Zinc levels and malaria severity in children below five years in Dar-es-salaam, Tanzania

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Abstract

Introduction: Interventions which will decrease the morbidity and mortality related to Malaria are still being sought in order to improve the state of children in developing countries. Zinc is recognized to improve child health by improving immunity growth, weight and reducing episodes of infectious disease. The relation of Zinc and Malaria is still not very well understood.

Objective: This cross-sectional study was undertaken to assess the Zinc levels and Malaria severity in children below 5 years in Dar-es-Salaam, Tanzania.

Methods: Fifty children each with severe malaria, non-complicated malaria and without malaria were studied and their plasma Zinc levels assessed at one time point on admission to study. Zinc levels were assessed using Atomic Absorption Spectrometry. Levels of Zinc below 10mmols/dl were taken as low.

Results: There was no statistical difference in their baseline characteristics in terms of age and gender. There was a statistically significant difference in the plasma Zinc levels between those with un-complicated and severe malaria, with an Odds ratio of 3.8 (CI 1.5-10; and p value 0.0007). The difference between those without malaria and severe malaria was slightly higher with Odds ratio of 4.1 (CI 1.6-11, and p value of 0.004).

Conclusion: In conclusion, In general almost half of all children in the study had significantly low levels of Plasma Zinc (<10mmol/dL), indicating a major public health problem of Zinc micronutrient problem. Severe malaria was significantly asoiated with plasma Zinc levels.

Key words: Malaria, Zinc deficiency, under fives, Tanzania.

Background

About 500 million clinical cases of malaria and 1.5 to 2.7 million malaria related deaths occur worldwide annually. Malaria alone kills more than 1 million children (2,800 per day or 2 children per second) each year in Africa alone. The Sub Sahara Africa has the highest malaria infection rate with a mortality of at least 1 million people each year.[1]

In Tanzania, sixteen million people, which are almost 40% of the population, suffer acute malarial illness and about 100,000 die due to malaria alone annually. Most malaria deaths occur among under fives and pregnant women. In a hospital based survey in Tanzania, morbidity trend for malaria had increased by 8 folds while mortality rate had risen by 6 folds. Malaria is affecting more children today than it used to be 25 years ago as noted from escalating trend in this hospital. [2]

Several interventions in treatment and prevention are being undertaken, of which the role of micronutrients, especially Zinc is being sought. Several studies have indicated that zinc supplementation may reduce the incidence of clinical attacks of malaria in children. [(3)] Zinc promotes normal growth and development and maintains an effective immune system. Zinc supplementation in malnourished children has been found useful in the improvement of weight and height and is also found to reduce the severity and duration of diarrhea in children under the age of five years [4].

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In recent years malaria has undergone a dramatic resurgence due to a number of reasons including increasing resistance of the mosquito to insecticides, widespread resistance of the parasite to many anti malarial drugs, the breakdown of malaria control programs due to war and political instability and climate changes and natural disasters. In our own setting, admissions due to malaria over the last two decades have shown an increasing trend despite several measures to curb the same. This indicates that there are many other factors which need to be addressed, such as immunity and micronutrient supplementation. [(2, 5)]

It is possible that combinations of above factors and the deficiency of essential trace element zinc, could lead to a dramatic increase in the number of severe malaria cases. Worldwide including Tanzania there are few studies on the relationship between malaria and plasma zinc.

Currently in Tanzania and other countries, the Ministry of health and Social welfare has now officially implemented zinc supplementation to children with diarrhea, irrespective of their zinc status [(6)] Therefore it is important that we sought the relationship between Zinc and malaria. This study was conducted to document the different plasma zinc levels in children less than five years of age with and those without malaria and determine any association to malaria severity.

Objective

To assess the plasma zinc and malaria severity in children less than five years of age in Dar es Salaam, Tanzania.

Methodology

This unmatched case-control study was conducted at three sites, the general pediatric wards (GPW), general pediatric OPD clinic of MNH and Mnazi Mmoja MCH clinic for under fives. The GPW contributed all malaria cases (n=50) and some uncomplicated malaria children (n=36). Fourteen (n=14) uncomplicated malaria children were recruited from general pediatric OPD. The general pediatric wards were chosen because laboratory investigation facilities were available. The hospital has four general pediatric wards that admitted children aged one month to seven years.

Children without malaria were recruited from Mnazi Mmoja MCH clinic. The Mnazi Mmoja MCH clinic was chosen because it is at the centre of Dar es Salaam city and had a laboratory for all study investigations and plasma zinc storage facilities. Plasma zinc levels examination was carried out at the Chemistry department of the University of Dar es Salaam while other laboratory investigations were performed either at MNH or Mnazi Mmoja MCH clinic.

