International Journal of Biochemistry Research & Review
4(4): 322-332, 2014

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Serum Zinc and Copper Levels in Children with *Plasmodium falciparum* Infection in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CAO conceived and designed the study, conducted some of the laboratory investigations and drafted the manuscript. Author MS supervised the study and reviewed the manuscript. Author ACO conducted some of the laboratory investigations and reviewed the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 13th November 2013
Accepted 2nd January 2014
Published 19th March 2014

ABSTRACT

Background: An estimated 2 million children die of malaria yearly, primarily because of *Plasmodium falciparum* and its complications. *Plasmodium falciparum* infection could lead to imbalance in micronutrient levels. Micronutrients such as zinc and copper are essential for immune functions. In this study, we determined the baseline serum zinc and copper levels of children with or without malaria in Jos, Nigeria. This is essential because its result will give us a proper insight whether there is a need for a further study on zinc supplementation in these patients or not.
Study Design: This was an analytical case-control study.
Place and Duration of Study: This study was conducted between August and November 2011 in various hospitals in Jos, North Central Nigeria.
Methodology: The blood samples of 600 children aged zero to18 years from various hospitals in Jos were analyzed for malaria parasite (MP), zinc, copper, albumin and total protein. All statistical analysis were done using SPSS version 17. The results were expressed as means, standard deviation and percentages.
Result: Out of 600 children, 306(51%) had malaria infection. Three hundred and thirteen

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(52.2%) were females. The overall prevalence of malaria was 51% while that of congenital and neonatal malaria were 2.0% and 1.5% respectively There was no significant difference in the serum zinc and copper levels of children with or without malaria (p=0.404 and p=0.559). Serum zinc and copper levels were significantly higher in males (p=0.001). There was a significant positive relationship between parasite density and serum zinc and copper levels (p=000). Serum zinc, copper levels and temperature were not predictors of malaria.

Conclusion: There was no significant difference in the serum zinc and copper levels of children with or without malaria. This implies that there may not be need to supplement children with malaria with zinc or copper, as this may not be necessary in solving the menace of malaria morbidity.

Keywords: Plasmodium falciparum; childhood malaria; serum zinc; copper, Nigerian.

1. INTRODUCTION

Malaria, a global health challenge is still endemic in the tropics and subtropics claiming the lives of many African children [1]. In Nigeria, statistics show that malaria accounts for 25% of under five mortality, 30% of childhood mortality and 11% of maternal mortality [2].

Some studies have provided data on the possible efficacy of zinc supplementation in reducing morbidity from malaria [3,4]. Zinc (Zn) and copper (Cu) are involved in numerous aspects of cellular metabolism [5]. They are required for the catalytic function of several enzymes, play essential roles in immune function and act as antioxidants [6,7]. Zn also plays a role in wound healing [7], protein synthesis, DNA synthesis and cell division [8]. It supports normal growth in childhood [9]. Maintaining a proper balance of copper and zinc is important as excess Zn impairs Cu absorption [10]. The ratio of Cu to Zn is clinically more important than the concentration of either of them [11]. Copper is essential also for maintaining the strength of the skin, blood vessels, epithelial and connective tissues. It plays a role in production of haemoglobin, myelin and melanin [10].

Apart from these well-known functions, Zn and Cu also play a critical role in host-pathogen interactions. Zn is required for each step of cell cycle in microorganisms [12]. Cu is an essential component of cuprozinc superoxide dismutase; an enzyme in the erythrocytes essential for host defense as well as parasite growth [13].

The metabolic pathways of plasmodium require several enzyme cofactors such as iron-sulphur clusters [14] and possibly zinc and copper, this may lead to deficiency of these nutrients in the host. Zn and Cu deficiencies are associated with changes in cellular function, changes in growth motor development, behaviour and cognitive functions [15]. These alterations in cellular and humoral functions may increase host susceptibility to *P. falciparum* [16,17].

