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Chimah OU
Abhulimhen-Iyoha BI
Ibadin MO
Abiodun P O.

Levels of serum zinc and severity of malaria in under-fives: any relationship? Experience from Benin, Edo State.

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Chimah OU (✉)
Department of Paediatrics,
Federal Medical Center, Asaba,
Delta State, Nigeria.
E-mail: osatomah@yahoo.com
Tel: +234-8037198966.

Abhulimhen-Iyoha BI
Ibadin MO, Abiodun P O
Department of Child Health,
University of Benin Teaching
Hospital, Benin City Nigeria.

Abstract *Background:* It remains uncertain why some individuals infected with *Plasmodium falciparum* develop severe disease while others do not. This may be due to differences in immunological status of the individuals. Zinc levels may play some roles in the immune competence of such individuals as manifested in its effects on some clinical and laboratory parameters.
Objective: To determine the relationship between serum zinc levels and some laboratory and clinical parameters in under-five children with malaria.
Methods: The study was conducted at the University of Benin Teaching Hospital, Benin City between March and November 2003 and involved 640 under-five children. Of these, 384 children had parasitologically proven malaria while 256 were healthy controls. Determination of zinc in sera was done using the Atomic Absorption Spectrophotometry.
Results: Mean serum zinc level of patients with severe/complicated malaria (13.7 ± 9.4µmol/l) was significantly lower than that obtained in non-severe/uncomplicated malaria (17.1±8.0 µmol/l; t =3.67; p =0.000). There was a negative correlation between malaria parasite density and serum zinc levels (r = -0.101, $p < 0.05$). A similar trend was observed between zinc levels and degree of pyrexia (r = -0.120; $p < 0.05$).
Conclusion: Patients with severe malaria presenting with hyperpyrexia and hyperparasitaemia tended to have lower levels of serum zinc. Hypozincaemia is associated with severity of the disease either as a cause or effect.
Recommendation: Similar studies should be conducted in other centres to validate the findings.

Key Words: Serum Zinc, Malaria parasite density, Malaria, Under-fives.

Introduction

Malaria is a major cause of morbidity and mortality especially amongst children in tropical Africa.[1,2] In Nigeria, it remains the commonest cause of morbidity and mortality as well as the leading cause of childhood hospital admissions.[2,3] In rural areas in The Gambia, Greenwood *et al*[3] found malaria to be the commonest cause of childhood deaths in children aged under five years.

Despite various malaria control programmes, transmission of malaria in Nigeria is intense and stable all year round.[2] In the under-five population, infection rate for *Plasmodium falciparum* is said to rise from zero to two percent during the first three months of life to 90% by one year. Thereafter it persists at a high level during early childhood until school age when considerable immunity is acquired. In endemic areas, the under-five population, known to suffer more from its morbidity and mortality, is known to have low and unsustained levels of

immunity.[4] In recent times, studies have substantiated the need for zinc supplementation in boosting general immunity of individuals.[5]

Zinc is a crucial micronutrient required for normal development of the immune system and its maintenance. It is thus a critical modulator of host resistance to infections caused by viruses, bacteria, fungi and protozoa.[5,6] It is involved in both specific and non-specific aspects of immunity.[5,6] as buttressed by several studies on zinc supplementation in children with diarrhoea and pneumonia.[7]

It is largely unknown why some individuals infected with *Plasmodium falciparum* in endemic regions develop severe disease while others experience only mild cases.[8] Possible explanations for this scenario could include differences in immunological status of the individuals. Inadequate immunity results in rapid increase in the parasite load and development of complications.[9] In the early months of life the manifestations of malaria are usually mild, probably because of substantial immunity acquired from immune mothers. Mortality rates in hyperendemic areas are however, highest during the first two to four years of life when passive immunity has waned and acquired immunity is low.[8]

The exact role(s) of zinc in malaria immunity is not well documented. Suggested mechanisms include its immune modulating or antioxidant properties or a combination of both.[7] It is hypothesized that in severe disease values of serum zinc would be low either as a predisposition or effects. The study therefore seeks to evaluate the association between malaria severity and levels of serum zinc as seen in children with malaria.

Patients and Methods

The study was a case-control, cross-sectional one carried out at the Children Emergency Room (CHER), Paediatric Casualty Unit, Consultant Out-Patient Clinic (COPC) and Well Baby Clinic of the University of Benin Teaching Hospital (UBTH), Benin City between March and November 2003.