Study Population

Cases

- Children aged 1 to 59 months of age admitted in general pediatric wards with a WHO clinical diagnosis of severe malaria.
- Must have any positive blood slide for malaria parasites.
- Children who had not received blood transfusion in the past 4 months.

Controls

- First arm of controls- Uncomplicated Malaria:
  - Children aged 1 to 59 months attending General Paediatric OPD clinic or admitted in the GPW with a WHO clinical diagnosis of uncomplicated malaria.
  - Must have a non- heavy malarial parasiteamia blood slide (of < 200,000 asexual parasites/µL of blood).
- Second arm of control- Children without malaria:
  - Children aged 1 to 59 months attending Dar es Salaam city MCH clinic at Mnazi Mmoja for routine growth monitoring and immunizations.
  - Normal temperature, normal respiratory rate and weight for age ≥ 80%.
  - Must have negative blood slides for malaria parasites.

Exclusion criteria

- All children below 1 month and above 5 years of age.
- Unwillingness of the parents/guardians to participate in the study.
- Children who had a history of oral zinc supplements in the past 4 Weeks.
- Children who were jaundiced.

3.5 Sample size estimation

Sample size estimation was calculated using EPI INFO version 6-computer program software. The prevalence of severe malaria for children admitted in GPW from previous studies was 10%, significance level of 5% (0.05) and a power of 80%. The proportion of exposure among controls was calculated at 35%. A relative Risk of 3.5, Z_α of 1.96 and Z_β of 1.24 were used. The sample obtained was 48 in each group. Thus a total of 150 children were studied.

Sampling technique and recruitment

Recruitment was done only on weekdays during working hours. All children with severe malaria and uncomplicated malaria from the GPW were recruited into the study on a daily basis until sample size was met, while those with no malaria were obtained by simple random method from the Mnazi Mmoja MCH clinic on Tuesdays and Fridays until the sample size was met.

Data

A thorough demographic and clinical history and physical examination that included symptoms and signs of malaria disease according to WHO criteria was obtained and entered in a questionnaire. Demographic data included the use of Insecticide Treated Nets (ITNs).

Body weight was measured using Salter scale or Seca beam balance recorded to the nearest 10 grams. Temperature was recorded from the armpit using mercuric thermometer to the nearest 0.1 degree centigrade.

Palmar pallor was assessed using both hands to serve as a clinical guideline to severity of anemia and were recorded as no Palmar pallor, some or severe palmar pallor, as a guide used in primary health care settings. [7]

Blood slides for malaria parasites

A single sterile pinprick was performed on left index finger after a thorough cleaning with 70% alcohol. A capillary tube was used to draw 0.05 mls of blood .The tube was sealed on one side for hemoglobin examination (details below). Three thick blood smears from the same sterile pinprick were prepared. These were left to dry on air or using Stiebel Electronic machine that took 1 minute to dry.

The slides were stained with Giemsa stain and after 30 minutes, they were rinsed using tap-water and left again to dry for another ten minutes. The films were then examined on electric microscope under oil immersion using 100x power magnification lens. A positive malaria slide was recorded as*: Any Number of asexual parasites per 200 white blood cells.* The parasite density (number of asexual parasites per µLitre of blood) was calculated multiplying by 40. A parasite density of ≥200,000 asexual parasites/µL of blood was indicative of severe malaria.

A negative slide was reported if all three blood slides smears were negative for malaria parasites. To ensure accuracy, a senior laboratory scientist with an experience in malaria research examined all blood slide smears.

Hemoglobin level examination

The sealed capillary tube was automatically centrifuged for five minutes using Centurion – 8000 Series machine at 3000 revolutions per minute. A Hawksley Micro-Heamatocrit reader machine was used to read and record the Packed Cell Volume in percentage. PCV value was converted into hemoglobin levels (g/dl) after dividing by 3 (a constant Factor).

Plasma Zinc

Inpatients and OPD patients: Following aseptic techniques and using a 5 millilitre disposable plastic syringe with stainless needles, 4 millilitres of venous blood was drawn either from anterior cubital area or dorsum of the hand. Whole blood was transferred to a plastic vacutainer with an anticoagulant (EDTA) and mixed thorough. The gloves, syringes and the

vacutainers (Eppendorf tubes- Starstedt Inc, Princeton, NJ) were all special zinc free. The specimens were sent to the laboratory for separation of plasma, which was deep frozen at temperature -30°C until zinc analysis was done. The plasma was analysed within 2 weeks of blood collection by use of *Standard Atomic Absorption Spectrophotometer (SAAS) for zinc. Zinc atoms absorbed light of specific wavelength. The absorbed light was* directly proportional to the concentration of metal zinc in plasma.[8] The Principal Laboratory Scientist performed the SAAS analysis method for zinc at the Department of Chemistry of the University of Dar es Salaam.