Several studies [18,19,20] have reported that zinc is low in children with malaria in developing countries. Chimah et al. [21] in a study in Benin city, South- South Nigeria reported that patients with severe malaria presenting with hyperpyrexia and hyperparasitaemia tended to have lower levels of serum zinc. The authors also reported a negative trend between parasite density and serum zinc levels. Muller et al. [22] reported that zinc supplementation has no effect on morbidity from falciparum malaria in children in rural West Africa. In Tanzania, Hofs et al. [23] concluded that supplementing young

Tanzania children with zinc either alone or with other multi-nutrients does not protect against malaria.

Due to zinc's crucial role in immune system function, and recent evidence that zinc supplementation appears to reduce malaria morbidity; we determined the baseline serum zinc and copper levels of children with or without malaria in Jos, Nigeria. This is essential because its result will give us a proper insight whether there is a need for a further study on zinc supplementation in these patients or not. We also determined the serum albumin and globulin levels of these children; to ascertain the effect of malaria on albumin since it is the Zn carrier protein.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was an analytical case- control study conducted between August and November 2011 in various hospitals in Jos, North Central Nigeria. The hospitals were: Jos University Teaching Hospital (JUTH) which is a reference hospital, Bukuru Central (BC), Bukuru Express (BE) and Rayfield (RF) Primary Health Care Centers all in the urban areas of the state.

2.2 Study Population

The study was conducted among children attending the Emergency Paeditaric Unit, Special Care Baby Unit, Paediatric Outpatient Department including the immunization unit of JUTH and the primary health care centers (PHCs) including those who came to the PHCs solely for the purpose of the study.

Children who met the study's inclusion criteria were recruited for the study. The inclusion criteria were: (1) Children who came to the health centers for the purpose of receiving treatment or solely to participate in the study. (2) Consent of children or parent/ caregiver. (3) Presence or absence of clinical signs/symptoms of malaria such as: fever, cough, diarrhea, pallor, jaundice, vomiting, chill and others. (4) Children without any history of treatment with anti-malaria drugs in the past 1 to 2 weeks and (5) Children aged 0 to18years old. The coverage of the total age range (0-18 years) for biological definition of a child (UNESCO) [24] gives this study a relative edge over several studies that limit similar studies to under five years. Children with positive blood slides for malaria parasite were grouped as case while the negative ones were the controls.

Children with any other diagnosed illness apart from malaria, children receiving zinc or copper supplements, children older than 18 years, preterm babies and non-consenting individuals/parents/caregivers were excluded from the study.

Children or Parents/guardians of eligible children gave written informed consent to allow their children to participate in the study. Participants were consecutively recruited into the study until the sample size of 600 was reached. A qualified health personnel used a pre-tested English questionnaire to collect patient's demographic information and the reasons why he/she was brought to the health center. The axillary body temperature was measured using a digital clinical thermometer. Fever was defined as body temperature ≥37.5ºC.

Children that were positive for malaria within 0-7days and 8-28daysof age were grouped as congenital and neonatal malaria group respectively [25].

3. METHODOLOGY

Taking asceptic precautions, 2ml of blood from venipuncture using 23 guage sterile needle, was collected both from case and control groups into metal-free plain tubes and on pre-cleaned slides for analysis of the biochemical parameters and malaria diagnosis respectively. The samples collected in plain tubes were centrifuged for 5minutes at 3000rpm using bench centrifuge, serum was obtained and preserved at 2-8ºC in sterile deionised plain vials. Analysis of the biochemical parameters was carried out within 7 days of sample collection by experienced clinical biochemists.

3.1 Malaria Diagnosis

Double slides of thick and thin blood films of respective subject were made. Blood films were air-dried without convection for one hour and stained with 30% freshly prepared Giemsa stain.

Thin blood films were fixed with 100% methanol prior to staining. Quality controlled Giemsa stain; dust-free microscopy glass slides and phosphate buffer pH 7.2 were used.

