ABO blood types followed the order A>AB>B>O. Copper/zinc ratio also followed similar order A>AB>B>O. Serum level of copper was significantly higher ($p<0.001$) and calcium lower in individuals with blood group A compared with other blood group types.

Keywords: ABO blood group, calcium, copper, magnesium and zinc

Introduction

The determinants of the ABO blood group systems are complex carbohydrate molecules found not only on the extracellular surface of erythrocyte membranes but on the surface of other cells and tissues of the body. Such cells include epithelium, platelet, sensory neurons and vascular endothelium (Storry and Olsson, 2009; Eastlund, 1998). The addition of specific monosaccharides to the common core precursor antigen H distinguishes A from B antigens. Molecularly, the transferase A (α -1,3-N-acetylgalactosaminyltransferase) and transferase B (β -1,3-N-acetylgalactosaminyltransferase) genes encode proteins associated with ABO blood group system (Zhang *et al.*, 2012). Blood group O consists of the core basic H antigen only without the addition of monosaccharide due to the deletion of guanine-258 in the coding region of the protein (Storry and Olsson, 2009; Jenkins and O'Donnell, 2006). A total of 35 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT). The two most important ones are ABO and the Rh D antigen. Together, they determine an individual's blood type (A, B, AB and O, with positive (+), negative (-) or Null denoting Rh D status) (McMahon and Cousins, 1998).

The clinical importance of ABO blood system extends beyond blood transfusion and studies have

shown that it could be a risk factor in the development of cardiovascular, cancers and other diseases (Emokpae and Akpologun, 2015; Franchini and Lippi, 2015 and Franchini *et al.*, 2007). We previously reported higher atherogenic indices among non-O blood groups than blood group O (Emokpae and Akpologun, 2015). It was suggested that, there are personality and behavioural characteristics that are common to blood types (Wazirali *et al.*, 2005). Evidence suggests that ABO blood group may be subjected to malaria-related selection pressure (Wolofsky *et al.*, 2012). The susceptibility of individuals to malaria infection was attributed to imbalance in the concentrations of micro-elements such as copper, zinc, copper/zinc ratio, magnesium and iron (Muller and Kappes, 2007; Nussemblatt and Semba, 2002). These suppositions may have essential elements or micronutrients involvement.

Serum copper, zinc, magnesium and calcium play significant roles in the body which include cell differentiation, replication and immune system regulation. Copper and zinc act as cofactors for anti-oxidant enzymes involved in mopping up of free radicals produced in the body. Trace elements are also involved in defense strategies of organism against infections (Faryadi and Mohebal, 2003; Barber and Cousins, 1988). One of the clinical manifestations of low zinc level is increased susceptibility to infection (Modaresinejab *et al.*, 2016; Davachi *et al.*, 2009). Zinc is involved in brain function, gene expression, immune function and immune signalling. Association between serum copper/zinc ratio and inflammatory biomarkers has been reported (Malavolta *et al.*, 2010; Jumaan, 2008). The concentrations of these elements may be modulated according to ABO blood types (Wiener *et al.*, 1973; Pfeiffer and Iliev, 1972). Wiener *et al.* (1973) reported significant differences in copper levels among the three blood types in sheep. Type BB sheep had significantly higher circulating copper levels than type AB. Since these data suggest a differing biochemical balance among blood types, Bonnett *et al.* (1972) undertook a similar study on human schizophrenics and reported increased levels in the study patients with blood group A. It is not clear whether such differences based on blood group

types are affected by race and geographical location. No study to the best of our knowledge in Nigeria that compares serum levels of these metallic elements among subjects with different ABO blood group types. The aim of this study was to evaluate the serum levels of zinc, copper, copper/zinc ratio, magnesium and calcium among undergraduate students of a tertiary institution with different ABO blood group phenotypes. The possible implications for human health are also discussed.

Materials and Methods:

Study population

This is cross sectional study involving 100 males (aged range 18-26 years) and 100 females (aged 18-25 years) undergraduate students of a tertiary institution in Benin City, Edo State, Nigeria. They were apparently healthy subjects without symptoms of any disease who were recruited from the Halls of residence of the university.

