

Association between Malnutrition and Immunoglobulin G Responses to Crude Asexual *P. falciparum* schizont Lysates in Children Attending Ishiara Hospital, Embu County, Kenya

Mungiria Juster^{1*}, Gicheru Michael¹, David Kavili¹ and Mburu David²

¹Department of Zoological Sciences, Kenyatta University, Nairobi, Kenya.

²Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MJ, GM, DK and MD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MJ, DK and GM managed the analyses of the study. Authors MJ and MD managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2017/31041

Editor(s):

(1) Rohit Saluja, Department of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, Germany.

Reviewers:

(1) Jaffu Othniel Chilongola, Kilimanjaro Christian Medical University College, Tumaini University, Tanzania.

(2) M. C. O. Ezeibe, Michael Okpara University of Agriculture, Umudike, Nigeria.

(3) Abdullahi M. Nuhu, Kaduna polytechnic, Kaduna, Nigeria.

Complete Peer review History: <http://www.sciencedomain.org/review-history/18491>

Original Research Article

Received 16th December 2016

Accepted 31st March 2017

Published 4th April 2017

ABSTRACT

Aim: The aim of this study was to determine levels of *Plasmodium falciparum* parasitaemia in children and relationship between IgG responses against the parasite and nutritional status of children.

Study Design: Cross sectional study of children diagnosed positive for *P. faciparum* in Ishiara District Hospital, Embu county, Kenya was done between August 2011 and June 2012.

Methodology: A total of 380 children, under 5 years who tested positive to *P. faciparum* infection were used for the study. Children with less than -2 Z score of height-for-age were classified as stunted and those with less than -2 Z score of weight-for-height were classified as wasted. Malnourished children were identified by the criteria of WHO. *P. faciparum* parasitaemia was determined by microscopy, using Giemsa stained thin and thick blood smears while the parasite's IgG antibody responses were assessed by the Enzyme linked immune-sorbent assay.

*Corresponding author: E-mail: justerm@yahoo.com;

Results: Of the 380 children 70% were malnourished. Of the malnourished children 30% were stunted, 37% wasted while 3% were both stunted and wasted. Well nourished children had mean parasitaemia of 530 per micro litre which was lower ($P < 0.05$) than mean parasitaemia 590 per micro litre of the malnourished children. There was no correlation between parasitaemia and wasting ($r = 0.4$, $P > 0.05$). IgG responses of well nourished children (1.9) was higher than 1.2 of wasted children ($r = 0.7$, $P < 0.05$), this implies a negative correlation between wasting and IgG response. The same pattern of correlation was observed between well nourished children and the stunted ($r = -0.6$; $P < 0.05$) and also between the well-nourished and those both stunted and wasted ($r = -0.8$; $P < 0.05$).

Conclusion: This study showed that malnutrition reduces IgG responses of children below 5 years to *P. falciparum* infection and predisposes them to malaria.

Keywords: Malaria; morbidity; nutrition; immunity; anthropometry; schizont extract; antibodies; plasmodium.

1. INTRODUCTION

Children under five years are predominantly susceptible to *P. falciparum* infection. Each year, about 800,000 children die of malaria, and 75% of these deaths occur in African children [1,2,3]. According to UNICEF [1], in sub-Saharan Africa, 38% of children under five years of age suffer from chronic malnutrition or stunting (height-for-age z-score below -2 of an international growth reference) while 9% suffer from acute malnutrition or wasting (weight-for-height z-score below -2). The relations between malaria and malnutrition interaction has been investigated for many years. It is now broadly documented that malnutrition and malaria share certain consequences, including cognitive impairment and decreased school performance [4,5,6].

Although many studies have shown a deleterious effect of malaria on nutritional status, it is still unclear as to whether malnutrition, influences malaria morbidity [7,8,9]. Many earlier studies based on hospital admissions for severe malaria showed lower risk of malaria infection among undernourished children [10,11,12]. However, results of recent community-based studies are conflicting: two studies showed that stunting increased the risk of malaria morbidity among rural children in Gambia [13,14], while a trial in Papua New Guinea indicated that stunting protected children from *P. falciparum* malaria [15]. Additionally, several studies have found no significant association between stunting or height-for-age z-score and malaria morbidity [16-20]. With regard to wasting, some studies showed a trend to lower malaria-related morbidity among wasted children [13,16,21]. Altogether, these studies point to the importance of taking into account the kind of child malnutrition (stunting, wasting,) in relationship between malaria and malnutrition. It has been

proven that malnutrition down-regulates immune functioning, resulting to infection [22,23]. Research reports have shown that micronutrients are modulators of anti-pathogen immunity [24,25]. Few research reports have examined the relationship between malaria, specific immunity and malnutrition [26,27,28]. Among these studies, two showed no impact of malnutrition on the antibody (Ab) response to *P. falciparum* [26,28]. One survey indicated lower specific Ab levels in children who suffered from malnutrition [27]. A trial in Papua New Guinea showed that the IgG antibody response to *P. falciparum* schizont extract was lower in wasted children than in well-nourished children [15].

Contradictory findings justify further studies on the impact of child malnutrition on anti-malaria immunity. Thus, it would be interesting to determine whether child malnutrition modulates the overall anti-*P. falciparum* immune response. It has been previously shown that Ab levels directed to wide antigens of *P. falciparum*, such as schizont antigens, were associated with *P. falciparum* infection [29] and, therefore, represented an overall view of anti-malarial immunity [30,31]. This study sought to investigate the impact of childhood malnutrition, on the overall anti-*P. falciparum* immune response, on children under five years visiting Ishiara District Hospital.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Ishiara Hospital in Mbeere District in Eastern province of Kenya. Mbeere District in Eastern Kenya is a semi arid region with unreliable rainfall, and has an estimated population of 183,166 persons [32].

Ishiara is a malaria endemic region and malnutrition cases are also prevalent [33].

