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The Relationship between zinc and copper in children with malaria

[1]Caroline A. Okoli and [2]Mariam Solomon
[1]Research Laboratory Unit, Department of Paediatrics University of Jos, Jos, Nigeria
[2]Department of Biochemistry, University of Jos, Jos, Nigeria
Email: caroalph@yahoo.com, danladis2003@yahoo.com

ABSTRACT

BACKGROUND
Zinc (Zn) and copper (Cu) are among the most important trace elements that are required for proper growth and health.

OBJECTIVE
To determine the effect Zn and Cu have on each other in children with malaria in order to deduce whether the serum levels of zinc and copper could play a role in the pathogenesis of malaria.

HYPOTHESIS
There will be a significant negative relationship between zinc and copper in children with malaria.

METHOD
The blood samples of 600 children aged zero to18years from various hospitals in Jos were analyzed for; malaria parasite (MP) using Giemsa staining technique and zinc and copper using colorimetric method.

RESULT
There was a non-significant positive relationship between zinc and copper in children with malaria (p=0.607). The strength of the relationship was beta=0.030. There was a significant negative relationship between zinc and copper in the control children (p=0.003). The strength of the relationship was beta =-0.159. Using the beta function of the model to compare the strength of the effect of the micronutrients on each other, it was discovered that zinc and copper have a stronger effect in the control subjects (beta=-0.159) than in the parasitaemic children (beta=0.030). There was a significant positive relationship between parasite density and serum zinc and copper levels (p=0.000).

CONCLUSION

Serum zinc and copper do not have significant effect on each other in the pathogenesis of malaria in children. However, it may be necessary to monitor the levels of these minerals in such patients.

Keywords: Zinc, Copper, Relationship, Malaria, Children

INTRODUCTION

Zinc (Zn) and copper (Cu) are among the most important trace elements that are required for proper growth and health. They are involved in numerous aspects of cellular metabolism and immune functions [1]. They are required for the catalytic function of several enzymes and act as antioxidants [2, 3]. Zn and Cu play a critical role in host-pathogen interactions [4]. Zn also plays a role in wound healing [5], protein synthesis, DNA synthesis and cell division [6] .Zn is required for each step of cell cycle in microorganisms [4]. Copper is essential also for maintaining the strength of the skin, blood vessels, epithelial and connective tissues. It is an essential component of cuprozinc superoxide dismutase, an enzyme in the erythrocytes essential for host defense as well as parasite growth [7]. It plays a role in production of haemoglobin, myelin, and melanin [8]. However, copper is well documented to induce several toxic effects in the body, when elevated. Because copper is a pro-oxidant when free and unbound, it can quickly generate free radicals. Role of zinc in cases of acute respiratory tract infections, chronic diarrhea and severe protein energy malnutrition has been repeatedly proven in multiple studies [9] but there are only very few studies on zinc and copper status in cases of malaria in children. The metabolic pathways of plasmodium require several enzyme cofactors such as iron-sulphur clusters [10] 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 [11]. These alterations in cellular and humoral functions may increase host susceptibility to *P. falciparum* [12, 13]. Maintaining a proper balance of copper and zinc is important as excess Zn impairs Cu absorption [8]. They are antagonists. Copper and zinc levels are regulated by metallothionein, a short linear protein composed of 61 amino acid units synthesized in the liver. When this protein fails to perform its necessary functions, abnormal levels of nutrient metals such as zinc, copper can result. In this study, we

determined the effect of zinc and copper on each other in children with malaria in order to deduce whether the serum levels of zinc and copper could play a role in the pathogenesis of malaria.

MATERIALS AND METHODS

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.

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) [14] 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 18years, 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 consequently recruited into the study until the sample size of 600 was reached. 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 ≥ 37.5°C.

Ethical Considerations

The University of Jos Teaching Hospital Ethical Clearance Committee (Reference Number JUTH/DCS/ADM/127/XIX/4688) 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 guage 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^{O}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) objectives 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)[15]. Parasite density was by the number of

parasites per microlitre of blood (thick film) method[15]. 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:

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

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

DETERMINATION OF SERUM ZINC AND COPPER,

Serum zinc and copper were colorimetrically determined using 5-Br-PAPS (Johnsen and Eliasson ,1987)[16] and Didrom PAESA (Ade et al, 1989)[17]methods respectively. Centronic GmbH Germany (www.centronic-gmbh.com) manufactured test kits.

Quality control

Duplicate tubes of sample, control and standard solution were used for analysis of Zn and Cu. 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). A p-value of 5% as a test of significance was adopted. The results were expressed as means, standard deviation and percentages.