Ethical consideration

The protocol for this study was reviewed and approved by the ethical committee of the Edo State Ministry of Health, Benin City and all participants gave written informed consent before samples were collected.

Sample collection

Four millilitres (4mL) of blood sample was collected from the cubital fossa of each subject by an experienced Phlebotomist using aseptic collection procedure as described by Cheesbrough (Baker and Silverson, 1976), dispensed into plain sample container and allowed to clot. The blood sample was centrifuged at 3000g for 10 minutes and the serum was separated. The separated serum was stored at -20°C until analyzed. The red blood cell was used to determine the blood group.

Blood group determination

Tile method was used to determine the blood group types using antisera supplied by Biotec (England) and the reversed serum grouping done using patient's serum and known washed red blood cells as



described by control as described by Ochei and Kolhatkar (2000).

Serum copper, zinc, magnesium and calcium were assayed colorimetrically using reagents supplied by Centronic (Germany) and Teco Diagnostics (USA) respectively.

The determination was based on the following principles: copper forms with 4-(3,5-Dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline a chelate complex, which is read at 580nm. Zinc forms with 2-(5-Brom-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol, a red chelate complex. The absorbance was read at 560nm and the absorbance is proportional to the concentration of total zinc. Serum magnesium reacts with xylydyl blue to form a coloured compound in alkaline solution. The intensity of the colour formed is proportional to the concentration of magnesium in the sample. Calcium in serum reacts at mildly acidic pH with metallochromogen Arsenic III to form a colour complex which was read at 650nm. Control sera were included in the assays to ensure accuracy and precision of assays.

Statistical Analysis

Statistical analysis including descriptive statistics was carried out using the Statistical Package for Social Sciences (SPSS) version 20.0. All values were expressed as Mean \pm Standard Error of the Mean. Student's t-test and Analysis of variance

(ANOVA) were used to determine significant differences in measured parameters. Confidence limit was set at 95% with level of significance ($p \leq 0.05$).

Results

Table 1 shows comparison of serum levels of measured parameters in individuals with different blood group types. Serum level of copper was significantly higher ($p < 0.001$) in blood group A while serum calcium was lower in blood group A than other blood group types. Serum calcium level was higher in subjects with blood groups B and AB than other blood group types. Serum zinc level was higher in blood group O and lower in blood groups A and AB than blood group B. Serum magnesium was higher in blood group AB and lower in blood group O than blood groups A and B. These differences were not statistically significant. Serum copper was significantly lower ($p < 0.001$) while calcium was significantly higher in subjects with blood group O compared with individuals with blood group A. Conversely, calcium was significantly lower ($p < 0.001$) while copper was higher in subjects with blood group O compared to individuals with blood group B (table 3). There were no significant differences in the levels of the measured parameters between subjects with blood groups O and AB (table 4).

Table 1: Comparison of the levels of measured parameters in subjects with ABO blood group types.

	Mean ± SEM				p-value
	Group O n=80	Group A n=56	Group B n=52	Group AB n=12	
Calcium (mmol/L) (2.02-2.60)	2.42±0.02 ^a	2.27±0.02 ^b	2.44±0.02 ^a	2.44±0.04 ^a	0.001
Zinc (µg/L) (46-150)	68.60±3.93 ^a	65.83±2.86 ^a	66.12±3.23 ^a	65.93±10.23 ^a	0.934
Magnesium (mmol/L) (0.7-1.3)	0.85±0.03 ^a	0.86±0.00 ^a	0.87±0.03 ^a	0.97±0.06 ^a	0.298
Copper (µg/L) (70-150)	139.33±2.51 ^a	151.04±1.29 ^b	139.65±2.37 ^a	143.33±5.47 ^a	0.001
Copper/ Zinc ratio	2.03±0.01 ^a	2.29±0.02 ^b	2.11±0.01 ^b	2.18±0.08 ^b	0.001

b=p<0.001; a=p>0.05. Reference ranges are in parenthesis.

