International Journal of Community Research http://www.arpjournals.com
ISSN: 2315 – 6562 E-ISSN: 2384 - 6828

ASSESSMENT OF SOME SERUM MICRONUTRIENT LEVELS IN MALARIA PARASITE INFECTED SUBJECTS IN EKPOMA AND ENVIRONS, NIGERIA

[1]Dic-Ijiewere, O. E.,[2]Elekhebor, J. E.,[3]Airhomwanbor, K.O., [1]Ehimare, R. I., [3]Idehen, I. C.,[4]Osarobo, E., [3]Okparaku, S. O.

[1]Department of Chemical Pathology, Faculty of Clinical Sciences, College of Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria. [2]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, University of Benin, Benin City, Nigeria. [3]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria.[4]Department of Haematology, Faculty of Clinical Sciences, College of Medical Sciences, Ambrose Alli University, Ekpoma
Correspondence: ebenexar@gmail.com

ABSTRACT

This study was carried out to determine the impact of malaria parasitaemia on some serum micronutrient levels. A total of one hundred and five (105) malaria parasite positive male and female subjects, and sixty (60) apparently healthy subjects were recruited for this study. Malaria parasite diagnosis was done by microscopic examination of Thick and Thin Stained Blood Films. Results showed that malaria parasitaemia subject samples, when compared with control sample values, were significantly lower for Calcium, Cobalt, Iron and Zinc ($p<0.05$). The serum Copper levels was significantly higher for the malaria parasitaemia subjects in comparison with control values ($p<0.05$). Serum Magnesium levels were reduced in malaria parasitaemia subjects though it was not statistically significant in comparison with the control subjects ($p>0.05$). Because of the observed steady decrease in Calcium, Cobalt, Iron, Magnesium and Zinc serum levels and increased Copper levels with increase in severity of malaria parasitaemia, there is need for antimalarial treatment before supplementation with these micronutrients or controlled supplementation during treatment to prevent either rapid multiplication of malaria parasites or conditions associated with deficiency of these micronutrients.

Keywords: Plasmodium, Malaria, Micronutrient, Parasitaemia, Serum

Published: 31st July, 2017

INTRODUCTION

Micronutrients or micro minerals are required by humans and other organisms throughout life in small quantities to orchestrate a range of physiological functions. In humans generally, they are required in amounts less than 100 milligrams/day - as opposed to macro minerals which are required in larger quantities. The micronutrients include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, molybdenum, strontium, Nickel and Silicon (Lieberman *et al.*, 1990). Calcium (Ca^{2+}) is one of the most abundant mineral components (i.e. the fifth of the common elements) in the body. Dietary sources include: animal milk and its products such as cheese, yoghurt; canned sardines, soya milk, orange juice. Other important sources include animal bones. Cereals (in form of whole grains) contain small quantity of calcium. However, being consumed frequently makes them a significant source of calcium (Straub, 2007).

In the body of a normal healthy adult subject, the total calcium level is about 1 to 2 kilograms (Burtis*etal*., 2006). Total plasma concentration (i.e. total ionized fraction plus the unionized fractions) is about 2.2 to 2.62 mmol per litre (8.8 to 10.4 mg per decilitre). Calcium is found in the plasma in three forms - protein bound (primarily to albumin but is also bound to some degree to alpha, beta, and gamma globulins), complexed as un-dissociated salts; and free ionized form. About 45% of calcium in plasma is ionized or free calcium, another 45% is protein-bound and the remaining 10% is complexed, only the free ionized form of calcium ions is biologically active (Bolarin,

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

2012). About 99 % of free calcium is found in the skeletal system in form of hydroxyapatite and in small quantity as amorphous calcium salts. The activity of biologically active calcium is seen in three of the body's major compartments: the skeletal system, soft tissues, and the extracellular fluid. Calcium phosphate in the bone is not an inert part of the skeletal system but is in constant active balance with the calcium ion and phosphate ion of the body fluids. Only 1% of total body calcium is in the extracellular fluid, while 0.1% is in the intracellular fluid (Bolarin, 2012).

Calcium is very important in body metabolic processes, both intracellular and extracellular. These include: Nerve action or conduction (Elisa*et al*., 1997), many enzymes regulation (Kaplan *et al.*, 1995), Hormone release and action - Ca++ ion acts as intracellular second messenger for many hormones, paracrine factors and neurotransmitters. It is involved in the action of cAMP or other intracellular messengers including inositol triphospate (Burtis*etal*., 2006). Muscle contraction. Excitation and contraction in smooth muscle and myocardium is also Ca++ dependent (Navarro *etal*., 1994) and also in Blood coagulation.

Hypocalcaemia is an abnormal reduction in plasma ionized calcium level. This condition exists when plasma calcium level is less than 8.8 mg per decilitre or 2.20mmol per litre (<2.20/l) in the presence of normal protein concentration (Bolarin, 2012). Thyroid disorders, kidney failure, severe burns, sepsis, and medications such as heparin and glucagon can deplete blood calcium levels (Elisa*et al.,* 1997). Another major cause of hypocalcaemia is Rickets and osteomalacia- common in most countries of the world. It may also be caused by malnutrition, malabsorption or Vitamin D deficiency (vitamin D deficiency is rare in the tropics due to constant sun light). Osteomalacia is the defect in the mineralization of osteoid (bone matrix) in adult while in children it is rickets. The demineralization results in a loss of bone mineral density and the strength of the bone. There is diffuse bone pain, bowing of the long bones of the legs (Burtis*etal*., 2006).

