

Original Research Article

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

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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 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 29 days to 11.9 months 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

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

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64 ▲ **Keywords:** *Plasmodium falciparum*, childhood malaria, serum zinc, copper, Nigerian,▲

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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 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, 2012 [21] in a study in Benin city, South- South Nigeria reported that patients with severe malaria presenting with hyperpyrexia and hyperparasitaemia tended to have

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108 lower levels of serum zinc. The authors also reported a negative trend between parasite density and
109 serum zinc levels. Muller et al, 2001[22] reported that zinc supplementation has no effect on morbidity
110 from falciparum malaria in children in rural West Africa. In Tanzania, Hofs et al, 2011[23] concluded
111 that supplementing young Tanzania children with zinc either alone or with other multi-nutrients does
112 not protect against malaria. ▲

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

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▲ MATERIALS AND METHOD ▲

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▲ Study design and setting ▲

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125 This was an analytical case- control study conducted between August and November 2011

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126 in various hospitals in Jos, North Central Nigeria. The hospitals were: Jos University Teaching
127 Hospital (JUTH) which is a reference hospital, Bukuru Central (BC), Bukuru Express (BE)and
128 Rayfield (RF) Primary Health Care Centers all in the urban areas of the state. ▲

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136 Study population

137 The study was conducted among children attending the Emergency Paediatric Unit, Special Care

138 Baby Unit, Paediatric Outpatient Department including the immunization unit of JUTH and the primary

139 health care centers (PHCs) including those who came to the PHCs solely for the purpose of the study.

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140 Children who met the study's inclusion criteria were recruited for the study. The inclusion criteria were:

141 (1) children who came to the health centers for the purpose of receiving treatment or solely to

142 participate in the study. (2) Consent of children or parent/ caregiver. (3) Presence or absence of

143 clinical signs/symptoms of malaria such as: fever, cough, diarrhea, pallor, jaundice, vomiting, chill

144 and others. (4) Children without any history of treatment with anti-malaria drugs in the past 1 to 2

145 weeks and (5) Children aged 0 to 18 years old. The coverage of the total age range (0-18 years) for

146 biological definition of a child (UNESCO) [24] gives this study a relative edge over several studies that

147 limit similar studies to under five years. Children with positive blood slides for malaria parasite were

148 grouped as case while the negative ones were the controls.

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150 Children with any other diagnosed illness apart from malaria, children receiving zinc or copper

151 supplements, children older than 18 years, preterm babies and non-consenting

152 individuals/parents/caregivers were excluded from the study.

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153 Children or Parents/guardians of eligible children gave written informed consent to allow their

154 children to participate in the study. Participants were consequently recruited into the study until the

155 sample size of 600 was reached. A qualified health personnel used a pre-tested English questionnaire

156 to collect patient's demographic information and the reasons why he/she was brought to the health

157 center. The axillary body temperature was measured using a digital clinical thermometer. Fever was

158 defined as body temperature $\geq 37.5^{\circ}\text{C}$. Children that were positive for malaria within 0-7 days and 8-

159 28 days of age were grouped as congenital and neonatal malaria group respectively [25].

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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 5minutes at 3000rpm using bench centrifuge, serum was obtained and preserved at 2-8°C in sterile deionised plain vials. Analysis of the biochemical parameters was carried out within 7 days of sample collection by experienced clinical biochemists.

Malaria diagnosis

Double slides of thick and thin blood films of respective subject were made from EDTA anti-coagulated blood less than 1 hour after the blood was drawn. 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

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192 reported as 'malaria parasite not seen' i.e. negative after examining about 100 fields. Thin films were
 193 used to identify species and stages of Plasmodium or other blood -borne pathogens (WHO 2007) [26].
 194 Parasite density was by the number of parasites per microlitre of blood (thick film) method [26]. The
 195 number of asexual parasitic forms (trophozoites and schizonts) present in as many microscopic fields
 196 as possible necessary to count 200 leucocytes was recorded. The standard value of 8000WBC/ μ l was
 197 assumed as a multiplier in the parasitaemia expression below:

198 $\text{Parasite}/\mu\text{l of blood (parasite density)} = N \times \text{total WBC counts}/\mu\text{l (8000)}$

199
$$\frac{\text{Leucocyte count (200)}}{\text{Where N= number of asexual parasitic forms present in as many microscopic fields as possible to count 200 leucocytes.}}$$

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

204 Determination of serum zinc, copper, albumin, total protein

205 Chemistry auto-analyzer, model; Mispa Excel version 2.1e lite, (Open System) 2006 , by
 206 Mispa Biosystems India was used to colorimetrically analyze the biochemical parameters. Serum zinc
 207 , copper , albumin and total protein(globulin = total protein- albumin level) were respectively
 208 determined using 5-Br-PAPS (Johnsen and Eliasson ,1987)[27] , Didrom PAESA (Ade et al,
 209 1989)[28],the Bromocresol green (Tietz 1986)[29] and Direct Biuret (Layne, 1957)[30] methods.
 210 Centronic GmbH Germany (www.centronic-gmbh.com) manufactured the zinc and copper test kits
 211 while Agape Diagnostics Ltd India (www.agappe.com) manufactured the test kits for albumin and total
 212 protein.