Table 2: Comparison of serum levels of measured parameters in blood groups O and A.

Measured parameters	Mean ± SEM		p-value
	Group O N=80	Group A N=56	
Calcium mmol/L	2.42± 0.02	2.27±0.02	0.001
Zinc (µg/L)	69.0±3.93	66.0±2.45	0.605
Magnesium mmol/L	0.85±0.03	0.86±0.00	0.804
Copper (µg/L)	139.3±1.23	151.0±1.02	0.001

**Table 3: Serum levels of measured parameters in blood groups A and B.**

Measured parameters	Mean \pm SEM		p-value
	Group A N=56	Group B N=52	
Calcium mmol/L	2.27 \pm 0.02	2.44 \pm 0.02	0.001
Zinc (μ g/L)	66.0 \pm 2.45	65.9 \pm 3.23	0.963
Magnesium mmol/L	0.86 \pm 0.00	0.87 \pm 0.03	0.660
Copper (μ g/L)	151.0 \pm 1.02	139.7 \pm 1.56	0.001

Table 4: Comparison of serum levels of measured parameters in blood groups O and AB.

Measured parameters	Mean \pm SEM		p-value
	Group O N=80	Group AB N=12	
Calcium (mmol/L)	2.42 \pm 0.02	2.44 \pm 0.04	0.760
Zinc (μ g/L)	68.6 \pm 3.93	65.9 \pm 6.29	0.793
Magnesium (mmol/L)	0.85 \pm 0.03	0.97 \pm 0.14	0.123
Copper (μ g/L)	139.3 \pm 1.23	141.3 \pm 2.17	0.545

Discussion

Besides ABO blood group role in immunohaematology, there are accumulating evidence that the ABO blood group system also plays key role in various human diseases such as cardiovascular, neurological and infectious diseases. On the other hand, essential metallic elements are needed for healthy growth and development as well as in the maintenance of biochemical processes in the body. Deficiency and high levels of essential

metallic elements may be associated with ABO blood group types.

The data obtained from this study indicates that subjects with blood group A had significantly lower level of calcium ($p < 0.001$) and higher ($p < 0.001$) level of copper when compared with other blood group types. These observations are in conformity with previous studies that observed an increased level of copper in blood group A in patients with

schizophrenia (Pfeiffer and Iliev, 1972). Although, chronic copper toxicity does not normally occur in humans because of transport systems that regulate absorption and excretion (Turnlund *et al.*, 2005), it was explained that the probable cause of an increased copper level is the inability of blood group A individuals to clear copper from the body (Georgopoulos *et al.*, 2001; Pfeiffer and Iliev, 1972). Since excess copper is excreted through bile, copper toxicity is most likely to occur in individuals with liver disease or other medical condition in which the excretion of bile is compromised (Araya *et al.*, 2006). Hypercupremia rather than deficiency of copper is most relevant in human disease. High blood levels of copper are associated with mental and neurological diseases such as Schizophrenia, autism, depression, tardive dyskinesia and memory loss (Pfeiffer and Mailloux, 1987). According to the American Academy of Sciences, clinical conditions due to copper deficiency are rare and may not occur in ambulatory or apparently healthy individuals. Copper intoxication coupled with zinc deficiency was reported in large population study (Pfeiffer and Mailloux, 1987). Again, this is consistent with our observation. It is therefore important for physicians to be familiar with clinical signs and symptoms especially in subjects with blood group A. Such individuals should be counselled to avoid lifestyle that may predispose them to copper toxicity. High copper intake does occur through the ingestion of contaminated drinking water either from reservoirs in which copper sulphate is used to control the growth of algae or galvanized steel water pipes. In addition, daily intake of multivitamin supplements and cigarette smoking has been incriminated in copper intoxication. List of multivitamins that contain high doses of copper was published in the Physician's Desk Reference (Barnhart, 1985). A stick of cigarette contains up to 0.19 μ g of copper and inhalation of cigarette smoke may lead to the accumulation of copper in the body (Creason *et al.*, 1976). Our study participants eat and drink water from the same source. Even though some admitted to cigarette smoking, the number of smokers cut across all blood group types. It is possible that the clearance rate may vary with blood group types.