2.2 Sample Size Estimation

Sample size was estimated at 5% significance [3]. The formulae

$$n = \frac{z^2 pq}{d^2}$$

Where:

n = the desired minimum sample size.

z= the standard normal deviation, set at 1.96, which corresponds to 95% Confidence level.

p= Estimate for *p* on the study by Fillol et al. [20] in a rural district of Senegal where a 55% decrease in anti-plasmodium IgG antibody response to *P. falciparum* infection was found in severely stunted children.

d = degree of accuracy desired, here set at 0.05.

q= 1-p

$$n = \frac{1.96^2 \cdot 0.55 \cdot (1 - 0.55)}{(0.05)^2} = 380$$

At least 190 participants for the experimental and similar number for the control are minimum cases recommended for the study.

2.3 Study Participants

A total of 380 participants positive for *P. falciparum* malaria were recruited for the study. The participants were children under five years of age (6 months to 5 years).

2.4 Study Design

This was a comparative cross-sectional study with two groups. One group consisted of children with malnutrition and malaria and the control group consists of children with malaria and no malnutrition. Identification of patients with malnutrition was as per the World Health Organization criteria [34]. Participants were admitted into the study after meeting the following inclusion criteria; Age of 2 months to 5 years, positive for *falciparum* malaria, History of fever in the preceding 24 hours at a temperature of 37.5c°, informed consent by the legal representative of the subject (the parent, if possible) and participant should be a resident of

the study area for a duration of at least 4 weeks. The participants with obvious congenital malformations and for whom parental/guardian written informed consent was not given were not included into the study.

2.5 Ethical Issues /Considerations

The study was approved by the Kenyatta University Graduate School. Ethical permission was obtained from Kenyatta university ethical committee after submitting the research proposal for the study and Ministry of Medical Services through Ishiara District Hospital. Informed consent was obtained from each parent/guardian prior to the enrolment exercise. A pre-consent counseling of the parent/guardian was carried out. Patients' names and other identifying characteristics were not documented and records were encoded to ensure anonymity and confidentiality during data collection and reporting.

2.6 Study Flow

Participants were recruited for the study consecutively until the sampling size was achieved. The patients were seen by the study clinician as they arrived in the hospital. The same was done for those in the pediatric wards and pediatric filter clinic in the hospital. Medical history was taken, vital signs were checked, and axillary temperature was measured with a standard mercury thermometer. Thick and thin blood smears were prepared from the finger prick for microscopic examination, Veni puncture were performed to monitor the IgG antibody levels specific for *P. falciparum* malaria.

2.7 Laboratory Procedures

2.7.1 Parasite density assessment

Dried thick and thin blood smears were stained with 10% giemsa solutions at a P^H of 7.2 for 10 min. Parasite species were indentified using standard morphologic characteristics, and the parasite density was calculated using standard procedure in which parasite were counted per 200 white blood cells multiplied by a standard count of 8000 leukocytes/*ul* [35].

2.7.2 Sample collection/determination of serum IgG antibody levels

About 2 mls of venous blood was collected by veni-puncture into a plain container. The samples

were spun in centrifuge at a speed of 2500 rps to separate serum from red blood cells. The serum obtained was stored in a freezer at a temperature of -20°C. The IgG antibody levels were estimated using enzyme linked immunosorbent assay as used by Fillol et al. [3]; 2 µg/ml of schizont extracts were coated on flat-bottom micro titer plates [3] with 100 µL/well for 2 h 30 min at 37°C. Plate wells were then blocked for 30 min at room temperature with 200 µL of blocking buffer, pH 6.6 (phosphate-buffered saline, with 0.5% gelatin [3] and washed one time with PBS, pH 7.2, 0.1% Tween 20 (Sigma, Individual sera were incubated in duplicate at 4°C overnight at a 1/100 dilution (in PBS-Tween-0.1%).

This dilution was determined as optimal after several preliminary experiments. For detecting human IgG, plates were incubated for 90 min at 37°C with 100 µl of mouse biotinylated mAb to human IgG [3] diluted 1/1000 in PBS-Tween 20, 0.1%, after washing three times with PBS-Tween 20, 0.1%. Plate wells were then washed four times with PBS-Tween 20 and incubated for 30 min at room temperature with 100 µl of peroxidase-conjugated streptavidin [3]. After washing six times with PBS-Tween 20, colorimetric development was carried out using ABTS (2,2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) diammonium; Sigma) in 50 mM citrate buffer (Sigma, pH = 4, containing 0.003% H₂O₂), and absorbance (optical density, OD) was measured at a wavelength of 405 nm. Individual results were expressed as ΔOD value calculated according to the formula: ΔOD = OD_x - OD_n, where OD_x was the individual OD value and OD_n was the individual OD value for each serum without antigen. Negative controls were used for each assay (OD_{neg}). The Schizont extract was

provided by Prof S. Longacre of Pasteur Institute, Paris.

2.8 Data Management and Statistical Analysis

Anthropometry was analyzed with nut Stat software of Epi info 2000 [36], which calculated Height- for- age and weight- for- age scores for each individual to compare with NCHS reference values. If the scores were less than 2 Z-score (SD units) below the reference median for the indices, the participants were classified as malnourished, [37]. Low height forage was considered an indicator of stunting. Low weight-for- height is considered an indicator of wasting and generally associated with failure to gain weight or a loss of weight [37]. Weight- for- age anthropometric index was not used in this study since it fails to distinguish tall, thin children from those who are short with adequate weight [38]. Statistical analyses of the data were performed using statistical software package. Statistical package for social sciences (SPSS) version 18.0. Analysis of variance (ANOVA) was used to compare the means of laboratory data. Bonferroni correction was done for multiple comparisons. Pearsons correlation test was also used to establish the relationship between variables such as parasite density and IgG antibody levels specific for *P. falciparum* malaria. The statistical significance level was set at 95% confidence interval and P. value <0.05 was considered significant

3. RESULTS

Out of 380 participants, 266 (70%) had malnutrition and malaria while 114 (30%) had malaria with no malnutrition. Table 1, shows the age distribution of the participants.