Subjects consisted of well-nourished children (six months to 59 months) with clinically and parasitologically proven malaria. Excluded from the study were children with suspected malaria without *Plasmodium falciparum* parasitaemia, and children with conditions associated with hypozincaemia like in overtly malnourished children (marasmus, marasmickwashiorkor, kwashiorkor),[10] children with sickle cell anaemia[11] and patients with proven concomitant bacterial infection or localizing signs of infections like bronchopneumonia, otitis media, viral exanthem or enanthem.[12] Controls comprised 256 apparently healthy children matched for age, sex and nutritional status seen in the paediatric COPC and Well Baby Clinic of UBTH. The presence of malaria parasite in an apparently healthy child excluded him/her from being recruited as control.

Patients were recruited consecutively into the study. Severity of malaria in each patient was classified based on WHO criteria[8] for severe/complicated malaria. A detailed history was obtained from accompanying parent(s) or guardian(s) for each child. This was followed by a thorough physical examination which was directed at excluding any focus of infection. Height of study subjects obtained were compared to the Jane's standards.[13] Nutritional status was assessed according to the Wellcome classification.[14] Protein Energy Malnutrition was defined as presence of any of the following: Wasting as weight for age less than 80% of expected with or without oedema, stunting as height or length less than 90% of expected for age or less than the third centile and mid upper arm circumference less than 13.5cm.

Findings including temperature readings were recorded in a proforma designed for the study. Ethical approval was obtained from the Ethics Committee of UBTH and written informed consent was obtained from parents/caregivers of subjects and controls.

Four millilitres of blood collected in a plain bottle was used for serum zinc analysis in accordance with methods described by Deric.[15] Other investigation done for some patients as determined by clinical assessment and suspected form of severe malaria include blood glucose level, urinalysis, urine microscopy and culture, cerebrospinal fluid analysis.

Diagnosis of malaria was confirmed on the basis of the presence of asexual forms (trophozoites/ring forms) of malaria parasites.[8,16] Malaria parasite density was determined according to methods described by Bruce Chwatts.[16]

The data collected were entered into Statistical Package for Social Science (SSPS) version 10 software. Student t-test was used for comparison of means and p value of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Of the 384 subjects recruited into the study, 196 (51.0%) were males while 188 (49.0%) were females (M: F ratio of 1.04:1). The mean age of study population was 26 ± 16.0 months while the mean weight and height were 12.0 ± 3.0 kg and 86.3 ± 16.3 cm, respectively. Severe/complicated malaria accounted for 149 (38.8%) cases, while non-severe/uncomplicated malaria was found in 235 (61.2%) cases (Table 1, Fig 1).]

Table 1: Mean serum zinc levels in patients and controls

	Mean Serum zinc ± SD (µmol/l)		
	(n=256)		
Controls	18.2 ± 7.0a		
Patients	All patients	Uncomplicated/ non-severe malaria	Complicated severe malaria
	(n=384)	(n=235) 61.1%	(n=149) 38.8%
	15.8± 8.7b	17.1 ± 8.0c	13.7 ± 9.4d

a vs b; t = 2.694, p = 0.007
a vs c; p > 0.05
c vs d; t = 3.67, p = 0.000

Among the 149 subjects who had severe/complicated malaria, cerebral malaria occurred in isolation in 3(2.0%) patients, anaemia in 16 (10.0%) and hyperpyrexia in 29 (19.5%). Others were isolated hyperparasitaemia, which were identified in 28 (18.8%) and persistent vomiting in 10 (6.7%). Some of the severe/complicated malaria diagnostic criteria also occurred in combination with others.

In non-severe/uncomplicated cases, the mean serum zinc was 17.1±8.0mol/L while in severe/complicated malaria patients; it was 13.7 ± 9.4mol/L. The difference between the mean values of non-severe /uncomplicated malaria and severe/complicated was statistically significant. (t = 3.67; p = 0.000).

Mean serum zinc levels in 142 (37.0%) patients with + malaria parasite density was 15.9 ±7.4mol/l (median 14.8mol/l), in 158 patients with 2+ malaria parasite density of 15.2 ± 8.9mol/l, while that for 82 (21.4%) patients with 3+ was 13.5 ± 9.8µmol/l. However, the mean serum zinc level in two patients with heavy malaria parasite density of 4+ was 15.9µmol/l. With the exception of the elevated mean serum zinc in the only two patients with heavy malaria parasitaemia, serum zinc tended to decline with increasing malaria parasitaemia.(r = -0.0101; p-value = 0.01; Table 2).