Giemsa stained thick and thin blood films were examined for malaria parasite using x 100 (oil immersion) objective by an experienced medical microbiologist in the Paediatrics Research Laboratory of the University who was involved in the study. This served as the internal quality control. The slides were also cross-read by an experienced microscopist who was not otherwise involved in the study (independent reader) this served as the external quality control. The degree of variation in the results was determined and subjected to statistical analysis at 95% confidence limit to test for significance using SPSS version 17, 2008 (www.spss.com). Malaria diagnosis was based on identification of asexual stages of *Plasmodium falciparum* on the thick blood smears. Film was reported as 'malaria parasite not seen' i.e. negative after examining about 100 fields. Thin films were used to identify species and stages of Plasmodium or other blood -borne pathogens (WHO 2007) [26]. Parasite density was by the number of parasites per microlitre of blood (thick film) method [26]. The number of asexual parasitic forms (trophozoites and schizonts) present in as many microscopic fields as possible necessary to count 200 leucocytes was recorded. The standard value of 8000WBC/µl was assumed as a multiplier in the parasitaemia expression below:

$$\text{Parasite/µl of blood (parasite density)} = \frac{N \times \text{total WBC counts/µl (8000)}}{\text{Leucocyte count (200)}}$$

Where N = number of asexual parasitic forms present in as many microscopic fields as possible to count 200 leucocytes.

3.2 Determination of Serum Zinc, Copper, Albumin, Total Protein

Chemistry auto-analyzer, model; Mispa Excel version 2.1e lite, (Open System) 2006, by Mispa Biosystems India was used to colorimetrically analyze the biochemical parameters.

Serum zinc, copper, albumin and total protein (globulin = total protein- albumin level) were respectively determined using 5-Br-PAPS [27], Didrom PAESA [28], Bromocresol green [29] and Direct Biuret [30] methods. Centronic GmbH Germany (www.centronic-gmbh.com) manufactured the zinc and copper test kits while Agape Diagnostics Ltd India (www.agappe.com) manufactured the test kits for albumin and total protein.

3.3 Quality Control

Duplicate tubes of sample, control and standard solution were used for analysis of the biochemical parameters. High, normal and low levels quality control sera from Randox Laboratories Company United Kingdom (www.randox.com) were used both as intra-batch and inter-batch controls.

3.4 Statistical Analysis

All statistical analysis were done using SPSS version 17, 2008 (www.spss.com). Z-test for independent samples was used for comparison of means between the evaluated groups. Chi-square was used to test for significance between the categorical variables. Regression was used to show the relationships between parasite density and serum levels of the minerals. Binary logistic regression was used to show the effects of temperature, serum zinc and copper level on malaria. A p-value of 5% as a test of significance was adopted. The results were expressed as means, standard deviation and percentages.

4. RESULTS AND DISCUSSION

Table 1 shows the malaria infection by age according to the various hospitals. The overall prevalence of childhood malaria was 51% while that of congenital and neonatal malaria were 2.0% (13/600) and 1.5% (9/600) respectively.

Table 2 shows the serum zinc and copper levels by gender of all the subjects. Both Zn and Cu were significantly higher in males ($p<0.05$).

A comparison of the serum zinc and copper levels of the case and control subjects is shown in Table 3. Serum zinc and copper levels were relatively higher in children with malaria though, this was not significant. The Cu/Zn ratio of the patients and controls were 0.56 and 0.57 respectively.

Serum levels of zinc and copper as well as temperature had no significant effect on malaria ($p=0.485$, 0.471 and 0.067 respectively). These were not predictors of malaria ($p>0.05$) (Table).

The relationship between parasite density and serum levels of zinc and copper is shown in Table 5. There was a significant positive relationship between parasite density, and serum zinc and copper levels ($p<0.05$). The relationship was stronger between parasite density and zinc (beta=0.101) than it was with copper (beta=0.042).

Serum albumin level was relatively lower in children with malaria, though, this was not significant ($p>0.05$) (Table 6).

