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Association between serum transferrin receptor levels and malaria recurrence in a malaria endemic area in Tanzania

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Abstract

Background: The relationship between body iron levels and malaria presents a complex interaction that provide variable and contradicting results. We designed a study to investigate associations between concentrations of biomarkers of body iron and malaria recurrence among children.

Methods: We conducted a longitudinal descriptive community based study in a malaria endemic area in north- eastern Tanzania. The study involved 428 children of ≤5 years of age who were positive for malaria. Over a period of 6 months, sick children visited the study clinic for screening of malaria and measurement of iron storage biomarkers by serological assays. Correlations between levels of biomarkers and malaria was determined by Spearman correlations and Mann-Whitney U-Test. Associations between malaria recurrence and serum levels of iron biomarkers were determined by odds ratio (OR) with significance cut-off points of <0.15 in univariate and <0.05 in multivariate logistic regression analyses.

Results: Only serum Transferrin Receptor (sTfR) levels had a positive correlation with malaria recurrence. When Mann-Whitney U test was used higher Hepcidin, sTfR and Leptin levels were significantly associated with malaria recurrence when malaria incidence was grouped into 'once' versus 'more than once'. When malaria incidence was recategorised to 'up to twice' versus 'more than twice', only higher level of sTfR was associated with recurrence of malaria. With univariate regression analysis, only sTfR was found to be significantly associated with malaria recurrence, although this associated was not observed in Multivariate analyses.

Conclusion: Despite the absence of association in multivariate analyses, univariate analyses suggest elevated levels of sTFR as a likely predictor of *Plasmodium falciparum* re-infection.

Keywords: serum transferrin receptor, malaria, iron, anaemia, Tanzania

Introduction

Malaria is endemic in 106 countries where it leads to an estimated 216 million cases per year and 655,000 deaths, the majority of which are in sub-Saharan Africa (WHO 2015). Tanzania is heavily affected by malaria, which is one of the leading causes of morbidity and mortality in the country, accounting for over 30% of the national disease burden (Mboera et al., 2013; World Health Organization, 2016).

Iron deficiency is a public health problem worldwide and in Tanzania (Stoltzfus *et al.*, 1997; Mwanri *et al.*, 2000; Horton & Ross, 2003; Finkelstein *et al.*, 2012). The interaction between iron deficiency and malaria is common in sub-Saharan Africa, and is a complex phenomenon. Unfortunately, the relationship between body iron levels and malaria has mainly been studied on situations when iron has been given as a supplement against iron deficiency (Gera & Sachdev, 2002; Sazawal *et al.*, 2006; Berger *et al.*, 2000). A considerable number of previous studies have either associated iron supplementation with occurrence of severe malarial cases or changes in malaria incidence (Gwamaka *et al.*, 2012). Many of such studies had suggested an association between iron supplementation with increased risk of malaria (Murray *et al.*, 1975; Gwamaka *et al.*, 2012; Oppenheimer *et al.*, 1986a). Relatively little is known about the converse relationship between iron deficiency and the incidence of malaria especially in a dramatically changing climate and malaria epidemiology.

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With the current shift in age prevalence of malaria, previously observed associations between iron and malaria may not remain the same, and thus need to be re-assessed (Hawley *et al.,* 2003; Mawili-Mboumba *et al.,* 2013; Athanase *et al.,* 2016). In a more specific way, iron levels in the body have to be assessed for its role on malaria not only using a single parameter, but a panel of indices known to be biomarkers for iron metabolism. We therefore designed an observational longitudinal study to investigate associations between concentrations of known markers of body iron status and malaria incidence among children in a high malaria transmission area of north-eastern Tanzania.

Materials and Methods

Study area

The study was conducted in Bondo Ward (05° 19.447'S, 38°33.249'E) in Handeni District of north-eastern Tanzania. The study site is a coastal rural area located 309 m above sea level. The mean annual rainfall ranges from 600-800mm in most parts of Handeni district. The local populations predominantly are subsistence farmers growing maize and cassava, with oranges being produced seasonally as cash crops. It has a stable and perennial malaria transmission, although peak malaria transmission occurs after long rains from March to June and to some extent after the short rains from October to November (Athanase *et al.,* 2016).