Table 1. Age distribution of Children at Ishiara Hospital, Embu County, Kenya who were malnourished and sick of malaria and those who were well-nourished but sick of malaria

Age group (Months)	Malnourished children with malaria	% of malnourished with malaria	Well nourished children with malaria	% of well nourished with malaria	P-value
0-9	53	71	22	29	
10-19	111	79	29	21	
20-29	48	77	14	23	8.38718E-06
30-39	42	61	27	39	
40-49	8	32	17	68	
50-60	3	38	5	62	
Total	266		114		

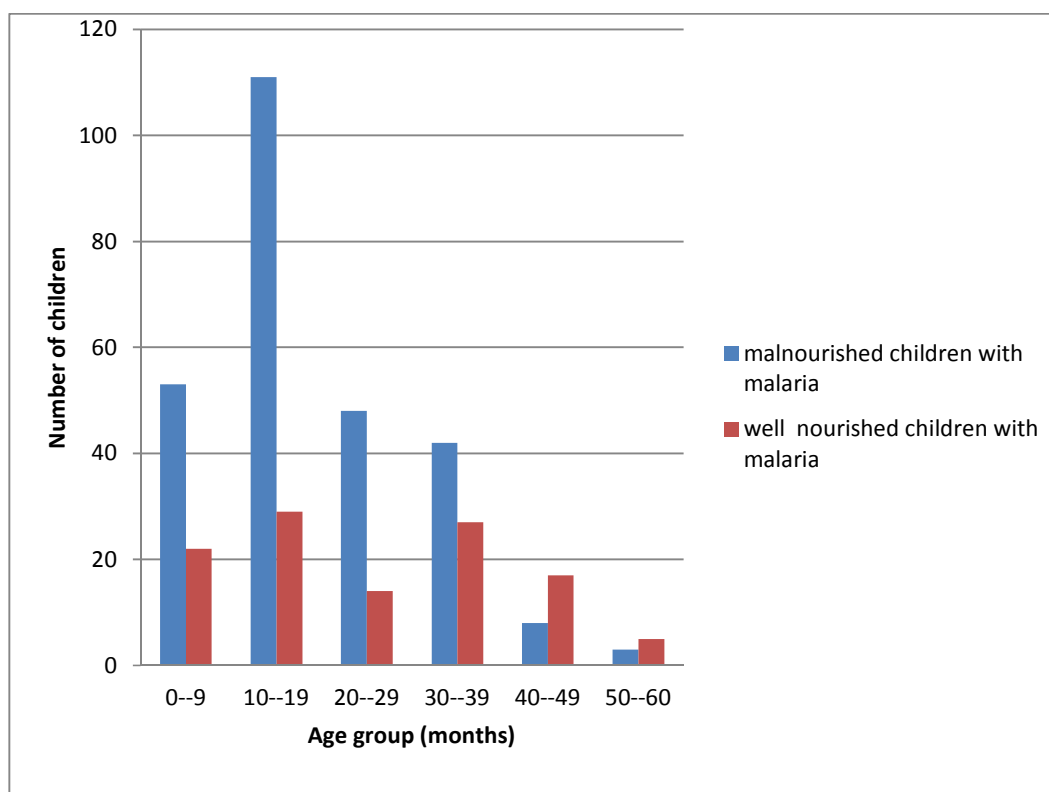


Fig. 1. Age distribution of children malnourished with malaria and those well-nourished without malaria at Ishiara

The highest number of children that had both malnutrition and malaria were in the 10-19 months age group (29%), while only 0.8% of the children in the 50-60 months age group had both malnutrition and malaria. The association between age and nutrition/malaria status was observed to be significant ($\chi^2 = 31.243$, $df=5$, $P < 0.01$).

Out of 380 participants, 198 (52%) of the sampled children were Males and 182 (48%) were females. Malnourished children were 266 while the well-nourished were 114. Out the 266 malnourished children, 112 were stunted (10% females and (20%) males), 142 were wasted while only 12 children were both stunted and wasted.

3.1 Mean Age of the Participants

The mean age of the study subjects was 22.9 ± 13.8 months. For the subjects with malaria and malnutrition the mean age was 23.4 ± 13.9 months while for those with malaria and without malnutrition was 21.4 ± 12 months (Table 2). The mean weight of the participants was 8.8 ± 3.56 kg, while the mean height was 78.7 ± 14.4 cm.

Table 3 shows the distribution of stunting among the participants involved in this study. When categorized by gender about 18% of the females were not stunted compared to only 12% males. In both cases stunting values ranged from 1SD to 3SD. In the females only 0.5% of the population had stunting values of 3SD while in males the values were a high value of 13%.

Table 2. Mean age of children in Ishiara Hospital, Embu County, Kenya who are malnourished and sick of malaria and those who are well-nourished but sick of malaria

Groups	N	Mean age	Std dev	Std error mean
Malnourished with Malaria	266	23	14	0.9
Well nourished with malaria	114	21	12	1.1

