

Original Research Article

Serum zinc and copper levels in children with Plasmodium falciparum infection in Nigeria

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.

Objective

To determine the serum zinc and copper levels in children with or without malaria.

Method

The blood samples of 600 children aged zero to 18 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 (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. Jos University Teaching Hospital had the

highest prevalence of malaria. All the hospitals recorded the highest rate of malaria among the 29days to 11.9months age group with the exception of Bukuru Express Primary Health Care Center. 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 ($p=0.000$). 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,

Introduction

Malaria in children is a major public health problem worldwide, with an estimated 2 million children dying of malaria yearly, primarily because of *Plasmodium falciparum* and its complications [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

50 plays a role in wound healing [7], protein synthesis, DNA synthesis and cell division [8]. It
 51 supports normal growth in childhood [9]. Maintaining a proper balance of copper and zinc is
 52 important as excess Zn impairs Cu absorption [10]. The ratio of Cu to Zn is clinically more
 53 important than the concentration of either of them [11]. Copper is essential also for
 54 maintaining the strength of the skin, blood vessels, epithelial and connective tissues. It plays
 55 a role in production of haemoglobin, myelin, and melanin [10].

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

60 The metabolic pathways of plasmodium require several enzyme cofactors such as iron-
 61 sulphur clusters [14] and possibly zinc and copper, this may lead to deficiency of these
 62 nutrients in the host. Zn and Cu deficiencies are associated with changes in cellular function,
 63 changes in growth motor development, behaviour and cognitive functions [15]. These
 64 alterations in cellular and humoral functions may increase host susceptibility to *P. falciparum*
 65 [16,17].

66 Several studies [18, 19,20] have reported that zinc is low in children with malaria in
 67 developing countries. Chimah et al, 2012 [21] in a study in Benin city, South- South Nigeria
 68 reported that patients with severe malaria presenting with hyperpyrexia and
 69 hyperparasitaemia tended to have lower levels of serum zinc. The authors also reported a
 70 negative trend between parasite density and serum zinc levels. Muller et al, 2001[22] reported
 71 that zinc supplementation has no effect on morbidity from falciparum malaria in children in
 72 rural West Africa. In Tanzania, Hofs et al, 2011[23] concluded that supplementing young

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

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

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86 **Materials and method**

87 *Study design and setting*

88 This was an analytical case- control study conducted between August and November
89 2011 in various hospitals in Jos, North Central Nigeria. The hospitals were: Jos University
90 Teaching Hospital (JUTH) which is a reference hospital, Bukuru Central (BC), Bukuru
91 Express (BE)and Rayfield (RF) Primary Health Care Centers all in the urban areas of the
92 state.

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95 *Study population*

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

100 Children who met the study's inclusion criteria were recruited for the study. The inclusion
101 criteria were: (1) children who came to the health centers for the purpose of receiving
102 treatment or solely to participate in the study. (2) Consent of children or parent/ caregiver. (3)
103 Presence or absence of clinical signs/symptoms of malaria such as: fever, cough, diarrhea,
104 pallor, jaundice, vomiting, chill and others. (4) Children without any history of treatment
105 with anti-malaria drugs in the past 1 to 2 weeks and (5) Children aged 0 to 18 years old. The
106 coverage of the total age range (0-18 years) for biological definition of a child (UNESCO)
107 [24] gives this study a relative edge over several studies that limit similar studies to under
108 five years. Children with positive blood slides for malaria parasite were grouped as case
109 while the negative ones were the controls.

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

114 Children or Parents/guardians of eligible children gave written informed consent to allow
115 their children to participate in the study. Participants were consequently recruited into the
116 study until the sample size of 600 was reached. A qualified health personnel used a pre-tested
117 English questionnaire to collect patient's demographic information and the reasons why
118 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 $\geq 37.5^{\circ}\text{C}$. Children that were positive for malaria within 0-7days and 8-28days of age were grouped as congenital and neonatal malaria group respectively [25].

Ethical Considerations

The University of Jos Teaching Hospital Ethical Clearance Committee and the Director of Primary Health Care Bukuru gave the approval for this study.