Data collection

This was a longitudinal descriptive community based study. A baseline sampling involving children aged ≤5 years was done to screen for *Plasmodium falciparum* infection in children from March to April 2015. Children were recruited into this study through a comprehensive survey of households in Bondo ward. To be eligible for this study, children were screened for malaria parasites. Confirmation of malaria was based on two tests, the ParaHIT® malaria rapid diagnostic test (mRDTs) (Span Diagnostics, Gujarat, India) for rapid detection and microscopy, a temperature of ≥37.5°C. A 5-10 µl blood drop was used for this test and results read after 5 minutes. Microscopy was done by examining Giemsa stained blood films for malaria parasites by standard methods described previously (Hänscheid, 1999; Tangpukdee *et al.*, 2009). Children who were mRDT positive were managed according to the Tanzania guidelines for managing uncomplicated malaria and those negative for malaria were managed based on the diagnoses made. Children with symptoms of severe malaria were referred to the nearby district hospital in Korogwe. Only malaria positive children were included in the study. Children were followed for presence of malaria parasites by passive case detection for over a period of 6 months. During the 6-month follow-up, children visited the study clinic for examination whenever they were sick to confirm presence of malaria.

Serum levels of selected iron store biomarkers including soluble Transferrin Receptor (sTFR), Dehydroepiandrosterone sulfate (DHEAS), C - reactive protein (CRP), Leptin (Lpt), Hepcidin (Hep) and Ferritin (Fer) were determined at baseline and each time the child had malaria for a period of six months. Determination of serum levels of Ferritin, C-reactive protein (CRP), hepcidin, and soluble transferrin receptor (sTfR) were done as described previously (Coutinho *et al.*, 2005).

Data analysis

Univariate analyses were performed using Spearman correlation analysis and Mann-Whitney U-test. In Mann-Whitney U-test, all values were ranked from low to high and then comparison was made based on the mean ranks. These nonparametric procedures were employed because the data did not meet the normality assumption necessary for parametric tests. The degree of association between malaria recurrence and iron storage biomarkers was measured by odds ratio (OR) with 95% confidence interval (CI). Variables with a p value of <0.15 in univariate logistic regression were considered possible predictors and were included in multivariate logistic regression. All tests were two tailed, and a p value of <0.05 was considered significant.

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Ethical considerations

Ethical approval for this study was granted by the Kilimanjaro Christian Medical University College Ethics and Research Committee with a certificate No. 553. All ethical issues were strictly adhered to during the conduct of this study.

Results

General characteristics of blood samples

Blood samples were collected from a total of 428 patients. Four out of six biomarkers that were studied (Soluble Transferrin Receptor, Dehydroepiandrosterone Sulfate, C - reactive protein and Ferritin) had a response rate above of 96%. Hepcidin and Leptin had response rates of 50.9% and 88.8%, respectively. Analysis was done based on complete cases in each biomarker. Table 1 below summarizes the distribution and characteristics of body iron storage biomarkers among participants.

Table 1: Iron storage biomarkers characteristics among participants, N= 428

Iron storage biomarkers	N (%)	Median	IQR
Hepcidin[a] (ng/ml)	218(50.9)	378.1	226.4 – 533.4
Soluble Transferrin Receptor[b] (ng/ml)	414(96.7)	3678.5	2399.0 – 4865.0
Dehydroepiandrosterone Sulfate [c] (ug/dl)	412(96.3)	660.6	374.3 – 981.4
C-Reactive Protein[d] (mg/dl)	380(88.8)	19.0	8.9 – 40.5
Leptin[e] (pg/ml)	418(96.7)	1844.5	756.4 – 4462.3
Ferritin [f] (mg/dl)	423(98.8)	16.4	8.1 – 46.6