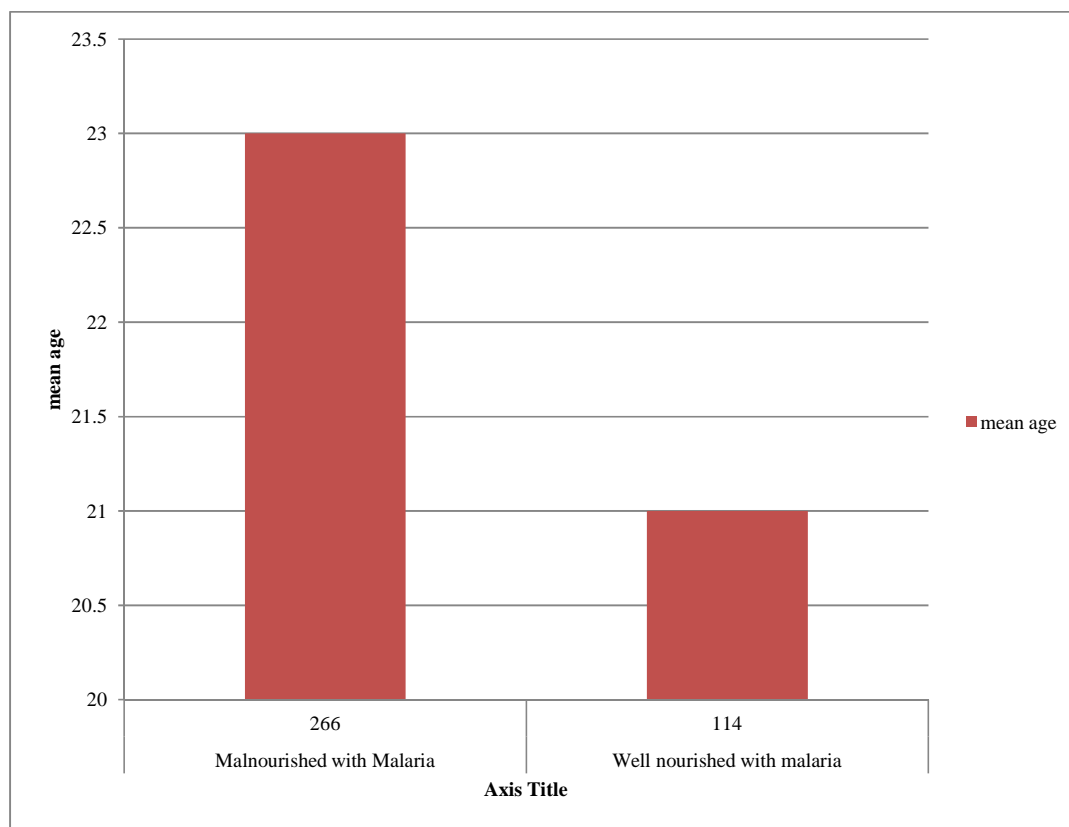


Fig. 2. Mean Age of children Malnourished and with malaria and well nourished without malaria at Ishiara

Table 3. Distribution of varying levels of stunting of the children at Ishiara Hospital

Sex	HAZ scores for stunting				Total
	0SD	-1SD	-2SD	-3SD	
Females	68	36	1	2	107
Males	46	11	15	47	119
Total	114	47	16	49	226

0SD=not stunted;-1SD=mild stunting; -2SD=moderate stunting;-3SD= severe stunting

142 participants were wasted, (13% females and 24% males) (Table 4). When categorized by gender about 18% of the females were not wasted while 13% of the females were wasted.

Considering males, 12% were not wasted while 24% of males were wasted. Comparing males and females who were wasted; the males (24%) registered a higher percentage than the females (13%).Wasting values ranging from -1SD to -4SD.

12 participants had both stunting and wasting types of malnutrition. When categorized by gender, 18% of the females were not wasted nor stunted while 1% of the females were wasted and stunted. About 12% of the males were neither wasted nor stunted while 2 % males had both stunting and wasting (Table 5).

.Table 4. Distribution of varying levels of wasting of the Children at Ishiara Hospital

Sex	WHZ Scores for wasting					Total
	0SD	-1SD	-2SD	-3SD	-4SD	
Females	68	5	3	18	25	119
Males	46	47	21	18	5	137
Total	114	52	24	36	30	256

0SD= not wasted;-1 SD=mild wasting; -2 SD= moderate wasting; -3 SD= severe wasting;-4 SD=Most severe wasting.

Table 5. Distribution of varying levels of wasting and stunting of the Children at Ishiara Hospital

Sex	WHZ/HAZ Scores for stunting and wasting				Total
	0/0SD	-1SD/-2SD	-2SD/-3SD	-3SD/-4SD	
Females	46	2	2	-	50
Males	68	-	6	2	76
Total	114	2	8	2	126

0SD /0 SD= not wasted nor stunted; -1 SD/-2 SD= mild wasting and stunting; -2 SD/-3 SD= moderate wasting and stunting; -3SD/-4SD =severe wasting and stunting.

The participants were checked for the presence of any other condition apart from malaria and malnutrition. The comorbidities observed in children under five years who visited Ishiara District Hospital during the study period and those who meet the inclusion criteria. Out of 380 participants it was observed that 77% had no associated comorbidities, 22.7% had diarrhea and 0.3% had pneumonia.

3.2 Age Groups and Malnutrition

Stunting and wasting may be influenced by the age of the child. It was observed that 266 children had malnutrition and malaria.

Table 6 shows how age influence nutritional status of a child (Table 6).

Table 6. Age groups and different types of malnutrition

Age group	Wasting	Stunting	Wasting and stunting
0-9	28	23	2
10-19	48	41	4
20-29	21	25	2
30-39	23	16	4
40-49	19	7	-
50-60	3	0	-
Total	142	112	12

Out of the 380 malnourished children positive for *P. falciparum* malaria, 13% were mildly wasted, 6 %were moderately wasted and 18% were severely wasted. Among all the levels of wasting, severe and most severe wasting combined had a higher percentage of 18% while moderate wasting had the least percentage of 6%. Majority of the study subjects were wasted with *P. falciparum* malaria infection 37%, compared with those who had other forms of malnutrition (stunting). Out of all participants of the study, 112 (30%) were *P. falciparum* positive and with malnourishment, and of these, 13% were mildly stunted, 6% moderately stunted, and 11% had severe stunting (Table 7).

Table 7. Distribution of participants in various types of malnutrition

Nutritional status	Stunting	Wasting	Wasting and stunting
0SD(control)	114	114	114
-1SD	48	50	4
-2SD	24	21	5
-3SD	40	40	3
-4SD	-	31	-

0 SD= not stunted nor wasted; -1 SD= mild stunting or wasted; -2 SD= moderate stunting or wasted; -3 SD= severe stunting or wasted.

It was observed that, only 1% of the children were mildly stunted and wasted, while 1.3% were moderately stunted and wasted, and 0.8% had severe stunting and wasting (Table 7). A total of 3% of the children were both wasted and stunted.

Children that were well-nourished had a lower mean parasite density (530) parasites density per microlitre of blood) compared to those who were severely wasted (590) parasites density per microlitre of blood) Findings show that severely wasted and stunted had very high mean parasite density of 880 parasite density per ul of blood when compared to the well-nourished participants that had 530 parasite densities per ul of blood (Table 8).

The relationship between the Parasite density, in the participants who were both stunted and wasted was compared to those who were well-nourished. Findings revealed a strong positive correlation between the varying levels of wasting and stunting ($r = 0.7$). This analysis revealed significant correlation in parasite density in the varying levels of both stunting and wasting (Table 9, $P < 0.01$).