Children from MCH clinic:

The Chief Investigator and a laboratory technician visited Mnazi Mmoja MCH clinic. Venous blood for plasma zinc sample was drawn only after meeting inclusion criteria (age group, normal temperature, normal respiratory rate, WFA ≥ 80%, and negative blood slides for malaria parasites). Using aseptic techniques 4 millilitres of venous blood were drawn from the anterior cubital area or the dorsum of the hand at any time of the working days and processed as described above. Plasma was separated using glass pipettes cleansed in HCl acid and stored in Eppendorf tubes. Aliquots were deep frozen at a temperature -30^0 C, and when they were ready to be analysed they were transferred in cold boxes to the Chemistry Department of the University of Dar es salaam.
Plasma zinc levels less than *10.0 µmols/L* was regarded as zinc deficient.

Ethical issues/clearance

Parents or guardians were explained thoroughly the aim of this study and that both informed verbal and written consent in either English or Swahili version were signed. Parents or guardian were informed the usefulness of the data. The ethical clearance and permission were requested and granted by MUHAS Research and Publication Committee.

Management

The clinical management of these children was done according to the management protocols of the pediatric wards. The normal children from Mnazi Mmoja were asked to return for results and follow their usual MCH clinic attendance.

Quality assurance

Certified materials were used for blood analysis. A two day training of three research assistants was carried out. The training focused on proper use of research tools, proper blood collection and storage before final analysis using zinc free containers. Data entry was performed with assistance from an experienced computer program and data analyst.

Data Analysis

Data analysis was done using EPI INFO- version 6 program. Statistical significant tests by odds ratios (OR), where indicated were calculated using a two-tailed p-value (Fishers). Mean ages were analyzed using analysis of variances (ANOVA). A p -value of < 0.05 was considered statistically significant.

Results

A total of 2074 children were admitted in the general pediatric wards of the Muhimbili National Hospital during the 4 month study period . One hundred mothers or caretakers of children with severe and uncomplicated malaria fro the hospital and 50 mothers from the Mnazi Mmoja clinic agreed and signed written consent forms on behalf of their children to participate in the study.
A total of one hundred and fifty children (43.3% females) aged 1 to 59 months were enrolled for the study. Thirty-two (64%) from severe, twenty-seven (54%) from uncomplicated and twenty-six (52%) from no malaria children groups were males. Twenty-nine (58%) of all severe malaria, thirty-nine (78%) from uncomplicated and forty-three (86%) from no malaria groups were ≤ 24 months of age. There were no statistical significance differences on population distribution on age groups and sex with a p- value of > 0.6. In the studied groups;

- Forty-two (84%) of all severe malaria, twenty-nine (58%) of all uncomplicated malaria and twenty-eight (56%) of all children without malaria had low plasma zinc levels.
- There was a significant association on low plasma zinc levels and malaria severity in all studied groups.
- The differences between groups were statistically significant. The odds ratio on low plasma zinc levels between severe malaria and uncomplicated malaria was 3.8 (95% CI 1.5,10) with a p-value of 0.007.
- The odds ratio on low plasma zinc levels between severe malaria and children without malaria was 4.1(95% CI 1.6,110) with a p value of 0.004.

The mean plasma zinc levels for severe malaria was 7.019 (±4.052) while 11.031 (±5.43) for uncomplicated and 12.490 (±8.31) for children without malaria groups.

Age

For the purpose of this study, the two age groups were children ≤ 24 months of age, and children > 24 months of age. One hundred and eleven (74%) of all children studied were ≤ 24 months of age. Sixty-seven (45%) of all children ≤ 24 months of age and thirty-two (82%) of all > 24 months of age had low plasma zinc levels. Twenty-three (46%) of all children aged ≤ 24 months with severe malaria and twenty-two (44%) of all children aged ≤ 24 months with uncomplicated had low plasma zinc levels. Twenty-two (44%) of all children aged ≤ 24 months without malaria had low plasma zinc

levels. The differences were not statistically significant on low plasma zinc levels and malaria severity according to age groups. The OR for low plasma zinc levels between severe malaria and uncomplicated malaria for children ≤ 24 months age groups was 0.39 (95% CI 0.2, 1) with a p- value of 0.129. The OR for low plasma zinc levels between severe malaria and without malaria children ≤ 24 months age groups was 0.3 (95% CI 0.1,1) with a p-value of 0.105.