RESULTS

Table1 shows the serum zinc and copper levels by age of the case and control subjects. Serum zinc level was relatively higher in the parasitaemic children aged zero to 9.9years than in the control. The reverse was the case in children aged 10 to 18.9years. The effect of zinc and copper on each other in children with malaria using Pearson correlation is shown in Table 2. There was a non-significant positive relationship between zinc and copper in children with malaria (p=0.607).

Table2b shows the effect of zinc and copper on each other in children with malaria using a regression model. The strength of the relationship was beta=0.030. The effect of zinc and copper on each other in the control subjects using Pearson correlation is shown in Table 3. There was a significant negative relationship between zinc and copper in the control children (p=-0.003). Thus, in apparently healthy children an increase in one of the elements leads to a decrease in the other. Table 3b shows the effect of zinc

and copper on each other in the control subjects using a linear regression model. The strength of the relationship was beta =-0.159. Using the beta function of the model to compare the strength of the effect of the micronutrients on each other, it was discovered that zinc and copper have a stronger effect in the control subjects (beta=0.159) than in the parasitaemic children (beta=0.030). Table 4 shows serum zinc levels by parasite density using correlation. The highest parasite density (901-1000) was at zinc level 101-200 µg/dl while the parasite count range with the highest frequency (101-200parasites/µl) was at zinc level 101-300µg/dl. There was a significant positive relationship between parasite density and serum zinc level (p=0.000). For Cu the highest parasite density (801-900) was at copper level 0-100µg/dl while the parasite count range with the highest frequency (101-200parasites/µl) was at Cu concentration 0-200µg/dl. There was a significant positive relationship between parasite density and serum copper level (p=0.000) (Table 5).

Table1. Serum Zinc and Copper Levels by Age of the Case and Control Subjects

Age range	No.	Case-Zinc(µg/dl) (Mean±SD)	Subjects Copper (µg/dl) (Mean±SD)	Control-Zinc(µg/dl) (Mean±SD)	Subjects Copper (µg/dl) (Mean±SD)
0-4years	402	249.68±131.668	131.44 ± 90.152	229.39± 122.45	132.10 ± 116.489
5-9years	134	224.76±144.412	123.91 ±77.341	203.72 ± 113.893	144.55± 68.023
10-14years	38	255.93± 91.794	177.32 ±129.156	258.33± 150.965	106.24± 42.110
14-19years	26	218.50± 73.832	144.68 ± 56.250	226.72± 109.938	115.12± 85.883
Total	**600**	**237.08±134.239**	**133.46±91.784**	**227.93±133.908**	**129.09±91.533**

Table 2: The Effect of Zinc and Copper on Each Other in children with malaria using Pearson Correlation

Pearson correlation	Zn(µg/dl)	Cu(µg/dl)	p-value
Zn(µg/dl)	1	0.030	0.607
Cu(µg/dl)	0.03	1	
No.	306	306	

Table 2b: The Effect Zinc and Copper on Each Other in children with malaria using a Linear Regression

Model	B	Std. Error	Beta	Z	Sig.
(Constant)	128.677	10.675		12.054	.000
Zn	.020	.039	.030	.515	.607
Dependent variable: Cu					
Constant)	231.317	13.574		17.041	.000
Cu	.043	.084	.030	.515	.607
Dependent Variable: Zn					

B: coefficient

z: calculated z

Table 3: The Effect of Zinc and Copper on Each Other in the Control Subjects using Correlation

Pearson Correlation		Cu	Zn	Sig
	Cu	1.000	-.159	-0.003
	Zn	-.159	1.000	
	N	294		

Table 3b: The Effect Zinc and Copper on Each Other in the Control Subjects using a linear Regression

Model	B	Std. Error	Beta	z	Sig.
(Constant)	258.011	13.367		19.301	.000
Cu	-.233	.085	-.159	-2.757	.006
Dependent Variable: Zn					
(Constant)	153.907	10.435		14.749	.000
Zn	-.109	.039	.159	-2.757	.006
Dependent Variable: Cu					

B: coefficient

z: calculated z

Table 4: The Relationship between Serum Zinc Level and Parasite Density using Correlation

	Zinc Level (µg/dl)							
Parasite Density	001-100	101-200	201-300	301-400	401-500	501-600	601-700	Total
1-100	6	18	18	10	0	4	2	58
	10.3%	31.0%	31.0%	17.2%	.0%	6.9%	3.4%	100.0%
101-200	26	58	53	31	0	10	2	180
	14.4%	32.2%	29.4%	17.2%	.0%	5.6%	1.1%	100.0%
201-300	2	2	10	6	2	0	0	22
	9.1%	9.1%	45.5%	27.3%	9.1%	.0%	.0%	100.0%
301-400	2	4	4	2	0	0	0	12
	16.7%	33.3%	33.3%	16.7%	.0%	.0%	.0%	100.0%
401-500	0	2	2	0	0	0	0	4
	.0%	50.0%	50.0%	.0%	.0%	.0%	.0%	100.0%
501-600	0	2	2	6	0	4	0	14
	.0%	14.3%	14.3%	42.9%	.0%	28.6%	.0%	100.0%
601-700	0	0	2	0	0	0	0	2
	.0%	.0%	100.0%	.0%	.0%	.0%	.0%	100.0%
801-900	0	0	0	0	0	2	0	2
	.0%	.0%	.0%	.0%	.0%	100.0%	.0%	100.0%
901-1000	0	4	0	0	0	0	0	4
	.0%	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
Total	36	90	91	55	2	20	4	298
	12.1%	30.2%	30.5%	18.5%	.7%	6.7%	1.3%	100.0%
p-value =0.000								