Cobaltis an important micronutrient which participates in the reactions of vitamin B12. As an essential constituent of vitamin B12, cobalt is very important for haemopoesis (Nielson, 1996). Dietary sources of cobalt include meat and whole-grain products as well as some fruits like grape juice, orange juice, red wine and banana. Vegetables like green beans, leafy vegetables, broccoli, and spices are relatively good sources. In contrast, foods high in simple sugars (like sucrose and fructose) are low in cobalt. Cobalt is present in the plasma or serum at a concentration of less than 10mmol/l. Average intake of more than 35micro g/day and 25micro g/day for male and female adults respectively is considered to be adequate. Deficiency leads to anaemia in Children (Chiba, 1996).

Copper (Cu)is present indietary sources such are molasses, cocoa, oil-bearing nuts, wheat germ, oysters, liver. Shellfish etc (Milne *et al*., 1990).Normal plasma or serum level of copper is 12 to 26micromole/litre. Daily requirement is about 3mg (Milne, 1994). In mammals, copper is absorbed in the stomach and small intestine. Copper released from the intestinal cells moves to the serosal (thin membrane lining) capillaries where it binds to albumin, glutathione and amino acids in the portal blood. Several or all of these copper binding molecules may participate in copper serum transport. Copper from portal circulation is primarily taken up by the liver. Once in the liver, copper is incorporated into copper requiring proteins which are subsequently secreted into the blood. Most of the copper excreted by the liver is incorporated into Caeruloplasmin which is an alpha- 2 globulin. Caeruloplasmin is the main copper carrier in the blood transporting about 95% of total serum copper. Other transporters of copper are albumin and amino acids. Copper excretion is into the bile (Nielsen, 1996).

Copper is a constituent of some enzymes (co factors) and very essential in their activity, e.g. Tyrosinase, lysyl oxidase (essential for cross- linkage in collagen polypeptides and elastin), monoamine oxidase, catalase, cytochrome, etc. Very important for erythropoiesis (as a component of enzymes of iron metabolism), It facilitates the absorption of iron. It is essential in the formation of haemoglobin (Bolarin, 2012). Dopamine monooxygenase is an enzyme which requires copper as a co factor and the enzyme converts dopamine to noradrenaline or norepinephrine an important neurotransmitter (Milne *etal*., 1990). Antioxidant function- copper metalloenzyme superoxide dismutase protects against free radical damage intracellularly and extracellularly in blood

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

plasma by converting superoxide radicals to hydrogen peroxide and this is then removed (Stoeker, 1996).

Iron (Fe) has several vital functions. Examples include as a carrier of oxygen to the tissues from the lungs in the form of haemoglobin, as a transport medium for electrons within the cells in the form of cytochromes, and as an integral part of enzyme reactions in various tissues (enzymes such as the cytochrome oxidase, peroxidase, and catalase). It is essential cofactor for the hydroxylation of proline and lysine (Bolarin, 2012). The daily requirement for male is 10 mg, for female is 10 to 50 mg (Bolarin, 2012). Iron has a wide distribution except in dairy products. It is found in soybean, flour, beef, liver, kidney, beans, peaches etc. Iron is present in all cells in the human body. 65% of the iron in the body is bound up in haemoglobin molecules in red blood cells. About 4% is bound up in myoglobin molecules. Around 30% of the iron in the body is stored as ferritin or hemosiderin in the spleen, the bone marrow and the liver, in blood plasma, iron is carried tightly bound to the protein transferrin. . Small amounts of iron can be found in other molecules in cells throughout the body.

Normal range of plasma iron is - 14 to 32 micro moles per litre for males and 10 to 28 micro moles per litre for females. Common causes of iron deficiency include; Impaired absorption - this may be due to malabsorption, abdominal surgery, etc, Severe loss -in female due to menstrual loss, in pregnancy due to greater demand by the developing embryo, Gastrointestinal bleeding, tumours, parasitic infections, tuberculosis, salicylate ingestion, etc (Allen, 2002). Symptoms of iron deficiency are not unique to iron deficiency. Iron is needed for many enzymes to function normally, so a wide range of symptoms may eventually emerge, either as the secondary result of the anemia, or as other primary results of iron deficiency (Allen, 2002).

Magnesium (Mg)is the fourth most abundant cation in the body after Na+, K+ and Ca2+ and is second only to K+ in the cell. It is usually referred to as the fifth but forgotten electrolyte. It is not technically a trace element (Elin, 1994). Richest dietary sources of magnesium include: cocoa and cocoa products, Nuts, barley, Oat and oat meal, soya flour and beans, Green vegetables, etc .It is about 0.35 gm per kg of body weight in an adult subject, and the reference or normal plasma or serum range is 0.70 to 1.1 mmol per litre (Bolarin, 2012). More than 60 percent of magnesium in the body is found combined with calcium and phosphate in the complex salts of the bone and it is not readily exchangeable with other body compartments. The remainder is in the soft tissues and body fluids. It is the fourth most abundant cation in the body and very essential to the various biochemical processes. It is mainly an intracellular cation and it is second to potassium ion in the body.