Diets high in calcium and phosphate causes a greater faecal loss of copper, the mechanism for the antagonism is not clearly understood (Solomons, 1985) however, metal interactions could be suspected. This may explain the reduction in calcium level with an elevated copper level observed in blood group A. A model explanation for blood group A poor clearance or excretion of copper was described in Wilson's disease, a recessive disorder characterised by impaired transport and excessive accumulation of copper in the liver, brain, and other tissues (Popevic *et al.*, 2011). The condition is due to mutations in the Wilson disease protein of ATP7B gene (located on chromosome 13q14.3) (Bull *et al.*, 1993), by impaired copper incorporation into ceruloplasmin and biliary copper excretion. It is characterized by a hepatic cirrhosis, neurological manifestations (dystonia, dysarthria, muscle weakness, vertigo), psychiatric manifestations, renal disease, and copper deposition in the cornea (Strausak *et al.*, 2001).

Susceptibility of individuals to malaria infection has been attributed to deficiency of micro-nutrients such as copper, zinc, magnesium and iron concentrations in children (Muller and Kappes, 2007; Nusseblatt and Semba, 2002) and blood groups A and B were reported to be susceptible to malaria infection which is commonest parasitic infection in our setting. The implications of high levels of copper coupled with low zinc observed in individuals with blood group A to malaria-related selection pressure cannot be over emphasized. Pathogenic infections in humans bring about the generation of reactive oxidants factor that assist a nuclear factor (NF-kB), that also modulate gene expression in immune response against pathogenic organisms (Owuor and Kong, 2002). A low zinc level may reduce about 50% activation of nuclear factor. Zinc protects against lipid peroxidation of membrane (UNESCO, 2003). The reactive oxidants factors produced in course of infection which are deleterious for the plasmodium and modulate expression of structural genes encoding for antioxidant enzymes (Holgrem, 2003). Zinc and copper may be used by plasmodium to fight the oxidant flow generated during infection (M'boh *et al.*, 2010). This may explain their levels are changed in infected individuals. Zinc participates



in the maintenance of immune function and its low levels may enhance the virulence of malaria infection since parasites found it easier to express its metabolism and virulence in reduced zinc levels (Nusseblatt and Semba, 2002; Prasad, 2000). The low zinc level can lead to imbalance in the Cu/Zn ratio and increase in oxidative stress in subjects which may subsequently worsen the pathophysiological state of affected individuals (Mezzetti et al., 1998). The human body has intricate or tortuous system for managing and modulating the levels of essential elements in the circulation and stored cells. Nutrient metals from the diet are integrated into blood if blood levels are depleted, incorporated into cells if cellular levels are inadequate or excreted if blood or cellular levels are sufficient or overload. High copper/zinc ratio has been implicated in impaired DNA, RNA and protein synthesis during brain development. In children, high copper-zinc ratio was associated with lower learning ability, apathy, lethargy and mental retardation. In adults, alcoholism, schizophrenia and brain disorders have been linked to imbalance in copper-zinc levels. High ratio has also been associated with hyperactivity, attention deficit disorders, behaviour disorders and depression (Osredkar and Sustar, 2011).

Significant ($p < 0.05$) correlation was observed between copper and zinc in blood groups A, O and B. This is consistent with previous studies (Hall et al., 1979; Bremner et al., 1976). Increased zinc supply has been shown to inhibit intestinal absorption, hepatic accumulation and placental transfer of copper, as well as to induce clinical and biochemical signs of copper deficiency while in blood group AB the correlation between copper and zinc was not significant ($p > 0.05$) (Yardick et al., 1989; L'Abbe et al., 1984).

Conclusion

Individuals with blood group A had higher level of copper and lower level of zinc and calcium than other blood group types. This may have health implications for individuals with blood group A phenotype.

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