Gender

There were eighty-five males (56.7%) in the studied population. Sixty-two (73 %) of all males and thirty-seven (56.9%) of all females had low plasma zinc levels. Twenty-eight (56%) of all males from severe malaria, eighteen of all males (36 %) from uncomplicated and sixteen of all males (32%) from children without malaria groups had plasma low zinc levels. There were more males in all three groups of children studied who had lower plasma zinc levels than females but the differences were not statistically significant.The OR for low plasma zinc levels on sex groups between severe malaria and uncomplicated malaria was 1.2 (95% CI 0.5, 3.3) with a of p-value of 0.801. The OR for low plasma zinc levels between severe malaria and without malaria children groups was 1.5 (95% CI 0.6, 4) with a p-value of 0.548 on sex groups.

Table 1: Association between plasma Zinc status and severity of malaria

Plasma Zinc	**Severe Malaria**	**Uncompl Malaria**	**No Malaria**	**Total**	**Odds ratio & 95%CI**	**P-value**
	n (%)	**N (%)**	**n (%)**	**n (%)**		
Low (<10µmol)	42 (84)	29 (58)	28 (56)	99 (66)	+ 3.8 (1.5,10)	+ 0.007
Normal	8 (16)	21 (42)	22 (44)	51 (34)	* 4.1 (1.6,11)	* 0 .004
Mean Zinc level in µmol(±SD)	7.019 (±4.052)	11.031 (±5.43)	12.490 (±8.31)			

+ Indicate, Severe malaria Vs Uncomplicated malaria. * Indicate, severe malaria Vs No malaria children

Table 2: Association between plasma zinc levels, malaria severity and the use of insecticide treated bed nets (ITNs).

Plasma Zinc	Severe Malaria n (%)	Uncompl Malaria n (%)	No Malaria N (%)	Total N (%)	Odds ratio & 95%CI	p-value
Normal	1 (9)	11 (61)	12 (46)	24 (44)	+ 0.1 (0.01,0.6)	+ 0.002
Low	10 (91)	7 (39)	14 (54)	31 (56)	* 0.12 (0.01,1)	* 0.005
Total	11	18	26	55		

NB: Twenty-six (47.3%) of children without malaria, eighteen (32.7%) from uncomplicated and eleven (20%) from severe malaria groups used ITNs.
+ Indicate, severe malaria Vs Uncomplicated malaria children., * Indicate, Severe malaria Vs No malaria children.

Table 3: Association between plasma zinc levels, severity of malaria and nutritional status

Plasma Zinc	WFA	Severe Malaria (%)	Uncompl Malaria N (%)	Total n (%)	Odds ratio & 95% CI	p-value
Low (<10*µmol)*	<80	15 (30)	10 (20)	25 (25)		
	≥80	27 (54)	19 (38)	46 (46)	+1.1 (0.4,2.9)	+1.000
Normal	<80	5 (10)	4 (8)	9 (9)		
	≥80	3 (6)	17 (34)	20 (20)	+7.1 (7.1,43)	+0.667

- Fifty-five (39%) of all mosquito net users reported use of insecticide treated mosquito bed nets (ITNs).
- One (9%) from severe, eleven (61%) from uncomplicated and twelve (46%) without malaria group children who used ITNs had normal plasma levels.
- The differences for low plasma zinc levels and malaria severity on use of ITNs were statistically significant among groups studied.
- The OR on low plasma zinc levels for use of ITNs between severe malaria and uncomplicated malaria was 0.1 (95% CI 0.01, 0.6) with a p-value of 0.002.
- The OR on low plasma zinc levels for use of ITNs between severe malaria and without malaria children was 0.12 (95% CI 0.01,1) with a p-value of 0.005.
- Low OR here meant that ITNs were protective (90%)with lesser chances of low plasma zinc levels. Table 3.

+ Indicate, Severe malaria Vs Uncomplicated malaria children. Using Tanzania MCH card number one, a child whose weight for age was < 80% was considered as malnourished while a child with weight for age ≥ 80% was considered as normal.

- Thirty-four (34%) out of all 100 children with malaria had malnutrition.
- Twenty-five (73.5%) out of all 34 malnourished children had low plasma zinc levels. Malnourished children with low plasma zinc levels were further stratified.
- Severe malaria children who were malnourished with low plasma zinc levels were fifteen (30%) while children who had uncomplicated malaria and malnourished were ten (20%).
- However there was no statistical significant difference on low plasma zinc levels and malaria severity in relation to the nutritional status using WFA.
- The OR for low plasma levels between severe malaria and uncomplicated malaria regarding nutritional status was 1.1 (95% CI 0.4, 2.9) with a p-value of 1.000.