Table 1. Hospital-specific malaria infection by age

Hospitals		Age categories							Total
	MI	0-7days	8-28 days	29 days-11 months	1-5years	6-10 years	11-15 years	16-20 years	
BE PHC	Test	0(0.0%)	2(100.0%)	13(35.1%)	28(46.7%)	26(57.8%)	5(55.6%)	6(54.5%)	80(48.8%)
	Control	0(0.0%)	0(0.0%)	24(64.9%)	32(53.3%)	19(42.2%)	4(44.4%)	5(45.5%)	84(51.2%)
	Total	0(100.0)	2(100.0%)	37(100.0%)	60(100.0%)	45(100.0%)	9(100.0%)	11(100.0%)	164(100.0%)
BC PHC	Test	4(28.6%)	4(80.0%)	17(33.3%)	9(18.8%)	10(38.5%)	4(36.4%)	1(14.3%)	113(69.8%)
	Control	10(71.4%)	1(20.0%)	34(66.7%)	39(81.2%)	16(61.5%)	7(63.6%)	6(85.7%)	49(30.2%)
	Total	14(100.0%)	5(100.0%)	51(100.0%)	48(100.0%)	26(100.0%)	11(100.0%)	7(100.0%)	162(100.0%)
BR PHC	Test	9(64.3%)	2(40.0%)	28(65.1%)	19(57.6%)	16(76.2%)	5(50.0%)	1(50.0%)	80(62.5%)
	Control	5(35.7%)	3(60.0%)	15(34.9%)	14(42.4%)	5(23.8%)	5(50.0%)	1(50.0%)	48(37.5%)
	Total	14(100.0%)	5(100.0%)	43(100.0%)	33(100.0%)	21(100.0%)	10(100.0%)	2(100.0%)	128(100.0%)
JUTH	Test	0(0.0%)	1(50.0%)	32(82.1%)	30(61.2%)	27(64.3%)	4(50.0%)	3(50.0%)	97(66.4%)
	Control	0(0.0%)	1(50.0%)	7(17.9%)	19(38.8%)	15(35.7%)	4(50.0%)	3(50.0%)	49(33.6%)
	Total	0(0.0%)	2(100.0%)	39(100.0%)	49(100.0%)	42(100.0)	8(100.0%)	6(100.0%)	146(100.0%)

Table 2. Serum zinc and copper levels by gender of all the subjects

Micronutrients (µg/dl)	Gender	No	Mean±SD	p- value
Zn	Male	287	251.10±129.098	0.001
	Female	313	215.63±136.43	
Cu	Male	287	138.70±102.561	0.059
	Female	313	124.55±79.839	

Table 3. Comparison of serum zinc and copper levels of the case and control subjects

Micronutrients (µg/dl)	Subjects	No.	Mean±SD	p-value
Zinc	Test	306	237.08±134.239	0.404
	Control	294	227.93±133.908	
Copper	Test	306	133.46±91.784	0.559
	Control	294	129.09±91.533	

Table 4. The effects of zinc, copper and temperature on malaria using binary logistic regression

Variables	B	S.E.	Wald	Df	Sig.	Exp(B)
Zn	.000	.001	.487	1	.485	1.000
Cu	.001	.001	.520	1	.471	1.001
Temp	.112	.061	3.362	1	.067	1.118
Constant	-4.231	2.234	3.587	1	.058	.015

S.E= standard error, B=coefficient, df= degree of freedom

Table 5. Regression model of zinc, copper and parasite density

Model	B	Std. Error	Beta	Z	Sig.
(Constant)	148.088	22.677		6.530	.000
Cu	.074	.100	.042	.739	.460
Zn	.121	.069	.101	1.762	.079

B: coefficient, Z: calculated z

Table 6. Comparison of serum levels of albumin and globulin in the case and control subjects

Protein fraction	Subjects	No.	Mean ± SD	p-value
Albumin	Case	306	3.934±0.661	P=0.658
	Control	294	4.056±0.472	
Globulin	Case	306	3.719±0.890	P=0.235
	Control	294	2.874±0.420	