Magnesiumis essential for all enzymatic processes involving ATP (including phosphatases, adenosine triphosphatase, and alkaline phosphatase, which are magnesium- activated and magnesium- dependent).It is very important in muscle contractions or neuromuscular integrity and stabilization of the macromolecular structure of DNA, RNA and ribosomes (Bolarin, 2012). Hypermagnesemia is an elevation of serum or plasma magnesium levels above 1.1 mmol per litre, it is seldom an important clinical condition and rarely needs treatment unless it is severe (i.e. More than 7.5 mmol/l) (Shils, 1996). Excessive magnesium levels may occur with end-stage renal disease, Addison's disease, or an overdose of magnesium salts and haemodialysis (Kies, 1994). Lethargy, hypotension, decreased heart and respiratory rate (Gottlieb *et al*., 1993), Muscle weakness and diminished tendon reflexes (Swan and Kaplan-Machillis, 1999).

Zinc (Zn) is an essential component of several enzyme systems (co factor), especially those concerned with protein and nucleic acid synthesis- DNA and RNA polymerases, including carbonic anhydrase and ALA dehydratase. Zinc is essential in wound healing, growth and sexual maturation, and insulin contains zinc (Bolarin, 2012). Dietary sources include vegetables, wheat, whole bran, oysters, crab, shrimp, red meat and fish (Cousins, 1996). Normal serum or plasma zinc is 10 to 20micromole/l and daily requirement is about 15 mg. Zinc deficiency may be due to poor intake (parenteral nutrition), high quantity of dietary phytates or high utilization of zinc. Deficiency causes dermatitis, diarrhoea, mental disturbance and lethargy. In infants, deficiency causes skin rashes (Klug and Schwabe, 1995), chronic diarrhoea and intestinal malabsorption, growth retardation, and hypogonadism (Chiba, 1996).

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Although these micro nutrients are present in many diets and supplements, parasitic infections such as Malaria Parasitaemia could be a burden on the body micronutrient levels, either as a result of loss of appetite and anorexia or utilisation of these micronutrients by the parasites, which could lead to deficiency or lack of balance of the various micronutrients. Deficiency or lack of balance of the various micronutrients can in turn lead to a variety of disease conditions leading to poor health and failure to thrive (Bolarin, 2012). Hence, the need to evaluate the extent of micronutrient loss in malaria Parasitaemia, and recommend measures for micronutrient recovery in Malaria Parasitaemia.

MATERIALS AND METHODS

Research Design: This study was carried out among patients visiting the various health centres and general hospital laboratories in Ekpoma town and adjourning rural settlements. A total of one hundred and five (105) malaria parasite positive male and female subjects between the ages of 15-55 years were recruited for this study. A total of Sixty (60) apparently healthy male and female subjects between the ages of 15-55 years who tested negative for malaria parasite were recruited as control subjects for this study. Ethical approval was obtained and the study was conducted with informed consent of the patients.

Geographical Description of the study area: Ekpoma is a town in Edo state, Nigeria. It is the headquarters of the Esan West Local Government Area. It is a semi urban area with an estimated population of 125,842 people at the 2006 census (National Population Commission, 2006).The town is home to the Ambrose Alli University. Ekpoma has the following coordinates; 6º45'N 6º08'E (NIPOST, 2009).

Sample Collection; Seven mililitre (7ml) of venous blood samples were collected by vene-puncture into accurately labeled plain containers for both subjects and control (2ml of blood was collected into lithium heparin bottles for Calcium estimation). The test subjects were those that presented at the various health centres and General hospitals with symptoms associated with malaria infection. The signs and symptoms of malaria infection in humans are caused by the asexual blood stage of the parasite which includes: fever, headache, joint pains, abdominal upset, nausea, vomiting and digestive disorders (Dayachi*et al.*, 1991). Thick and thin stained blood films were made, and the remaining blood samples were centrifuged at 3500 rpm for ten minutes at room temperature within one hour of sample collection and the serum separated into clean plain containers which were kept frozen until required for analysis.

Malaria Parasite Diagnosis by Microscopic Examination of Thick and Thin Stained Blood Films: Giemsa's staining technique was used for the staining of the thick blood films for malaria parasite detection and malaria parasite count, while the thin blood films were stained with Leishman staining technique for plasmodium species identification as described by Monica Cheesbrough, (2005). The changes in parasitized red cells helped to identity plasmodium species and to detect mixed infection of malaria parasite. The number of asexual *Plasmodium falciparium* and other species per 200 leukocytes were counted and when ten or more parasites were identified, the number was recorded, a blood sample was regarded as negative when the examination of thick films failed to show the presence of asexual parasites. The parasite count in relation to the leukocyte count was converted to parasite per micro litre of blood using this mathematical formula;

$$\text{parasites / ul of blood} = \frac{\text{Number of observed asexual parasites}}{\text{200(Number of leucocytes counted)}} \times \text{total WBC count / ul}$$

Procedure for Staining Thick Blood Film: Thick blood films were made on clean grease free glass slides, allowed to air dry and stained with prepared Giemsa stain for 30mintues.The Giemsa stain was prepared by diluting stock Giemsa stain (commercially obtained) in buffered water immediately before use. Stained slides were rinsed in clean water and allowed to air dry before examination under a microscope using X100 objective lens. Chromatin of malaria parasite was stained dark red and cytoplasm stained blue with Giemsa's stain. The presence of malaria parasite, identification of the species of human parasites and relative malaria parasite count in each blood sample was determined from the Giemsa stained thick films and Leishman stained thin blood films.