[a] n= 210 (49.1%) missing Hepcidin; [b] n= 14 (3.3%) missing Soluble Transferrin Receptor; [c] n= 16 (3.7%) missing Dehydroepiandrosterone sulfate; [d]n = 48 (11.2%) missing C - reactive protein; [e] n = 10 (2.3%) missing Leptin; [f] n = 5 (1.2%) missing Ferritin

Correlation of iron storage markers with malaria occurrence

The only positive correlation was observed between sTfR and frequency of malaria ($p<0.05$), although the correlation was weak. Correlation between malaria infection and Dehydroepiandrosterone Sulfate, Leptin, Ferritin and log Ferritin was negative but did not reach statistical significance (Table 2). Scatter plots (Figure 1) give a pictorial view of distributions of some of iron storage biomarkers by number of admissions.

Table 2: Correlation analysis between frequency of malaria occurrence and iron storage biomarkers

	Hepcidin	sTfR	CRP	DHEAS	Leptin	Ferritin	sTfR / Ferritin	log(sTfR / Ferritin)	s/log Ferritin	log Ferritin
Malaria incidence	0.086	0.101*	0.016	-0.042	-0.079	-0.002	0.029	0.029	0.045	-0.002

The value expressed as Spearman rank correlation. *Significant at $p<0.05$

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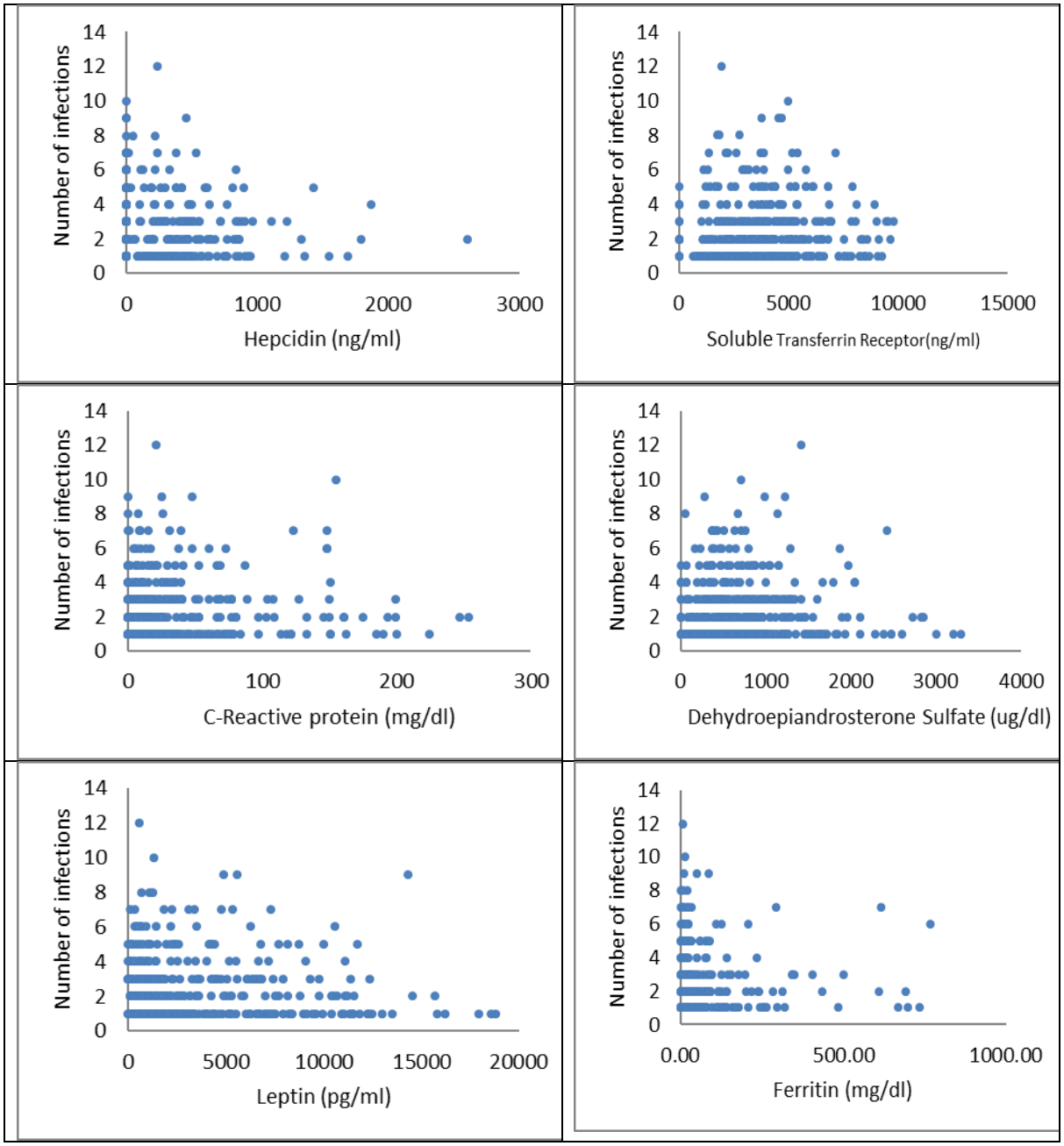