Table 5: The Relationship between Serum Copper Levels and Parasite Density using Correlation

	Copper level (µg/dl)					
Parasite Density	001-100	101-200	201-300	301-400	401-500	Total
1-100	22	30	4	0	2	58
	37.9%	51.7%	6.9%	.0%	3.4%	100.0%
101-200	63	93	16	2	10	184
	34.2%	50.5%	8.7%	1.1%	5.4%	100.0%
201-300	16	2	4	0	0	22
	72.7%	9.1%	18.2%	.0%	.0%	100.0%
301-400	4	6	0	2	0	12
	33.3%	50.0%	.0%	16.7%	.0%	100.0%
401-500	0	4	0	0	0	4
	.0%	100.0%	.0%	.0%	.0%	100.0%
501-600	4	4	4	0	0	12
	33.3%	33.3%	33.3%	.0%	.0%	100.0%
601-700	0	2	0	0	0	2
	.0%	100.0%	.0%	.0%	.0%	100.0%
801-900	2	0	0	0	0	2
	100.0%	.0%	.0%	.0%	.0%	100.0%
901-1000	0	4	0	0	0	4
	.0%	100.0%	.0%	.0%	.0%	100.0%
Total	111	145	28	4	12	300
	37.0%	48.3%	9.3%	1.3%	4.0%	100.0%

p-value=0.000

DISCUSSION

Zinc and copper are fundamental anti-oxidant elements able to reduce reactive oxidant factors generated in the course of infection in an organism. The result of this study showed that zinc in control subjects had a negative correlation with copper. In other words, increase in one leads to a decrease in the other. This is in conformity with an already established fact by multiple researchers [5, 18]. This negative relationship may be due to the antagonistic nature of copper to zinc absorption and vice versa. High zinc in circulation lowers copper bioavailability and vice versa [18]. This implies that maintaining the proper dietary balance of copper along with zinc is important. Cu can act as an antioxidant and a pro-oxidant. As a pro-oxidant, it can damage cell walls, interact with genetic materials and contribute to development of a number of health problems and diseases. In children with malaria, there was not a negative relationship between zinc and copper. This implies that an increase in serum Zn level does not lead to a decrease in serum Cu level and vice versa in children with malaria. Both minerals were also, relatively higher in the parasitaemic children. Thus, it is necessary to monitor the levels of these minerals in children with malaria because, though, the sera levels had no effect on the pathogenesis of malaria; copper in its pro-oxidant state could lead to increased oxidative stress in these children thereby worsening the ill health condition of the patients. Our report is similar to that of Baloch and colleagues, 2011[19]. The authors reported an increased serum copper concentration in malarial patients with comparison to the normal subjects. Our finding negates that of Melaine et al, 2010 [20] who reported significantly decreased zinc and increased copper levels in children with malaria compare to their aparasitaemic counterpart. Result from this study was actually contrary to our hypothesis, bearing in mind the antagonistic nature of these two elements on each other, a significant negative relationship was hypothesized to occur between zinc and copper in children with malaria. Presently, the reason for this result may not be conclusively established. However, it may be possible that the increase in serum copper level may be due to the host cells' bid to compensate for the low iron level evident in this infection. Copper is needed in iron transport in haemologbin. So host cells possibly used more of the copper to offset the imbalance in haemoglobin level (Osredlar and Sustar 2011)[5]. Furthermore, it is an already

established fact that copper and zinc levels are regulated by metallothionein, a short linear protein composed of 61 amino acid units. When this protein fails to perform its necessary functions, abnormal levels of nutrient metals such as zinc, copper can result [21]. Metallotheonein is synthesized in the liver, thus, the infection of the liver by the malaria parasites, possibly may have reduced the functionality of the liver, as well as the synthesis of metallothionein. This possible decease in the synthesis of this protein that regulates the levels of these micronutrients may have resulted to the increased levels of serum zinc and copper observed in children with malaria.

CONCLUSION

Serum zinc and copper do not have significant effect on each other in the pathogenesis of malaria in children. However, it may be necessary to monitor the levels of these minerals in such patients.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

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

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