Table 2: Correlation of mean serum zinc levels and malaria parasite density in patients with malaria.

Parasite density	N	Serum zinc± SD (mol/l)
+	142	15.9 ± 7.4
++	158	15.2 ± 8.9
+++	82	13.5 ± 9.8
++++	2	15.9

Correlation (r) = - 0.101 p= 0.01

Anaemia, hyperparasitaemia and hyperpyrexia as isolated diagnostic criteria for severe/complicated malaria were found in 16(10%), 28(18.8%) and 29(19.5%) patients respectively. The corresponding serum zinc values in these groups of patients was respectively 19.3 ± 13.0mol/l, 13.6 ± 7.5mol/l and 16.0 ± 9.5mol/l. In comparison with values obtained in controls, mean serum zinc in individuals with isolated hyperpyrexia (t = 8.77; p-value = 0.000) and hyperparasitaemia (t = 9.56; p-value = 0.000) were significantly lower. (Table 3).

Table 3: Mean serum zinc values in patients with isolated and combined diagnostic criteria for severe malaria

	Isolated criteria	Multiple criteria	p-value
Anaemia n =16	19.3 ±13.0	18.4 ± 13.2	0.76
Hyperpyrexia n = 29	16. 0 ± 9.5	11.1 ± 7.1	0.01
Hyperparasitaemia n =28	13.6 ± 7.5	12.2 ±9.3	0.45
Controls N = 256	18.2 ± 7.0		

The multiple criteria include various combinations of anaemia, hyperpyrexia, acidosis, hypoglycaemia, jaundice.

Mean serum zinc correlated negatively with malaria parasite density (barring the 4+ malaria density) and hyperpyrexia implying that serum zinc tended to decline with increasing malaria parasite density (r = - 0.101; p = 0.01) and increasing body temperature (t= 0.120; p = 0.04). There was, however, no correlation between serum zinc and haematocrit in the study subjects. (Table 4).

Table 4: Correlation of serum zinc with some laboratory and clinical parameters

Parameters of severity	r	p-value
Low Haematocrit	0.023	0.1
Parasite density	-0. 101	0.01
Hyperpyrexia	-0.120	0.04

Discussion

Serum zinc levels in patients with severe/complicated malaria varied significantly from those obtained in children with non-severe/complicated malaria while serum zinc concentration in controls was within the commonly quoted range.[12] The substantially low level of serum zinc in children with severe/complicated malaria in comparison with their counterparts who had non-severe/complicated may be attributed to increased consumption/utilization of zinc resulting from the enhanced production of tumour necrosis factor and other free radicals produced in the course of severe malaria. Kulkarni *et al*[17] had documented that there is enhanced production of these free radicals, in severe/complicated malaria. It has been suggested that lowered zinc level is mediated by exaggerated production of free radicals and may reflect a normal protective mechanism.[18] In addition to increased consumption/utilisatiion of zinc by free radicals and oxidants, another plausible explanation for lower zinc levels in severe/complicated malaria can be pre-existing zinc deficiency making the child more susceptible to severe/complicated malaria due to impaired immunity.[5,18] In 1998 Gibson *et al* [19] reported that zinc deficiency in Malawian pregnant women was associated with an increased prevalence of malaria.

In this study, with the exception of the two subjects with 4+ (which are, however, too few for inferential deduction), there was a reduction in the mean serum zinc levels in malaria patients with 3+ parasitaemia. Patients with 1+ and 2+ malaria parasitaemia had marginal differences in their mean serum zinc levels. This marginal difference could be as a result of early presentation at the health facility resulting in parasite clearance at presentation and a subsequent amelioration of free radicals production. This implies that serum zinc tended to decline with increasing malaria parasitaemia. In 2000, Shanker *et al*[20] reported 29% decline in overall health centre attendance, a 38% reduction in fever associated with *Plasmodium falciparum* parasitaemia, and a reduction in episodes of hyperparasitaemia with Densities greater than 100,000/L in zinc supplemented group suggesting that zinc supplementation might protect against severe forms of malaria.