Figure 1: Scatter plots showing the relationship between number of malaria re-infections *versus* iron storage biomarkers among study children

Association between iron storage biomarkers and frequency of malaria infection

Hepcidin, sTfR and Leptin were significantly associated with frequency of malaria occurrence ($p<0.05$) when this frequency was categorized to once and more than once. Higher mean ranks of Hepcidin and sTfR for those who had malaria more than once indicate that patients in this group had higher concentrations of Hepcidin and sTfR (Table 3a). For Leptin, participants who had malaria more than once had a lower mean rank meaning that they had lower concentrations of Leptin (Table 3a). When frequency of malaria occurrence was categorized as 'up to twice' and 'more than twice', only sTfR was significantly associated with it ($p<0.05$). Patients who had malaria more than twice had a higher mean rank of sTfR than those who had malaria up to twice, indicating higher concentration of sTfR in this group (Table 3b). Categorizing frequency of malaria occurrence into other groups of above 4 (i.e. ≤ 3 vs. > 3, ≤ 4 vs. >4, and ≤5 vs. > 5) did not add useful information.

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Table 3a: Relationship between occurrence of malaria more than once during the study period and iron storage biomarkers

	Frequency of malaria occurrence		
	Once	More than once	
Iron storage biomarkers	**Mean rank**	**Mean rank**	**MW-U**
Hepcidin	99.0	118.1	4853.0*
Soluble Transferrin Receptor(sTfR)	191.7	219.3	18177.5*
C-Reactive Protein	188.1	192.3	17296.0
Dehydroepiandrosterone Sulfate	212.6	202.0	19642.5
Leptin	225.5	199.5	18865.0*
Ferritin	203.8	217.9	20313.5
sTfR /Ferritin	207.3	203.3	20004.0
LG(sTfR /Ferritin)	207.3	203.3	20004.0
sTfR /log(Ferritin)	201.5	207.5	19840.0
log(Ferritin)	203.7	217.9	20310.0

*Significant at p<0.05. MW-U: Mann-Whitney U

Table 3b: Relationship between occurrence of malaria more than twice during the study period and iron storage biomarkers

	Frequency of malaria occurrence		
	Up to twice	More than twice	
Iron storage biomarkers	**Mean rank**	**Mean rank**	**MW-U**
Hepcidin	106.2	115.3	5036.0
Soluble transferrin Receptor(sTfR)	198.4	223.4	17450.5*
C-Reactive protein	191.1	189.5	16654.0
Dehydroepiandrosterone sulfate	209.5	201.4	18930.0
Leptin	214.5	203.4	19234.0
Ferritin	218.2	201.4	19177.0
sTfR /Ferritin	197.0	218.8	17350.0
LG(sTfR /Ferritin)	197.0	218.8	17350.0
sTfR /log(Ferritin)	197.6	217.7	17520.0
Log (Ferritin)	218.2	201.4	19180.0

*Significant at p<0.05; MW-U: Mann-Whitney U

Predictors of recurrence of malaria

In univariate regression analysis, the risk for malaria recurrence was significant with sTfR only (p = 0.041). A milligram increase in sTfR increased the chance of malaria recurrence by 10% (OR=1.1; 95%CI = 1.0 – 1.2). However, in multivariate analysis after controlling for Leptin and, there was no association between recurrence of malaria and sTFR (Table 4).