Malaria Parasitaemia was confirmed by microscopic examination using X100 objective lens (oil immersion lens). A slide was scored as negative when 100 high

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

power fields had been examined for about 30 minutes without seeing any parasites. The amount of relative parasite count (Occurrence) in positive smears was done using a simple code from one to four crosses (+ - ++++) (Dayachi*etal*., 1991), although none of the subjects had ++++. Malaria ParasitaemiaOccurrence was graded as; + = 1 – 10 parasites per 100 thick film field; ++ = 11 – 100 parasites per 100 thick film field; +++ = 1 – 10 parasites per single thick film field; ++++ = more than 10 parasites per single thick film field after staining for 30 minutes as described by Monica Cheesbrough, (2005).

Procedure for Staining Thin Blood Film; Thin blood films were made on clean grease free glass slide and stained using Leishman staining technique as described by Monica Cheesbrough, (2005). The films were allowed to air dry and covered with Leishman stain for four minutes. The stain on the slides were diluted with buffered distilled water and allowed to stain for ten minutes. Slides were rinsed with water, allowed to air dry and examined under microscope using X100 objective lens.

MalariaParasiteCount: Quantitative parasitaemia count (Parasite density) was determined by counting the number of asexual parasites (trophozoites, schizonts) present in as many microscopic fields (100x) necessary to count 200 leukocytes in each thick blood film and multiplies by the total white blood cells count of each blood sample. Parasitaemia was graded as low (parasite 1000-9,999 μL^{-1}) and high (>10,000 μL^{-1}) (Warhurst and Williams, 1996).

$$\text{parasites / ul of blood} = \frac{\text{Number of observed asexual parasites}}{\text{200(Number of leucocytes counted)}} \times \text{total WBC count / ul}$$

Biochemical Analysis; Measurement of total Calcium was done spectrophotometrically using the o-cresolphthaleincomplexone (CPC) alkaline solution. CPC formed a red chromophore with calcium, which was measured at 570 to 580 nm (Gitelman, 1967).
The serum concentrations of Cobalt, Copper, Iron, Magnesium and Zinc in the samples were determined using the atomic absorption spectrophotometer as described by Walsh, (1962). In practice, Atomic Absorption Spectrophotometry (AAS) methodology entails the aspiration of a sample into a flame, where it becomes atomized. A light beam is directed through the flame into a monochromator and onto a detector. The detector then measures the intensity of light absorbed by the atomized elements in the flame. Thus, the amount of light intensity absorbed in the flame is proportional to the element in the sample.

Statistical Analysis; The data generated from the study was subjected to basic statistical measurement for mean and standard deviation, and comparisons were carried out to test for significant differences, using parametric analysis of variance (ANOVA) with the aid of the computer SPSS 20.0 windows application. Statistical significance was accepted at $p < 0.05$.

RESULT

The statistics of the data obtained from the analyzed 1subjects and control samples are shown in Tables 1-5 below. Values from Control samples were compared with values from the test subjects, and multiple comparisons were carried out with the one-way ANOVA statistics between groups and within groups. Other than table 1, the control and test subject values are presented as mean ± Standard Deviation (SD).

Table 1 Shows the Demographic characteristic of the study population. Of the total of 105 test subjects used for this study, 59(56.18%) were males of which 18(17.14%) of them were within the ages of 15-25, those within the ages of; 26-35 were 16(15.23%), 36-45 were 14(13.33%), 46-55 were 11(10.48%). 28(26.60%) males had '+' occurrence of malaria parasitaemia, 24 (22.85%) had '++' occurrence of malaria parasitaemia and 7(06.60%) males had '+++' occurrence of malaria parasitaemia. A total of 46(43.81%) female test subjects were used for this study, within the ages of 15-55. Those within the ages of 15-25 were 14 (13.33%), within the ages of 26-35 were 15 (14.29%), within the ages of 36-45 were 08 (07.62%), and those within the ages of 46-55 were 09 (08.57%). 30 (28.51%) females had '+' occurrence of malaria parasitaemia, 14 (13.33%) had '++' occurrence of malaria parasitaemia and 03 (02.81%) females had '+++' occurrence of malaria parasitaemia.