Wasted and stunted participants showed a strong positive correlation ($r=0.7$), than wasted only ($r=0.4$) and stunted only ($r=0.3$) at $P < 0.05$ (Table 9).

Table 8. Mean parasite densities per microliter in the various types of malnutrition

Nutritional levels	Wasting only	Stunting only	Wasting and stunting
0SD(control)	530	534	530
-1SD	570	552	540
-2SD	578	554	700
-3SD	565	599	880
-4SD	590	-	-

0 SD= not wasted, nor stunting;-1 SD= mild wasting or stunting; -2 SD= moderate wasting or stunting; -3 SD= severe wasting or stunting;-4 SD=Most severe wasting or stunting

Anti-*P. falciparum* IgG responses were determined among malaria infected children with varying nutritional status. Children with a normal nutritional status had the highest mean IgG antibody level of 1.9 compared to other types of malnutrition It is clear that, IgG responses were low in children with wasting only, stunting only and much lower in those with both wasting and stunting (Table 10).

Table 9. Correlational relationship between parasite densities in the various forms of malnutrition

Parameter	Wasting only	Stunting only	Wasting and stunting
Parasite density	$r = 0.4$	$r = 0.3$	$r = 0.7$
Pearsons Correlation (2 tailed)	$P < 0.05$	$P < 0.05$	$P < 0.05^*$

$P < 0.05^* = \text{significant}$

Table 10. Mean IgG antibody levels (OD) in various types of malnutrition

Nutritional levels	Wasting only	Stunting only	Wasting and stunting
0SD	1.90	1.90	1.90
-1SD	1.87	1.30	1.40
-2SD	1.51	1.40	1.30
-3SD	1.32	1.50	1.20
-4SD	1.20	-	-

0SD= not wasted;-1 SD= mild wasting or stunting; -2 SD= moderate wasting or stunting; -3 SD=severe Wasting or stunting;-4 SD= Most severe wasting or stunting.

Children who are stunted and at the same time wasted have higher risk of malaria infection .This is indicated by very low mean IgG antibody responses, OD value of 1.2.There was a strong inverse correlation between stunting and wasting

and IgG response, with well-nourished having the highest IgG response and the stunted and wasted children having the least IgG response ($r = -0.8$).The data shows that there is a significant difference between all the levels of stunting and wasting in the participants with both parameters, when compared to the well-nourished children (Table 11, $P < 0.01$). The findings show that, when both parameters are considered, even much lower IgG responses are obtained when compared to those with either form of malnutrition. (Stunting or wasting).

Table 11. Correlational relationship between IgG Antibody levels in the various forms of malnutrition

Parameter	Wasting only	Stunting only	Wasting and stunting
IgG Antibody levels	$r = -0.7$	$r = -0.6$	$r = -0.845$
Pearsons Correlation (2 tailed)	$P < 0.05^*$	$P < 0.05^*$	$P < 0.01^*$

$P < 0.05^* = \text{significant}, P < 0.01^* = \text{significant}$

4. DISCUSSION

The aim of this hospital based cross-sectional study conducted in August 2011-June 2012 at Ishiara District Hospital, Embu County, Kenya; was to investigate whether child malnutrition could modulate malaria infection and the anti-*P. falciparum* antibody responses among pre-school children. In this study male and female respondents was 52% and 48%, respectively. It was noted that 46% of the males had malnutrition as compared to 24% of the females. This data is comparable with data presented by Ngare and Mutunga [38] on prevalence of malnutrition in Kenyan children where they reported rates of 51% in males and 49% in females in a cross-sectional study performed in the eight provinces in Kenya. In a study in Tanzania, nutritional status in under-five attending maternal and child health clinics found 51% to be males and 48% to be females [39]. Findings from both studies suggest that, intervention programmes need to consider all children suffering from malnutrition but more focus need to be given to the male child, since they seem to be more affected by malnutrition than the female children.

Studies on sexual preference on malaria and malnutrition in Kenya are lacking. In this study it

was noted that 46% of the males had malaria and malnutrition as compared to 24% of the females. The findings are comparable to those presented by Etuku et al. [40] on prescription pattern of anti-malaria drugs in children below 5 years in tertiary health institution in Nigeria which found rates of 58% males and to 44% females. In a study by Paul [41] in Singapore male sex respondents were more because of preference of male to the female child by the community where and male children were more readily brought to hospital compared to female children. It is likely that in Ishiara the same preference is accorded to the male child. Alternatively it is also possible that the male child is underfed or has lower immunity to *P. falciparum* which is the dominant species in this community, than the female child. Currently, there is no evidence to support or discount either. Lastly, it is also likely that in male child the high rate of malnutrition and malaria infection is by chance alone.

Considering age groups, the highest number of children with malnutrition and malaria were in the 10-19 months, while the smallest number was in 50-60 months. Weaning of children happens at this age group so it is most likely that the malnutrition observed is probably because the children are not well adjusted to new foods regimes. So there is need for special malnutrition control intervention to target children less than 3 years. Observations of this study concur with those of Ngare and Mutunga [38] which stated that malnutrition was highest among children of the 12-23 months age group. The findings also agree with those of Matee et al. [39] which found both stunting and wasting to be most serious between 11-25 months age group. Finally the study of Etuku et al. [40] was also consistent with the findings in that it observed that the most affected was between 12-24 months.

A total of 37% of the children seeking treatment at Ishiara District Hospital were wasted. The high prevalence of under-nourishment can be explained by the high level of food insecurity in the area. In addition, other factors that have been associated with under-nutrition in children include higher family size, maternal education, and poor feeding practices [42]. In this study, young children aged 10-19 months were more likely to have malnutrition and malaria as compared to older children, which is in concurrence with findings of Ngare and Mutunga [38].