The morbidities of hyperparasitaemia, hyperpyrexia and anaemia were associated with varying mean serum zinc levels. Most profound reductions were found in association with isolated hyperpyrexia and hyperparasitaemia. The apparent high levels of serum zinc in patients with anaemia might be as a result of the well known high intracellular zinc concentration in red cells which leaves the serum zinc at a relatively normal level following haemolysis of the red blood cells. It may, therefore, be suggested that children with hyperparasitaemia and hyperpyrexia are at greater risks of having markedly reduced levels of zinc and a probable attendant heightened propensity for complications. It was difficult to assess the relationship between cerebral malaria and serum zinc levels, as children with isolated cerebral malaria were too few for meaningful deductions to be made.

Conclusion

Patients with severe malaria presenting with hyperpyrexia and hyperparasitaemia tended to have lower levels of serum zinc. There were negative correlation between malaria parasite density and serum zinc in under-fives with malaria.

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References

1. Snow RW, Craig M., Deichmann U, Marsh K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull WHO 1999; 77:624-40.
2. Federal Ministry of Health Lagos. Malaria in Nigeria Epidemiology and control. *Nigeria Bulletin of Epidemiology 1991 Nov; 1: 1-19.*
3. Greenwood BM, Bradley AK, Greenwood AM. Mortality and morbidity from malaria among children in rural areas of Gambia, West Africa. *Trans R Soc Trop Med Hyg 1987; 81: 478- 86*

4. Sowunmi A, Akindele JA.Presumptive diagnosis of malaria in infants in an endemic area. Tran R Soc *Trop Med J 1984; 15: 69 78.*
5. Shanker AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutri. 1998; 68: 447S-63S.*
6. Abi B. What Does Zinc Do? *Bri Med Jour. 2002; 325(7372): 1062*
7. Bhutta ZA, Black RE, Brown KH, Gardner JM., Gore S, Hidayat A. Prevention of diarrhoea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized control trial. *J Paediatr 1999;135: 689 97.*
8. World Health Organisation: Action Programme on severe and complicated malaria Trans R Soc Trop Med Hyg. 2000; 94: pp190.
9. Warrel DA. Management of severe malaria. Parasitologia 41, 287- 94.
10. Whitehead RG, Paul AA. Nutritional needs of healthy infants In: Jellife, DB. Stanfield, JP.(eds). Diseases of children in the tropics and Subtropics. 3rd edition London: Edward Arnold Publishers 1991. pp344.
11. Bashir NA. Serum zinc, copper in sickle cell anaemia and beta thalasaemia in North Jordan. *Ann Trop Paediatr. 1998 Dec 15; **4:**291-3.*
12. Shakur S, Malek MA, Tarafder SA. Zinc status of Bangladeshi children suffering from respiratory tract infection. *Orion Med J 2000 Jan 5:20-4.*
13. Jane MD. Physical growth of Nigerian Yoruba children. *Trop Geogr Med 1974; 26:389-398.*
14. Classification of Protein Energy Malnutrition. Welcome Trust Working Party on classification of energy malnutrition. *Lancet 1970; 11:302-305.*
15. Mikac-Deric D. A new spectrophotometric method for the determination of zinc in serum and urine Clin. Chim Acta. 1969; 23: 499.
16. Gilles HM. Diagnostic methods in malaria. In: Gilles HM. Warrel AD (eds). Bruce Chwatts Essential Malariology. London: Arnold, 1993: 81 5.
17. Kulkarni AG, Suryakar AN, Sardeshmukh A, Rathi DB. Studies on biochemical changes with special references to oxidant and antioxidants in malaria patients. *Indian Journal of Clinical Biochemistry, 2003:136.*
18. Beck FWJ, Kaplan J, Fine N, Handschu W, Prasad AS. Decreased expression of CD 73 (ecto- 5'- nucleotidase) in the CD8+ subset is associated with zinc deficiency in human patients. *J Lab Clin Med 1997; **1**: 1226-1228.*
19. Gibson RS, Huddle J-M. Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. *American Journal of Clinical Nutrition. 1998; 67:702709.*
20. Shankar AH, Genton B, Baisor M, Paino J, Tamja S, Adiguma T, et al. The influence of zinc supplementation on morbidity due to Plasmodium falciparum: a randomized trial in preschool children in Papua New Guinea. *Am J Trop Med Hyg* 2000; 62:663 669.