Discussion

This study found significant low level of plasma zinc among all the children studied. The low levels were inversely proportional to the severity of malaria. Thus in severe Malaria the zinc levels were lower. This may indicate a linear relationship between absence of malaria, acquisition of malaria and severity of malaria. This trend of progressive decreasing levels of zinc leads us to speculate that low zinc levels are associated with severe malaria and may be related causally. There were 4.1 higher odds of low plasma zinc levels among children with severe malaria compared to those who had no malaria. While the odds of low plasma zinc levels among uncomplicated malaria and severe malaria were 3.8. Therefore the odds of low plasma zinc levels among severe malaria and those with uncomplicated malaria was almost four fold.

In this study zinc deficiency in otherwise healthy children without malaria was widely prevalent (56%). Findings from other developed countries indicate a prevalence of 38-50% zinc deficiency in children.[8] the higher prevalence of low plasma zinc levels in our population may be related to the frequent malaria episodes or other intercurrent infections.[9]

A high number of malaria cases in this part of the country predispose children to a constant low plasma zinc levels. It is also evident that other febrile illnesses have a negative effect on plasma zinc, and that zinc plays an important role in the acute phase reaction (APR). [10]

This case-control study supports the clinical trial studies which indicate that oral zinc supplementation in malaria endemic areas reduce frequency of health centre attendance due to illness and heavy parasiteamia due to P. falciparum malaria. [11-18.] although a causal pathway has not been elucidated. It is unclear whether low plasma zinc levels predispose to severity of malaria or whether severe malaria further depletes the already low levels of zinc remain. The prevalence of low zinc increased from 56% from children without malaria to 58 % and 84 % from uncomplicated and severe malaria respectively, while the mean plasma zinc levels declined from 12.49*μmols/l* (± 8.31) for children without malaria to 11.031 *μmolls/l* (±5.43) and 7.019 *μmols/l* (± 4.052) for uncomplicated and severe malaria children respectively. This trend of serial decline in plasma zinc levels may indicate an early warning sign of impending severe malaria attack and may herald a poor outcome. This might signify that prompt efforts should be put into effect to prevent these children from progressing to severe forms of plasmodium infection or to obtain a rapid recovery. Malnutrition might be responsible for low plasma zinc levels in children in both two age groups. This study did not go into the details of nutritional intake and breast-feeding details.

There was a high rate of mosquito net use response, ninety four percent of the population studied reported sleeping under mosquito nets at night. This was a good response but there could be some information bias, as the investigators had no means of verifying these reports. Children without malaria group had a 100% use of mosquito nets as compared to 92% and 90% for uncomplicated and severe malaria groups respectively. All nine (100%) children from non mosquito net users had low plasma zinc levels. There aren't studies to indicate a direct relation between mosquito nets use (plain or ITNs) and plasma zinc levels.

However other studies had concluded that mosquito nets lower the prevalence of malaria attacks and possibly severity.[19] in this context it could have a positive effect on plasma zinc levels indirectly. Fifty percent of children without malaria and had used ITNs, had normal plasma zinc levels as compared to uncomplicated and severe malaria groups (45.5% and 42% respectively). The differences on low plasma zinc levels and malaria on use of ITNs were statistically significant. The risk (OR) for using ITNs and getting low plasma zinc levels from uncomplicated malaria was 0.1 while that from without malaria was 0.12. It should be clear that for ITNs users, the lower the OR the higher the protection against severe malaria with an increase chances for normal plasma zinc

levels. The use of ITNs may be related to inequalities in health related to socio-economic status. In a study in Southern Tanzania, anemia, malaria severity and non-use of ITN was found to be significantly higher among the poorest as compared to the least poor. (personal communication) [20]

In conclusion

Low plasma zinc levels were significantly associated with malaria severity in children less than five years of age at MNH. Eighty four percent of severe malaria children less than five years of age admitted in GPW at MNH had low plasma zinc levels. While fifty eight percent of uncomplicated malaria at MNH had low plasma zinc levels.

More than a half (i.e.56%) of all children less than five years of age without malaria attending MHC clinic for routine growth monitoring and immunization had low plasma zinc levels.

ITNs use in children less than five years of age from Dar es Salaam had a protective effect of (1-0.1=9) 90% against severe malaria and low zinc levels. There is a need for large randomized control trials to confirm the causal relationship of malaria and zinc and recommend public health measures for zinc supplementations in malaria. This may provide a cheap intervention and complementary therapy for malaria, just as well as for other infectious diseases.

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