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Table 1: Demographic characteristic of the study population

Age (Years)	Male Subjects (%)	Female Subjects (%)
15-25	18 (17.14)	14 (13.33)
26-35	16 (15.23)	15 (14.29)
36-45	14 (13.33)	08 (07.62)
46-55	11 (10.48)	09 (08.57)
Total (100%)	**59 (56.18)**	**46 (43.81)**
Occurrence of Malaria Parasitaemia		
+	28 (26.60)	30 (28.51)
++	24 (22.85)	14 (13.33)
+++	07 (06.60)	03 (02.81)
Total (100%)	**56.00**	**44.00**

The mean values obtained from the analyzed parasitaemia subject samples as shown in Table 2 when compared with control sample values was significantly lower for Calcium (p=0.03), Cobalt (p=0.01), Iron (p=0.00)and Zinc (p=0.01).The plasma Copper levels was significantly higher for the malaria parasitaemia subjects (p=0.01) in comparison with control values, while the serum Magnesium levels was not statistically significant.

Table 2: Mean± standard deviation of serum micronutrients subjects with malaria parasite infection compared with healthy controls.

Parameters	Control Subjects n=60	Malaria Parasitaemia Subjects n=105	f-ratio	p-value
Ca (mg/dl)	10.11 ± 1.09	7.42 ± 1.01	15.23	0.03*
Co (µmol/l)	0.78+0.07	0.35+0.06	6.12	0.01*
Cu (µmol/l)	35.24 ± 6.03	46.12 ± 4.23	4.11	0.01*
Fe (µmol/l)	25.65 ± 2.19	14.38 ± 4.20	5.62	0.00*
Mg (mmol/l)	2.94 ± 0.16	2.89 ± 0.10	3.98	0.07
Zn (µmol/l)	18.70 ± 2.18	12.55 ± 1.18	3.17	0.01*

Key
Values are expressed as mean ± standard deviation.
Values are significantly different at $p<0.05$.
Ca = Calcium; Co = Cobalt; Cu = Copper; Fe = Iron; Mg = Magnesium; Zn = Zinc;
n = Number of samples;
µmol/l = Micro mole per litre;
mg/dl = Milligram per deciliter

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

As shown in Table 3, The mean values obtained from the analyzed male subjects when compared with male control subject values was significantly lower for Calcium (p=0.04), Cobalt (p=0.00), Iron (p=0.01) and Zinc (p=0.01). The plasma Copper levels was significantly higher for the malaria parasitaemia male subjects (p=0.03) in comparison with male control values, while the serum Magnesium levels was not statistically significant (p=0.12).

Table 3: Mean±standard deviation of serum micronutrients of Male subjects with malaria parasite infection compared with healthy Male controls.

Parameters	Male control Subjects n=30	Male Malaria Parasitaemia Subjects n=59	f-ratio	p-value
Ca2+ (mg/dl)	10.15± 0.90	7.02 ± 0.81	9.56	0.04*
Co (µmol/l)	0.80+0.11	0.41+0.12	6.89	0.00*
Cu (µmol/l)	36.21 ± 5.44	47.47 ± 3.23	11.89	0.03*
Fe2+ (µmol/l)	26.14 ± 1.18	16.27 ± 3.00	4.23	0.01*
Mg (mmol/l)	2.87 ± 0.20	2.81 ± 0.30	6.09	0.12
Zn (µmol/l)	18.03 ± 1.77	13.08 ± 0.99	3.56	0.01*

Keys:
Values are expressed as mean ± standard deviation.
Values are significantly different at $p<0.05$.
Ca = Calcium; Co = Cobalt; Cu = Copper; Fe = Iron; Mg = Magnesium; Zn = Zinc;
n = Number of samples;
µmol/l = Micro mole per litre;
mg/dl = Milligram per deciliter

As shown in Table 4, The mean values obtained from the analyzed female subjects when compared with female control subject values was significantly lower for Calcium (p=0.00), Cobalt (p=0.00), Iron (p=0.01) and Zinc (p=0.00). The plasma Copper levels was significantly higher for the malaria parasitaemia female subjects (p=0.04) in comparison with male control values, while the serum Magnesium levels was not statistically significant (p=0.06).

As shown in Table 5, the various levels of severity of malaria parasite in the test subjects when compared and multiple comparisons carried out with the one-way ANOVA statistics between groups and within groups, it was statistically significant for Calcium (p=0.03). The '+' severity of malaria parasite was not statistically significant in comparison with the '++' severity of malaria parasite, but it was significantly higher in comparison with the '+++' severity of malaria parasite. Values for Cobalt when compared was statistically significant (p=0.04). The '+' severity of malaria parasite was significantly higher in comparison with the '++' and '+++' occurrence of malaria parasite and the '++' was significantly higher in comparison with the '+++' severity of malaria parasite. Values for Iron and Zinc when compared was statistically significant (p=0.00) and (p=0.00) respectively. The '+' severity of malaria parasite was significantly higher in comparison with the '++' and '+++' severity of malaria parasite and the '++' was significantly higher in comparison with the '+++' severity of malaria parasite for both Serum levels of Iron and Zinc. The plasma Copper levels was significantly higher for the '+++' in comparison with the '+' and '++' severity of malaria parasite (p=0.02). Magnesium levels were not statistically significant (p=0.89).

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Table 4: Mean±standard deviation of serum micronutrients of Female subjects with malaria parasite infection compared with healthy Female controls.