In this study the prevalence of wasting was high of 37% and this contrasts that of Ngare and Mutunga [38] who found a low wasting prevalence of only 6%. Likewise Matee et al. [39] and Fillol et al.; [20] also reported a prevalence level of 2.9% and 9% respectively. The high prevalence of malnutrition in the current study could be explained by the fact that the study was hospital based and that most of the sick children attending hospital are likely to have an underlying malnutrition or an undiagnosed disease. In addition, in a study by Ngare and Mutunga [38], differences in prevalence rates were due to regional disparities with lowest rates of 23% being observed in Kiambu District in Central Province and highest rates of 57% being observed in Kwale District in Coast Province respectively.

A total of 29.5% of participants were stunted. This indicates that stunting just like wasting is still a serious problem in Ishiara community. These findings are comparable with other studies which include a study in sub-Saharan Africa by Fillol et al. [20] where stunting prevalence was about 38%, in a Kenyan study by Ngare and Mutunga [38] who reported a value of about 37% and in the Tanzanian where Matee et al. [39] reported stunting values of about 31.6%. Most of the current study subjects (77%) had no associated comorbidities, 22.3% had diarrhoea and 0.3% had pneumonia. The associated comorbidities could be explained by the fact that children with malaria can present with diarrhea and respiratory symptoms as part of the disease process. This implies that other diseases did not largely interfere with the results reported in the current study.

The parasite density in infected children was estimated using the protocol described by Nankabirwa et al. [35]. The findings from the current study show that the severely wasted children had a higher mean parasite density (590 parasites per μ l of blood) than those who are well-nourished (530 parasites per μ l of blood). Those with both forms of malnutrition had even higher mean parasite densities (880 parasites per μ l of blood). These findings are comparable to those observed in three other studies [13,14,43] that demonstrated that malnourished children have higher degree of malaria parasite density and are at risk of developing severe malaria. These findings demonstrate that malnourished children, both wasted and stunted, have a higher degree of parasite density than the well-nourished and

thus at risk of developing severe malaria infection. It also suggest that severe forms of malnutrition are associated with heavier parasite density and thus are more likely to experienced severe malaria as they are associated with high parasite densities. It also further suggests that the host resistance to malaria is poor as severe forms of malnutrition experience high parasite density

The intensity of infection could be a confounder in association between malnutrition and anti-*P. falciparum* antibody response. The type and level of malnutrition in a participant could be correlated to parasite density. Malnutrition reduces the antibody levels specific for *P. falciparum* therefore leading to an increase in the number of parasites per microliter of blood. In this study, severely malnourished children, both those stunted and wasted presented a higher parasite density than those moderately or mildly malnourished while lower parasite density was observed in the well-nourished children. Those with both types of malnutrition had even higher mean parasite density compared to all the other levels. This means that malnutrition is positively correlated with parasite density and thus affects immunity to *P. falciparum* malaria. A weak positive correlation was found between parasite density and wasting and stunting when compared with the well-nourished children ($r = 0.4$ and 0.3). However, a strong positive correlation ($r = 0.7$) was obtained when the parasite density was compared with malnourishment (stunted and wasted combined). This clearly shows that malnutrition predisposes children under five years to *P. falciparum* malaria, and this increases the risk of infection. This finding is consistent with previous finding of Samani et al. [44] who provided that malaria occurrence was positively associated with the degree of malnutrition in the Sudanese children. Also a study by Takarura et al. [45] whose study showed that malaria infection especially *P. falciparum* infection was associated with acute malnutrition in children and adolescents in Khammouane Province in Japan. Thus, to address such infections from this particular group, this study suggests that, malnourished children should limit infections through; feeding on balanced diet, use of insecticide treated nets, malaria chemopropylylaxis as well as empiric treatment of malaria in areas where access to malaria parasite diagnosis is difficult. It is also important that the issue of malnourishment should be addressed.

In this study the malnourished had a reduction in specific *P. falciparum* IgG responses. Children with a normal nutritional status had the highest mean IgG antibody level compared to the malnourished children. Wasted children presented a mean IgG antibody level of 1.2 while the well-nourished had 1.9. Stunted children presented mean IgG antibody levels 1.5 of which was low compared to the well-nourished who presented a value of 1.9. Wasted and stunted cases had much lower mean IgG antibody levels than any other group, and an OD value of 1.2 was recorded for the severely wasted and stunted participants compared to OD value of 1.9 for the well-nourished. These findings show that wasting affects the IgG responses more when compared to stunting. The observations indicate show that there is a significant difference ($P < 0.05$) in the anti-*P. falciparum* IgG levels in well-nourished and under-nourished children, both stunted and wasted groups. Thus, the null hypothesis that there is no significant difference in the anti-*P. falciparum* antibody between the well-nourished and the malnourished children is rejected.

Based on antibody responses the results suggest an association between malnutrition, malaria and specific immunity. The findings from this study indicate that both wasting and stunting reduce the mean IgG antibody levels specific for *P. falciparum*. These antibodies provide immunity to malaria infection [46], thus reduction in their levels may predispose this particular group to malaria infections. Hence, it is important to consider nutrition interventions in malaria control programmes if *P. falciparum* malaria in children under five years is to be managed. The findings of this study are comparable to a study by Dominguez and Alzate, [27], which showed that anti-malaria antibody response, was lower in malnourished Colombian children. Also a study by Genton et al. [15] showed that there is a lower antibody response in wasted children in Papua New Guinea. Similarly, Friedman et al. [12] showed that stunting increases the malaria morbidity in children under five years in western Kenya. Results from some other studies are conflicting, with some showing association; others show no association while others show protection against infection [20,26,28]. Indeed, no association was found between the specific Ab levels and acute or chronic under nutrition in Tanzanian children [26] whereas the anti-malarial Ab response was lower in

malnourished Colombian children [27] and wasted Papua New Guinean children [15] than in well-nourished children.

Child malnutrition can be evaluated using different definitions and this contributes to the discrepancies and conflicting results between the different studies. Indeed, in the Tanzanian study, children were classified as acutely undernourished based on reduced weight-for-age and normal height-for-age, whereas chronically undernourished children had normal height-for-weight and reduced weight-for-age. In the Colombian study, child malnutrition was defined according to Waterlow [47] classification (based on weight-for-age and height-for-age or the Gomez classification [48] based only on weight-for-age. In a more recent longitudinal study in Papua New Guinea [13], children were classified as stunted, wasted or underweight according to WHO [34]. These studies also differ in the age range of the children: from 10 to 120 months in Papua New Guinea and under six years of age in the Colombian study, whereas in Tanzania, subjects were school children.