The overall prevalence of childhood malaria in this study was 51% and that of congenital malaria was 2.0%.Malaria is a life threatening morbidity. *P. falciparum* infection is lethal leading to different complications. From the study, serum zinc and copper levels were relatively higher in children with malaria than in their non-malaria counterparts, though, this was not significant. This implies that malaria has no significant effect on serum levels of Zn and Cu. In addition, the Cu/Zn ratio of the patients compared well with the controls (0.56 and 0.57 respectively). The serum levels of these trace minerals had no significant effect on

plasmodium falciparum infection, thus; they were not predictors of the infection. These imply that supplementation of these micronutrients in children suffering from malaria may not be necessary in solving the menace of malaria morbidity. This is in consonance with the finding by Nidhi et al. [31] but in disagreement with the reports of some studies that have shown reduced serum levels of zinc in malaria infection to non-malaria controls (Das et al, 1996) [19].

Zinc and copper are ubiquitous essential trace elements found in almost all body tissues and fluids. They are necessary for the survival of all forms of life [32]. *Plasmodium falciparum* parasite thrives and multiplies using nutrients from the host [33]. In view of this, one would expect the serum levels of zinc and copper to be low in children with malaria compared with the uninfected group. These micronutrients were rather found to be higher in children with malaria. This finding could be explained from the fact that the liver is the point of call in the early stage of plasmodium infection [33]. The liver is the site for synthesis of body's carrier molecules such as albumin, ceruloplasmin. Therefore, the infection of the liver by the parasite could lead to decrease activity of the liver cells thereby resulting in reduced synthesis of these carrier molecules [33]. This may be the reason for the relatively low albumin concentration obtained in this study among children with malaria. In addition, illnesses such as malaria may result in increased catabolism of albumin, ceruloplasmin and other nitrogen containing molecules thereby leading to nitrogen loss as was earlier noted by Adeleke [33]. With the resultant decrease in the synthesis of these carrier molecules and possible increased catabolism, some of the zinc and copper in circulation will be unbounded and be in the free state, thereby leading to increased levels of these free elements [33].

Furthermore, in order to manage and regulate the amount of trace metals circulating in blood and stored in tissues, the body performs different homeostatic measures which include incorporating into the blood from cellular stores if blood levels are depleted [11]. Thus, the slightly increased zinc and copper levels obtained in this study among malaria infected children may also, be due to body's homeostatic system mobilizing from the cellular stores of zinc and copper, into peripheral circulation in order to make up for the possibly marginal zinc and copper depletion resulting from parasite usage to establish infection and by the host cells to fight infection [34]. Thus, in order to compensate for these possibly low levels, the system draws from body stores of these elements into the circulation, thereby leading to the slightly increased levels of the free elements in plasma of patients.

These micronutrients in their free state may encourage the virulence of pathogens [35]. Parasitaemia was found to significantly increase as zinc and copper levels increased. This implies that increase in free zinc and copper levels in children with malaria may increase the risk of multiplication of the parasite and intensity of malaria. This is similar to the findings by Boijker and colleague (2006) [36], who reported that increase in the intensity of exposure to *Plasmodium falciparum* leads to an increase in the infection. This suggests that there is a need for prompt; correct diagnosis and management of children with malaria infection to avoid the eminent morbidity and mortality associated with *Plasmodium falciparum* infection due to increasing parasitaemia, derangement in levels of biochemical parameters and the resultant complications.

Finally, zinc being in higher concentration in the males may be a physiologic means for the body's readiness for proper development of secondary sexual characters since zinc is needful in prostate functions.

5. CONCLUSION

There is no significant difference in the serum zinc and copper levels of children with or without malaria. This implies that there may not be need to supplement children with malaria with zinc or copper, as this may not be necessary in solving the menace of malaria morbidity.

ETHICAL CONSIDERATIONS

The Medical and Health Ethics Committee of Jos University Teaching Hospital and Primary Health Care Centers approved this study.

ACKNOWLEDGEMENTS

We acknowledge the entire staff of the Department of Paediatrics University of Jos Teaching Hospital, the primary health care centers and Kolac Medical and Research Laboratories Jos, Nigeria for their tremendous assistance to the success of this work.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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