Parameters	Female control subjects n=30	Female Malaria Parasitaemia Subjects n=46	f-ratio	p-value
Ca2+ (mg/dl)	9.34 ± 1.32	5.72 ± 0.71	13.44	0.00*
Co (µmol/l)	0.72+0.21	0.31+0.09	2.68	0.00*
Cu (µmol/l)	32.33 ± 3.24	41.46 ± 3.17	6.23	0.04*
Fe2+ (µmol/l)	24.79 ± 2.65	12.33 ± 1.98	16.02	0.01*
Mg (mmol/l)	2.74± 0.26	2.72 ± 0.41	4.23	0.06
Zn (µmol/l)	17.91 ± 1.58	11.37 ± 1.09	4.87	0.00*

Keys:
Values are expressed as mean ± standard deviation. Values are significantly different at p<0.05.
Ca = Calcium; Co = Cobalt; Cu = Copper; Fe = Iron; Mg = Magnesium; Zn = Zinc;
n = Number of samples; µmol/l = Micro mole per litre; mg/dl = Milligram per deciliter

Table 5: Comparison of Mean ± standard deviation of serum micronutrients of subjects with malaria parasite infection at various levels of severity/occurrence.

Parameters	+ severity of Malaria parasite n=57	++ severity of Malaria parasite n=38	+++ severity of Malaria parasite n=10	f-ratio	p-value
Ca2+ (mg/dl)	8.16± 1.05^{a}	6.04±0.44^{a}	4.64±0.08^{b}	8.99	0.03*
Co (µmol/l)	0.37+0.08^{a}	0.31±0.11^{b}	0.27±0.05^{c}	5.64	0.04*
Cu (µmol/l)	40.14 ± 4.05^{a}	45.32±3.87^{a}	49.24±9.32^{b}	6.45	0.02
Fe2+ (µmol/l)	19.14 ± 4.56^{a}	11.21±2.67^{b}	6.79±1.55^{c}	4.22	0.00*
Mg (mmol/l)	2.82 ± 0.08^{a}	2.67±1.03^{a}	2.62±1.11^{a}	3.12	0.89
Zn (µmol/l)	19.12 ± 1.05^{a}	13.43±0.78^{b}	8.19±1.03^{c}	8.23	0.00*

Keys:
Values are expressed as mean ± standard deviation.
Values in a row with different alphabetical superscript are significantly different at P<0.05.
Ca = Calcium; Co = Cobalt; Cu = Copper; Fe = Iron; Mg = Magnesium; Zn = Zinc;
n = Number of samples;
µmol/l = Micro mole per litre;
mg/dl = Milligram per deciliter

EndorsedBy: *Innovative Science Research Foundation* (***ISREF***) and *International Society of Science Researchers* (***ISSCIR***).
IndexedBy: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate*)

DISCUSSION

Results from this study indicated that the micronutrient levels of test subjects was affected by the malaria parasite infection as most of the micronutrients were significantly lower ($p<0.05$) in malaria parasitaemia subjects in comparison with the healthy control subjects. Significantly reduced plasma concentration of Calcium was observed in malaria parasitaemia (7.42 ± 1.01) when compared with that of control (10.11 ± 1.09), and lower levels were observed for females (5.72 ± 0.71) than males (7.02 ± 0.81). A significant decrease in calcium concentration with increase in the severity of malaria parasitaemia was observed which ranged from 8.16± 1.05 in '+' of malaria parasitaemia to as low as 4.64±0.08 in '+++' of malaria parasitaemia. According to Kon*et al*., (2003), the influx of calcium into the infected cells caused by changes in the form and fluidity of the erythrocytes membrane infected by plasmodium falciparum could also cause the reduction of calcium in circulation. The reduction in serum concentration of calcium in malaria parasitaemia could be due to erythrocytes parasites cytoadhering in the glomerular capillaries which may lead to urinary excretion of Calcium in malaria (Asaolu and Igbaakin, 2009).

Significantly reduced serum Cobalt levels were reported when compared with the healthy control, and significant gradual decline was observed with increase in severity/occurrence of the malaria parasitaemia. Cobalt is a component of the vitamin B12 molecule known as cobalamin, other than this it has no known function in humans (Farell*et al.*, 2010). Free cobalt cannot be incorporated in body's' vitamin B12 pool, hence diet has to supply body's B12 needs. Therefore, the significant decrease observed for cobalt in malaria parasitaemia subjects could suggest low dietary intake of vitamin B12 in malaria or parasitic depletion of plasma vitamin B12 as it was observed that significant decrease in serum cobalt occurred with increased severity of the malaria parasite.

From this study serum copper level of malaria parasitaemia subjects higher than that of healthy control was observed, this is in conformity with the study done by Baloch *et al.*, (2011) which reported increased serum copper (Cu) levels significantly higher in vivax malarial patients as compared to healthy subjects. Therefore increased level of copper in patients with malaria if not treated properly in time could result in Wilson's disease.Wilson's disease is a rare inherited disorder that causes copper to accumulate in your liver, brain and other vital organs. Most people with Wilson's disease are diagnosed between the ages of 5 and 35, but it can affect younger and older people, as well. Copper plays a key role in the development of healthy nerves, bones, collagen and the skin pigment melanin. Normally, copper is absorbed from your food, and excess is excreted through a substance produced in your liver (bile) (Bandmann, 2015).