Several discrepancies between the studies especially the methods used to determine levels of malnutrition could explain these conflicting results. While, others elements such as genetic factors, different history of malaria infection or self-medication might also explain the differences observed in specific antibody responses. It is generally agreed that active growth faltering occurs mainly during the first year of life [49]. Antibody levels directed to wide antigens of *P. falciparum* such as schizont antigens were associated with *P. falciparum* infection. In the current study, IgG antibody responses were measured by indirect ELISA assay. There were four categories of participants, those wasted, stunted, both stunted and wasted and well-nourished. The findings show lower mean levels of IgG antibody responses in the malnourished groups (1.3) compared to the well-nourished (1.9). A weak inverse correlation was found between wasting and stunting when compared separately with IgG responses ($r = -0.7$ and -0.6). The Lowest mean IgG antibody responses were noted in the group with both stunting and wasting (1.2). A strong inverse correlation was found between this group when compared to the well-nourished ($r = -0.845$). The differences in the antibody levels in the malnourished children and those well-nourished shows that malnourishment, both

wasting and stunting lowers the antibody response to *P. falciparum* malaria, thus contributing to an increase in malaria risk in the children under five years. To save this vulnerable group from severe *P. falciparum* malaria there is need to integrate nutritional programmes into malaria intervention programmes.

5. CONCLUSION

The findings suggest an association between malaria infection and child malnutrition. Malnourished children had more malaria parasites and lower IgG antibody levels than the well-nourished children, this suggest that malnutrition lowers the *anti-P. falciparum* antibodies levels, thus increasing child malaria morbidity. Therefore malaria prevention and control programs should consider nutrition interventions in management of malaria in children under five years.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. United Nations Children Fund (UNICEF). WHO Progress for Children. A World Fit for Children Statistical Review. 2007;6:19–24.
2. Breman J, The ears of the hippopotamus: Manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg.* 2001;64:1–11.
3. Fillol F, Sarr J, Boulanger D, Cisse B, Sokhna C, Riveau G, et al. Impact of child malnutrition on the specific anti-*Plasmodium falciparum* antibody response. *Malaria Journal.* 2009;8:116.
4. Martoreli R, Rivera J, Kaplowitz H, Pollitt E. Long-term consequences of growth retardation during early childhood. In *Human growth: Basic and clinical aspects*, Amsterdam. Elsevier Science Publishers B.V. 1992;143–149.
5. Branca F, Ferrari M. Impact of micronutrient deficiencies on growth: The stunting syndrome. *Ann Nutr Metab.* 2002; 46:8–17.
6. Fernard D, Wickremasinghe R, Mendis K, Wickremasinghe A. Cognitive performance

- at school entry of children living in malaria-endemic areas of Sri Lanka. *Trans R Soc Trop Med Hyg.* 2003;97:161–165.
7. Bradley-Moore A, Greenwood B, Bradley A, Kirkwood B and Gilles H. Malaria chemoprophylaxis with chloroquine in young Nigerian children. III. Its effect on nutrition. *Ann Trop Med Parasitol.* 1985; 79:575–584.
 8. Snow R, Molyneux C, Njeru E, Omumbo J, Nevill C, Muniu E, et al. The effects of malaria control on nutritional status in infancy. *Acta Trop.* 1997;65:1–10.
 9. Terlouw D, Phillips-Howard P, Hawley W, Friedman J, Kolczak M, Kariuki S, et al. Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey. *Am J Trop Med Hyg.* 2003;68:100–107.
 10. Hendrickse R, Hasan A, Olumide L, Akinkunmi A. Malaria in early childhood. An investigation of five hundred seriously ill children in whom a "clinical" diagnosis of malaria was made on admission to the children's emergency room at University College Hospital, Ibadan. *Ann Trop Med Parasitol.* 1971;65:1–20.
 11. Murray M, Murray A, Murray N, Murray M. Diet and cerebral malaria: The effect of famine and refeeding. *Am J Clin Nutr.* 1978;31:57–61.
 12. Ahmad S, Moonis R, Shahab T, Khan H and Jilani T. Effect of nutritional status on total parasite count in malaria. *Ind J Pediatr.* 1985;52:285–287.
 13. Deen J, Walraven G, Von Seidlein L. Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia. *J Trop Pediatr.* 2002;48:78–83.
 14. Friedman J, Kwena A, Mirel L, Kariuki S, Terlouw D, Phillips-Howard P, et al. Malaria and nutritional status among pre-school children: Results from cross-sectional surveys in western Kenya. *Am J Trop Med Hyg.* 2005;73:698–704.
 15. Genton B, Al-Yaman F, Ginny M, Taraika J, Alpers M. Relation of anthropometry to malaria morbidity and immunity in Papua New Guinean children. *Am J Clin Nutr.* 1998;68:734–741.
 16. Snow R, Byass P, Shenton F, Greenwood B. The relationship between anthropometric measurements and measurements of iron status and susceptibility to malaria in Gambian children. *Trans R Soc Trop Med Hyg.* 1991;85:584–589.
 17. Tshikuka J, Gray-Donald K, Scott M, Olela K. Relationship of childhood protein-energy malnutrition and parasite infections in an urban African setting. *Trop Med Int Health.* 1997;2:374–382.
 18. Muller O, Garenne M, Kouyate B, Becher H. The association between protein-energy malnutrition, malaria morbidity and all-cause mortality in West African children. *Trop Med Int Health.* 2003;8: 507–511.
 19. Nyakeriga A, Troye-Blomberg M, Dorfman J, Alexander N, Back R, Kortok M, et al. Iron deficiency and malaria among children living on the coast of Kenya. *J Infect Dis.* 2004;190:439–447.
 20. Fillol F, Cournil A, Boulanger D, Cisse B, Sokhna C, Targett G, et al. Influence of wasting and stunting at the onset of the rainy season on subsequent malaria morbidity among rural preschool children in Senegal. *Am J Trop Med Hyg.* 2009; 80:202–208.
 21. Mitangala N, Hennart P, D'Alessandro U, Donnen P, Porignon D, Bisimwa B, et al. Protein-energy malnutrition and malaria-related morbidity in children under 59 months in the Kivu region of the Democratic Republic of the Congo. *Med Trop.* 2008;68:51–57.
 22. Field C, Johnson I, Schley P. Nutrients and their role in host resistance to infection. *J Leukoc Biol.* 2002;71:16–32.
 23. Cunningham-Rundles S, Mc Neeley D, Moon A. Mechanisms of nutrient modulation of the immune response. *J Allergy Clin Immunol.* 2005;115:1119–1128.
 24. Scrimshaw N, Taylor C, Gordon J. Interactions of nutrition and infection. *Monogr Ser World Health Organ.* 1968; 57:3–329.
 25. Schaible U, Kaufmann S. Malnutrition and infection: Complex mechanisms and global impacts. *Plos Med.* 2007;4:e115.
 26. Carswell F, Hughes A, Palmer R, Higginson J, Harland P, Meakins R. Nutritional status, globulin titers, and parasitic infections of two populations of