213 Quality control

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

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218 Statistical Analysis

219 All statistical analysis were done using SPSS version 17, 2008(www.spss.com). Z-test for
220 independent samples was used for comparison of means between the evaluated groups. Chi-square
221 was used to test for significance between the categorical variables. Regression was used to show the
222 relationships between parasite density and serum levels of the minerals. Binary logistic regression
223 was used to show the effects of temperature, serum zinc and copper level on malaria. A p-value of
224 5% as a test of significance was adopted. The results were expressed as means, standard deviation
225 and percentages.

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228 RESULT AND DISCUSSION

229 Table 1 shows the malaria infection by age according to the various hospitals. The overall
230 prevalence of childhood malaria was 51% while that of congenital and neonatal malaria were 2.0%
231 (13/600) and 1.5% (9/600) respectively.

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232 Overall, all the hospitals recorded the highest rate of malaria infection among the 29days to
233 11.9months age group with the exception of Bukuru Express PHC which recorded it at 1-5 years age
234 group. It was only Bukuru Central- and Bukuru Rayfield PHCs that had participants of 0-7 day's age
235 group. The prevalence of congenital and neonatal malaria in Bukuru Central were 2.5% while in
236 Bukuru Rayfield PHC they were 7.0% and 1.6% respectively, this center also had the lowest
237 prevalence of malaria (1.5% at 1-5 years age group) while JUTH had the highest (21.9% at 29 days
238 to 11.9months age group).

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

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

244

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

248

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

252

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

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

259 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%)

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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	

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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	

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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

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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

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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	

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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].

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506 Zinc and copper are ubiquitous essential trace elements found in almost all body
507 tissues and fluids. They are necessary for the survival of all forms of life [33]. *Plasmodium*
508 *falciparum* parasite thrives and multiplies using nutrients from the host (Adeleke, 2007) [34].
509 In view of this, one would expect the serum levels of zinc and copper to be low in children
510 with malaria compared with the uninfected group. These micronutrients were rather found to
511 be higher in children with malaria. This finding could be explained from the fact that the
512 liver is the point of call in the early stage of plasmodium infection (Adeleke, 2007)[34]. The
513 liver is the site for synthesis of body's carrier molecules such as albumin, ceruloplasmin.
514 Therefore, the infection of the liver by the parasite could lead to decrease activity of the liver
515 cells thereby resulting in reduced synthesis of these carrier molecules (Adeleke, 2007) [34].
516 This may be the reason for the relatively low albumin concentration obtained in this study
517 among children with malaria. In addition, illnesses such as malaria may result in increased
518 catabolism of albumin, ceruloplasmin and other nitrogen containing molecules thereby
519 leading to nitrogen loss as was earlier noted by Adeleke,2007,[34]. With the resultant
520 decrease in the synthesis of these carrier molecules and possible increased catabolism, some
521 of the zinc and copper in circulation will be unbounded and be in the free state, thereby
522 leading to increased levels of these free elements(Adeleke, 2007)[34].

523 Furthermore, in order to manage and regulate the amount of trace metals circulating in
524 blood and stored in tissues, the body performs different homeostatic measures which include
525 incorporating into the blood from cellular stores if blood levels are depleted [11]. Thus, the
526 slightly increased zinc and copper levels obtained in this study among malaria infected
527 children may also, be due to body's homeostatic system mobilizing from the cellular stores of
528 zinc and copper, into peripheral circulation in order to make up for the possibly marginal zinc
529 and copper depletion resulting from parasite usage to establish infection and by the host cells

530 to fight infection (Shanker & Prasad,1998)[35]. Thus, in order to compensate for this possibly
531 low levels, the system draws from body stores of these elements into the circulation, thereby
532 leading to the slightly increased levels of the free elements in plasma of patients.

533 These micronutrients in their free state may encourage the virulence of pathogens
534 (Beales 1991)[36]. Parasitaemia was found to significantly increase as zinc and copper levels
535 increased. This implies that increase in free zinc and copper levels in children with malaria
536 may increase the risk of multiplication of the parasite and intensity of malaria . This is similar
537 to the findings by Bojker et al, 2006[37], who reported that increase in the intensity of
538 exposure to *Plasmodium falciparum* leads to an increase in the infection. This suggests that
539 there is a need for prompt; correct diagnosis and management of children with malaria
540 infection to avoid the eminent morbidity and mortality associated with *Plasmodium*
541 *falciparum* infection due to increasing parasitaemia, derangement in levels of biochemical
542 parameters and the resultant complications.

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

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549 **CONCLUSION**

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

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554 **CONFLICT OF INTEREST**

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The authors declare that they have no conflict of interest.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

CAO conceived and designed the study, conducted some of the laboratory investigations and drafted the manuscript. MDS supervised the study and reviewed the manuscript. ACO conducted some of the laboratory investigations and reviewed the manuscript.

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.

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