From this study, serum Iron level of malaria parasitaemia subjects (14.38 ± 4.20) was significantly lower than that of healthy control (25.65 ± 2.19), with females having a lower serum iron concentrations than male for both the malaria parasitaemia subjects and healthy control subjects. Baloch *et al.*, (2011) reported significantly lower Serum iron levels in vivax malarial patients, in their study the serum iron levels decreases 2.0708 times in vivax malarial patients. This agrees with this study in which a 1.7837 times decrease in serum iron was observed in malaria parasitaemia (though predominantly *Plasmodium falciparium)* subjects from the healthy control subjects. A steady decrease in serum iron concentration with increase in severity was observed for the malaria parasitaemia subjects, with the serum iron of '++' malaria parasitaemia subjects being 1.7074 times lesser than the '+' malaria parasitaemia and the '+++' malaria parasitaemia being 1.6510 times lesser than '++' malaria parasitaemia subjects. This decrease can be attributed to the digestion of hemoglobin by malaria parasites. Normally, the body contains about 3-4g of iron, more than half of which is in the form of hemoglobin responsible for the transport of oxygen from lungs to the tissues. Iron is also a constituent of a number of enzymes (Wester, 1987).

Serum Magnesium levels in malaria parasitaemia was slightly reduced in comparison with the control, also serum levels of Magnesium reduced with increase in severity, although the reduction was not statistically significant. This is in conformity with the study done byRani *et al.*, (2015) which reportedvariation in level of Mg depending on the type of parasite exposed to by the patients. They observed reduced Mg level in patients suffering from *P. falciparum* infection. Discordantly, the increased Mg level was observed in patients suffering from *P. vivax* infection. Magnesium

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

deficiency has been associated with reduced number of RBCs, which is a common feature of Malaria Parasite infection (Garba and Ubom, 2006).

From this study, serum Zinc level of malaria parasitaemia subjects (12.55 ± 1.18) was significantly lower than that of healthy control (18.70 ± 2.18), with females having lower serum zinc concentrations than male for both the malaria parasitaemia subjects and healthy control subjects. This agrees with the work done by Gouado*et al.*, (2007). A decrease in serum zinc concentration with increase in severity was observed for the malaria parasitaemia subjects, serum zinc levels for '++' malaria parasitaemia subjects was lesser than the '+' malaria parasitaemia subjects and the '+++' malaria parasitaemia was lesser than '++' malaria parasitaemia subjects.

Depletion of body micronutrients by malaria parasite (mainly *plasmodium falciparum*) was clearly discovered by way of steady decline in serum micronutrients levels which occurred in inverse proportion to severity. This queries the suitability of administering micronutrients supplements in malaria parasite infection without prior treatment of the infection, as it could provide enough micronutrients for parasite multiplication and possible resistance, although the possibility of resistance is subject to further research verification. Loss of appetite leading to poor nutrition which is common with malaria infection could also contribute to the decrease levels of micronutrients.

CONCLUSION

Calcium, Cobalt, Copper, Iron, Magnesium and Zinc plasma and serum levels were affected by Malaria parasitaemia infection. Severe malaria parasite infection is associated with reduced Calcium, Cobalt, Iron, Magnesium, Zinc levels and increased serum copper levels, hence the need for antimalarial treatment before supplementation of these micronutrients, or controlled supplementation during treatment.

ACKNOWLEDGEMENT

We are grateful to the health centres and general hospital laboratories in Ekpoma town and environs, Labheth Diagnostic and Research Group Edo state, and the Faculty of Clinical Science Laboratory, Ambrose Alli University Ekpoma, Nigeria for providing us the opportunity to conduct this study. We the authors declare that there is no conflict of interests.

REFERENCES

Allen, L.H. (2002): Supplement; Forging Effective Strategies to Combat Iron Deficiency, The American Society for Nutritional Sciences. *Journal of Nutrition*. 132:813S-819S.

Asaolu, M. F. and Igbaakin, P. A. (2009): Serum Levels of micronutrients and antioxidants during malaria in pregnant women In Ado-Ekiti, Ekiti State, Nigeria. *International Journal of Medicine and Medical Sciences.* Vol 1.(11) pp. 523-526.

Baloch, S., Memon,S. A., Gachal, G. S. and Baloch, M. (2011): Determination of Trace Metals Abnormalities in Patients with Vivax Malaria. *Iran J Parasitol.* 6(2): 54–59.

Bandmann, O.(2015): Wilson's disease and other neurological copper disorders. *The Lancet Neurology.* 14:103.

Bolarin, D.M. (2012): Bolarin's Aids to Chemical Pathology. New edition. O and A Publication. Ibadan, Nigeria. Pp. 691-727.

Burtis, C.A. and Ashwood, E.R. (2006): Tietz Fundamentals of Clinical Chemistry.5th edition. Philadelphia, W.B., Sanders. An imprint of Elsevier. The Curtis Centre. Independence Square West Philadelphia, Pennsylvania. 19106. Pp 797-800.

Chiba, M. (1996): Concentrations of essential trace elements in blood and introduction of analytical techniques. Nippon Rinsho.54; 179-185.

Cousins, R.J. (1996): Zinc. In Ziegler .E.E., Filer, F.F. Jr. (eds); Present Knowledge in Nutrition.7th edition. Washington DC,ILSI Press. Pp 256-264.