Table 4: Logistic regression analysis for malaria occurrence more than once

		Univariate analysis		Multivariate analysis	
Variables	**Level**	**OR(95%CI)**	**P-value**	**OR(95%CI)**	**P-value**
Hepcidin (ng/ml)	1 mg increase	2.2(0.9-9.2)	0.081	2.0(0.8-4.7)	0.126
Soluble Transferrin Receptor(sTfR) (ng/ml)	1 mg increase	1.1(1.0-1.2)	0.041	1.1(0.9-1.3)	0.243

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C-Reactive Protein (mg/dl)	100 mg increase	1.2(0.7-1.9)	0.445		
Dehydroepiandrosterone sulfate (ug/dl)	0.5 mg increase	0.9(0.7-1.1)	0.160		
Leptin (pg/ml)	4 ng increase	0.8(0.7-1.0)	0.096	1.0(0.7-1.4)	0.884
Ferritin (mg/dl)	100 mg increase	1.1(0.9-1.3)	0.522		
sTfR /Ferritin	500 units increase	1.0(0.9-1.2)	0.632		
log(sTfR /Ferritin)		1.0(0.9-1.1)	0.877		
sTfR /log(Ferritin)	3000 units increase	1.0(0.9-1.2)	0.487		
log(Ferritin)		1.1(0.9-1.2)	0.339		

Discussion

In the present study, three iron storage markers, hepcidin, sTFR and leptin were associated with malaria incidence when individuals who had malaria once were compared with those who had malaria more than once. While higher hepcidin and sTFR concentrations were associated having malaria more than once, children who had malaria more than once had lower leptin concentrations. We show sTFR to be consistently associated with higher malaria incidences even when individuals were differently categorized into those who had malaria twice versus those who had malaria more than twice. With this recategorization, associations between malaria incidence and leptin and hepcidin were lost. DHEAS concentrations did not show any useful associations with malaria. It has long been known that concentrations of sTfR are an indicator of iron status. Iron deficiency causes over expression of transferrin receptor and sTfR levels (Murray *et al.*, 1978; Speeckaert *et al.*, 2010). Transferrin receptor is expressed in cells with high iron demand. sTFR, a single 85 kDa polypeptide, is derived from transferrin receptor by proteolytic cleavage of its extracellular domain. Plasma concentration of sTfR is a reliable indicator of its density on cells and the number of cells expressing it and therefore its concentrations are indicative of both cellular iron demands and to erythropoiesis rate (Speeckaert *et al.*, 2010). Iron deficiency (ID) is among the leading risk factors for death and disability worldwide.

Many previous studies have determined the interactions of iron and malaria risk. Unfortunately, these studies present conflicting results. While some have associated higher iron levels with increased risk of malaria (Nyakeriga *et al.*, 2004; Oppenheimer *et al.*, 1986b), other studies have associated high body iron levels with low malaria risk (Gwamaka *et al.*, 2012) or no effect (Snow *et al.*, 1991). Each of these studies provides an explanation of what could have been the mechanisms for observed effects of body iron levels on malaria risk. Further, different sample sizes, study populations and methodologies used in these studies provide another variable that account for whatever discrepancies of findings. Amidst such conflicting results, a consensus on what is the precise body iron storage biomarker is lacking. While some studies have used Ferritin as the routine marker for measuring body iron levels, others have used sTFR, hepcidin and other markers. Many recent studies have attempted to combine sTFR and Ferritin levels to an index (sTFR/log10 Ferritin) and use the index as a measure of body iron levels.