- Tanzanian school children. *Am J Clin Nutr.* 1981;34:1292–1299.
27. Dominguez-Vazquez A, Alzate-Sanchez A. Nutritional status in children under 6 years of age and its relation to malaria and intestinal parasitism. *Salud Publica Mex.* 1990;32:52–63.
 28. Blair T, Alvarez S, Villa R, Carmona F, Rios O. Nutritional status and immunoglobulin and cytokine concentrations in children with malaria. *Ann Pediatr.* 2003;58:418–424.
 29. Sarr J, Remoue F, Samb B, Dia I, Guindo S, Sow C, et al. Evaluation of antibody response to *Plasmodium falciparum* in children according to exposure of *Anopheles gambiae* s.l or *Anopheles funestus* vectors. *Malar J.* 2007;6:117.
 30. Aribot G, Rogier C, Sarthou J, Trape J, Balde A, Druilhe P, et al. Pattern of immunoglobulin isotype response to *Plasmodium falciparum* blood-stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, West Africa). *Am J Trop Med Hyg.* 1996; 54:449–457.
 31. Perraut R, Guillotte M, Drame I, Diouf B, Molez J, Tall A, et al. Evaluation of anti-*Plasmodium falciparum* antibodies in Senegalese adults using different types of crude extracts from various strains of parasite. *Microbes Infect.* 2002; 4:31–35.
 32. Republic of Kenya. Census report; 1999. Available:www.aridgo.ke/semi-profiles/Mbeere-profiles.pdf
 33. Ministry of Medical Services Report. Intermittent preventive treatment of malaria and pregnancy; 2010.
 34. World Health Organization (WHO). Physical status: The use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser.* 1995;854:1–452.
 35. Nankabirwa V, Tylleskar T, Nankkunda J, Engebretsen I, Sommerfelt H, Tumwine J. Malaria parasitaemia among infants and its association with breast-feeding peer counseling and vitamin. A supplementation. *Plos One.* 2011; 6(70):e21862. DOI: 10.1371/journal.pone.0021862
 36. Dean A, Arner T, Sangam S. Epi info 2000, a data base and statistics program for public health professionals for use on windows 95,98, NT and 2000 computers. Atlanta, Georgia, USA: Center for Disease Control and Prevention; 2000.
 37. World Health Organization (WHO). Use and interpretation of anthropometric indicators of nutritional status. *Bull WHO.* 1986;64:929-41
 38. Ngare D, Mutunga J. Prevalence of malnutrition in Kenya. *East Afri Med. J.* 1999;76:378-80.
 39. Matee M, Msengi A, Simon E, Lyamuya E, Mwinula J, Mbeni E, et al. Nutritional status of under fives attending maternal and child health clinics in Dar es Salam, Tanzania. *East Afr Med J.* 1997;74:368-71
 40. Etuku E, Eguwa M, Muhammad A. Prescription pattern of antimalaria drugs in children below 5 years in tertiary health institution in Nigeria. *Ann Afri Med.* 2008; 7:24-28.
 41. Paul F. Nutritional disorders encountered in children in the department of paediatrics. *Singapore Med J.* 1974;15: 231-40.
 42. Mamabolo R, Alberts M, Steyn N, Delemarre-van de Waal H, Levitt N. Prevalence and determinants of stunting and overweight in 3-year-old black South African children residing in the Central Region of Limpopo Province, South Africa. *Public Health Nutr.* 2005;8:501–508.
 43. Saka A, Saka M, Adeboye M, Mokuolo O, Abu-Saeed M, Abu-Saeed K. Malaria parasitaemia in Children with protein Energy Malnutrition in North Central Nigeria: A case Control Study. *J Pharmacy and Biol Sci.* 2012;49-53:2278-3008.
 44. Samani F, Willet W, Ware J. Nutritional and Socio-demographic risk indicators of malaria in children under five. Across sectional study in Sudanese rural community. *J Trop Med Hyg.* 1987;90:69-78.
 45. Takarura M, Uza M, Sasaki Y, Nagahama N, Phompidas S, Bounyadeth S, et al. The relationship between Anthropometric Indicators of nutritional status and malaria infection among youths in Khammouane Province LAO PDR. *Trop Med Int Health.* 2001;32:2-6
 46. Chan J, Katherine B. Targets of antibodies against *plasmodium falciparum*- infected

- erythrocytes in malaria immunity. Amer soc clin inv. 2012;122:3227-3238.
47. Waterlow J. Classification and definition of protein-calorie malnutrition. Br Med J. 1972;3:566-569.
48. Gomez F, Galvan R, Cravioto J, Frenk S. Malnutrition in infancy and childhood, with special reference to kwashiorkor. Adv Pediatr. 1995;7:131-169.
49. Martoreli R, Khan L, Schroeder D. Reversibility of stunting: Epidemiological findings in children from developing countries. Eur J Clin Nutr. 1994;48:S45-57.

© 2017 Juster et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/18491>