Dayachi, F., Kabongo, L. and Ngoie, K. (1991): Decreased mortality from Malaria in children with symptomatic HIV infection. *Int. Cont. AIDS*. 2: 164.

Elin, R.J. (1994): Magnesium; the fifth but forgotten electrolyte. *Am J ClinPathol*; 102:616-622.

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Elisaf, M., Milionis, H. and Siamopoulos, K.C. (1997): Hypomagnesemichypokalemia and hypocalcaemia: clinical and laboratory characteristics. *Miner Electrolyte Metab*.23:105-112. Medline Web of Science

Farell, J.J. (2010): Digestion and Absorption of nutrients and vitamins. In: Feldman M, Friedman LS, Brandt LJ (eds). Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease 9th ed. New York. 2010:423-427.

Garba, I.H. and Ubom, G.A. (2006): Potential role of serum magnesium measurement as a biomarker of acute falciparum malaria infection in adult patients. *Biol Trace Elem Res*, 114 (1–3): 115– 120.

Gitelman, H. (1967): Clinical chemistry calcium –O-cresolpthaleincomplexone method. *AnalBiochem*. 20; 521.

Gouado, I., Lehman, L. G., Some, I. T., Mbouyap, Y., Pankoui, M. J. B., Ejoh A. R., Tchouanguep, M. F. (2007): Influence of malaria on the serum levels of vitamin A, zinc and calcium of children in Douala-Cameroon. *Afr J Biotechnol.* 6 (7):871–876.

Kies, C. (1994): Bioavailability of manganese. In: Klimis-Tavantzis D.L. (ed). Manganese in health and disease. Boca Raton: CRC Press, Inc;:39-58.

Klug, A. and Schwabe, J.W.(1995):Protein motifs 5.Zinc fingers. FASEB J; 9; 597-604.

Kon, Y.P., Czeizel, A.E., Festa, M.D. (2003): Controlled studies of calcium supplementation on pregnancyoutcomes. *Ann. N.Y. Acad. Sci*. 687: 266–275.

Lieberman, S. and Bruning, N. (1990): The Real Vitamin & Mineral Book. NY: Avery Group, 3. Monica Cheesbrough. (2005). Discrete Laboratory Practice in Tropical Countries Part 1, Cambridge Second Editions. Published by Press Syndicate of the University of Cambridge, chp. 5, page 247- 258.

Milne, D.B. (1994): Assessment of copper nutritional status. *ClinChem*; 40:1479-1484.

Milne, D.B., Johnson, D.E. and Klevay, L.M.(1990):Effect of copper intake on balance and absorption. *Nutr Res*.10;97.

National Population Commission (2006): Housing and population census result: Edo State Nationalpopulation Office, Benin City.

Navarro, J.F., Teruel, J.L., Montalban, C., Gallego, N. and Ortuno, J. (1994): Hypercalciuria secondary to chronic hypophosphatemia. *Miner Electrolyte Metab*.20:255-258.

Nielsen, F.H.(1996): Other trace elements. In Ziegler .E.E., Filer .F.F. Jr. eds); Present Knowledge in Nutrition.7th edition. Pp 353-377, Washington DC,ILSI press.

NIPOST (2009): Post Offices-with map of LGA. Archived from the original on 2009-10-07. Retrieved 2009-10-20.

Rani, A., Akhtar, S., Nawaz, S. K., Irfan, S., Azam, S., and Muhammad, A. (2015): Electrolyte Disturbance and the Type of Malarial Infection. *Iran Journal of Public Health*. 44(11): 1492–1497.

Shils, M.E.(1996):Magnesium.In Ziegler, E.E.,Filer, F.F.Jr(eds);Present Knowledge in Nutrition,7th edition,Pp 256-264,Washington,DC,ILSI Press.

Stoeker, B.J. (1996):Chromium. In Ziegler E.E., Filfer, F.F. Jr.(eds);Present Knowledge in Nutrition.7th edition.ILSIpress.Pp 344-352.

Straub, D.A. (2007): Calcium Supplementation in Clinical Practice: a review of forms, doses and indications. *NutrClinPract* ; 22: 286-96.

Swain, R. and Kaplan-Machlis, B. (1999): Magnesium for the next millenium. *South Med J; 92:1040-1047.*

Warhurst, D.C. and Williams, J. E. (1996): Acp Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *J. Clin. Pathol*. 49: 533-538.

Wester, P.O. (1987): Magnesium. *Am J ClinNutr*. 45(5 Suppl):1305–12.

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Walsh, A.(1955):The application of atomic absorption spectra to chemical analysis. *Spectrochim. Acta7.108-117.*

AUTHOR'S CONTRIBUTIONS

DIC-IJIEWERE, O.E. was responsible for the development of the design, and execution of the research. ELEKHEBOR, J. E. was involved in the design of the conceptual framework and ensured quality control.AIRHOMWANBOR, K.O. and EHIMARE, R. I., provided logistic framework and support.IDEHEN, I. C., Okparaku, S. O. and OSAROBO, E. were involved in resource and quality control. Manuscript preparation and Data analysis was carried out by DIC-IJIEWERE, O.E.

EndorsedBy: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***IndexedBy**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*