Iron deficiency in poverty-stricken populations has many aetiologies that range from nutritional, chronic infections, worm infestation and other types on infections (Stoltzfus *et al.*, 1997). Nutritional iron deficiency (ID) arises when physiological requirements cannot be met by iron absorption from diet. Infection and inflammation increase ferritin levels, an important iron storage biomarker which is an acute phase reactant. The interaction of the many ID causes brings in the challenge of establishing a direct causal effect association between ID and risk of malaria particularly in individuals living in tropical settings where multiple infections may lead to the presence of continuous inflammatory processes, which interfere with the body levels of some markers such as ferritin, hepcidin and transferrin (Małyszko *et al.*, 2005; Kuragano *et al.*, 2010; Skikne *et al.*, 2011). Moreover, chronic hookworm infestation is known to be a strong predictor of iron status (Layrisse & Roche, 1964; Stoltzfus *et al.*, 1997; Hopkins *et al.*, 1997; Dreyfuss *et al.*, 2000).

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Our findings of lower sTFR concentrations in individuals who had malaria more than once, suggests ID to be a risk factor for malaria. Majority of individuals with high likelihood of having multiple parasite infections are the same individuals who had malaria in the study site, both of which are known to modulate iron metabolism. Therefore, interpretations of presence or absence of associations between body iron levels and malaria risk needs extreme care and must inevitably involve screening for most known causes of ID. Although it has been proposed that iron supplementation might predispose children to malaria, a recent systematic review by Neuberger *et al.* (2011) shows that, in areas where malaria control services are sufficient, iron supplementation may reduce malaria incidence.

For about a decade now, the Tanzanian National Voucher Scheme (TNVS) has significantly scaled up availability and accessibility of insecticide-treated nets (ITNs) to particularly pregnant mothers and children by subsidizing costs of bed-nets (Renggli *et al.*, 2013). A number of similar programmes aimed at strategically controlling malaria such as Under-five Catch-up Campaign and Universal Coverage Campaign continued to provide an effective integrated malaria control environment through increased ITN use, subsidized tests, artemisin-based medicines and massive community sensitization (Bonner *et al.,* 2011). The net result of these strategies is reduction in incidence and child mortality rates in Tanzania (Renggli *et al.*, 2013).

As previously suggested by Verhoef et al. (2001), that elevated sTfR concentrations, a parameter thought to predict erythropoietic activity, suggests increased erythropoiesis in asymptomatic parasitemia. We show that higher sTFR levels, indicative of reduced body iron levels, were associated with higher malaria re-infection rates. Although no explanation has been provided by previous studies based on this argument, we speculate that by strict malaria control, individuals are left with other ID causes such as helminths in the absence of solid immunity to malaria. Helminth infections are thought to contribute to malnutrition which in turn can also predispose individuals to infections including malaria (Mwangi *et al*., 2006; Nacher et al., 2002). Associations between high sTFR and higher malaria incidence may imply a non-malarial iron deficiency predisposing study individuals with low anti-malarial immunity to more malaria infections.

Reduced hepcidin is indicative of physiological response to an iron deficit and together with other iron storage biomarkers such as ferritin and sTfR they reflect different aspects of iron metabolism. It is crucial that evaluation of these indices should be undertaken concomitantly in order to provide complementary clinical information. Further studies are required to evaluate the role of hepcidin in the diagnosis of iron deficiency in other groups of patients (Pasricha *et al.*, 2011).

Our study was limited by the short follow up of children and absence of other factors known to affect malaria incidence. However, we report valuable data regarding associations of studied parameters with elevated risk of *P. falciparum* re-infections. Since iron metabolism involves a complex physiology, we recommend that future studies that seek to evaluate the effect of body iron on malaria should also study distinct iron deficiency markers and causes of ID together and their independent influence on malaria.

Competing Interests

The authors declare that they have no competing interest

Authors' contributions

UD, EA, PI, CK, CM participated in data collection and analyses and made significant reviews of the manuscript. LM and PH provided critical advice on data analysis and manuscript writing. JC supervised the study and wrote the manuscript. All authors read and approved the final version of the manuscript.

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