Methodology

Taking aseptic precautions, 2ml of blood from venipuncture using 23 gauge sterile needle, was collected both from case and control groups into metal-free plain tubes and EDTA anti-coagulated tubes for analysis of the biochemical parameters and malaria diagnosis respectively. The samples collected in plain tubes were centrifuged for 5 minutes at 3000rpm using bench centrifuge, serum was obtained and preserved at $2-8^{\circ}\text{C}$ in sterile deionised plain vials. Analysis of the biochemical parameters was carried out within 7 days of sample collection by experienced clinical biochemists.

144 *Malaria diagnosis*

145 Double slides of thick and thin blood films of respective subject were made from
 146 EDTA anti-coagulated blood less than 1 hour after the blood was drawn. Blood films were
 147 air-dried without convection for one hour and stained with 30% freshly prepared Giemsa
 148 stain.

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

151 Giemsa stained thick and thin blood films were examined for malaria parasite using x 100
 152 (oil immersion) objective by an experienced medical microbiologist in the Paediatrics
 153 Research Laboratory of the University who was involved in the study. This served as the
 154 internal quality control. The slides were also cross-read by an experienced microscopist who
 155 was not otherwise involved in the study (independent reader) this served as the external
 156 quality control. The degree of variation in the results was determined and subjected to
 157 statistical analysis at 95% confidence limit to test for significance using SPSS version 17,
 158 2008(www.spss.com). Malaria diagnosis was based on identification of asexual stages of
 159 *Plasmodium falciparum* on the thick blood smears. Film was reported as ‘malaria parasite not
 160 seen’ i.e. negative after examining about 100 fields. Thin films were used to identify species
 161 and stages of Plasmodium or other blood -borne pathogens (WHO 2007) [26]. Parasite
 162 density was by the number of parasites per microlitre of blood (thick film) method [26]. The
 163 number of asexual parasitic forms (trophozoites and schizonts) present in as many
 164 microscopic fields as possible necessary to count 200 leucocytes was recorded. The standard
 165 value of 8000WBC/ μ l was assumed as a multiplier in the parasitaemia expression below:

166 $\text{Parasite}/\mu\text{l of blood (parasite density)} = N \times \text{total WBC counts}/\mu\text{l (8000)}$

167 -----

168 Leucocyte count (200)

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

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 (Johnsen and Eliasson ,1987)[27] , Didrom PAESA (Ade et al, 1989)[28],the Bromocresol green (Tietz 1986)[29] and Direct Biuret (Layne, 1957)[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.

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.

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.

RESULT

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.

All the hospitals recorded the highest rate of malaria infection among the 29days to 11.9months age group with the exception of Bukuru Express PHC which recorded it at 1-5 years age group. It was only Bukuru Express and Bukuru Rayfield PHCs that had participants of 0-7 day's age group. The prevalence of congenital and neonatal malaria in Bukuru Central were 2.5% while in Bukuru Rayfield PHC they were 7.0% and 1.6% respectively, this center also had the lowest prevalence of malaria (1.5% at 1-5 years age group) while JUTH had the highest (21.9% at 29 days to 11.9months age group).

Malaria infection by sex in the various hospitals had been reported in our previous study [31].

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

216 with malaria though, this was not significant. The Cu/Zn ratio of the patients and controls
217 were 0.56 and 0.57 respectively.

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

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

228 Serum albumin level was relatively lower in children with malaria, though, this was
229 not significant ($p>0.05$) (Table6).

230 **Table 1: Hospital-Specific Malaria infection by Age**

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Age categories									
Hospitals	MI	0-7days	8-28days	29days-11 months	1-5years	6-10years	11-15years	16-20years	Total
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%)

Table2. 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	

Table3. 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	

Table4. 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

Table5. 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

Table6. Comparison of serum levels of albumin and globulin in the case and control subjects.

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

Discussion

The overall prevalence of childhood malaria in this study was 51% and that of congenital malaria was 2.0% (these had been reported in the authors previous study [31].

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, 2011 [32] 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 [33]. *Plasmodium falciparum* parasite thrives and multiplies using nutrients from the host (Adeleke, 2007) [34]. 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 (Adeleke, 2007)[34]. 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 (Adeleke, 2007) [34]. 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,2007,[34]. 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(Adeleke, 2007)[34].

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 (Shanker & Prasad,1998)[35]. Thus, in order to compensate for this 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 (Beales 1991)[36]. 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 Bojker et al, 2006[37], 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.

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346 **Conclusion**

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

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352 **Conflict of interest**

353 The authors declare that they have no conflict of interest.

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358 **References**

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