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Relationship between exposure to malaria and haemoglobin level of children 2–9 years old in low malaria transmission settings

Zewdie Birhanu[a,*], Yemane Ye-ebiyo Yihdego[b], Daniel Emana[c], Damtew Feyissa[d], Silashi Kenate[d], Estifanos Kebede[c], Kefelegn Getahun[e], Delenasaw Yewhalaw[f]

[a] Department of Health Education and Behavioral Sciences, Institute of Health, Jimma University, P.O. Box 378, Ethiopia
[b] Abt Associates, Africa Indoor Residual Spraying, Accra, Ghana
[c] Department of Medical Laboratory Sciences and Pathology, Institute of Health, Jimma University, Ethiopia
[d] Oromia Regional Health Bureau, Jimma Zone Health Department, Jimma, Ethiopia
[e] College of Social Sciences and Humanities, Jimma University, Ethiopia
[f] Tropical and Infectious Diseases Research Center, Jimma University, Jimma, Ethiopia

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ABSTRACT

In the context of reduced transmission of malaria, it is essential to examine the association between exposure to malaria and haemoglobin level. This study measured the Haemoglobin level of children 2–9 years of age and examined its association with malariometric indices. A cross sectional study was conducted, during June 2016, on 763 children 2–9 years old, recruited from ten sites representing different malaria transmission settings in Ethiopia. Haemoglobin concentration was determined using HemoCue analyzer. Malariometric indices (splenomegaly rate, parasite rate and serological marker) were measured. The overall prevalence of anaemia was 17.3% (95% CI: 14.6–19.9) in the study population. Mild, moderate and severe anaemia accounted for 7.3%, 7.2% and 2.8% respectively. Of the children with anaemia (132), only 7 (5.3%) had malaria parasitaemia. The prevalence of malaria parasitaemia was 3.6% (2/56), 9.1% (5/55) and 0.0% (0/21) among children with mild, moderate and severe anaemia, respectively. Malaria reactive antibody and anaemia co-occurred in 3.13% (21/672) of the samples. Seroprevalence and parasitaemia did not have significant association with anaemia ($p > 0.05$). However, splenomegaly was significantly associated with increased risk of anaemia (AOR = 14.93; $p = 0.001$). Anaemia was significantly higher among children 2–4 years old (22.2%), and children living in households without any insecticide treated bed net (34.0%). The prevalence of anaemia was lower by 55.0% among children living in households with at least one net (AOR = 0.45, 95% CI: 0.21–0.96). Repeated exposure to malaria infections (seropositive) and parasitaemia was less likely to contribute to development of anaemia among children 2–9 years in this study setting. Thus, in low malaria endemic settings, anaemia prevention and control program required to reconsider the historical evidence that suggests malaria is one of the major risk factor for anaemia.

1. Introduction

Anaemia is a condition in which the haemoglobin concentration falls below an established cut-off value thereby consequently impairing the capacity of the blood to transport oxygen around the body (World Health Organization, 2014). In anaemic individuals, the number of red blood cells and their oxygen carrying capacity is inadequate to meet physiologic needs, resulting in an increased morbidity (World Health Organization, 2014, 2008). In developing countries, over half of preschool children are estimated to be anaemic (World Health Organization, 2014, 2008). In Ethiopia, 43.1% and 6.9% of the children under the age of five had mild and moderate anaemia, respectively (EHNRI, 2012). According to the Ethiopian Demographic and Health Survey (EDHS), about 44% of Ethiopian children were anaemic in which mild, moderate and severe anaemia accounted for 21%, 20% and 3% respectively. According to this report, the prevalence of anaemia was higher among rural children (45%) as compared to children urban settings (35%) (Central Statistical Agency of Ethiopia, 2011). Similar community based studies also reported a magnitude of anaemia that is as high as 66.6% (Gutema et al., 2014; Mesfin et al., 2015; Assefa et al., 2014; Deribew et al., 2010). According to the World Health Organization (WHO), anaemia is a major public health problem in Ethiopia

* Corresponding author.
E-mail addresses: zbkoricha@yahoo.com (Z. Birhanu), yemaneyy@gamil.com (Y.Y.-e. Yihdego), aga.danie2009@gmail.com (D. Emana), dfjebessa@gmail.com (D. Feyissa), siles2009@yahoo.com (S. Kenate), estifka@yahoo.com (E. Kebede), kefish2002@gmail.com (K. Getahun), delenasawye@yahoo.com (D. Yewhalaw).

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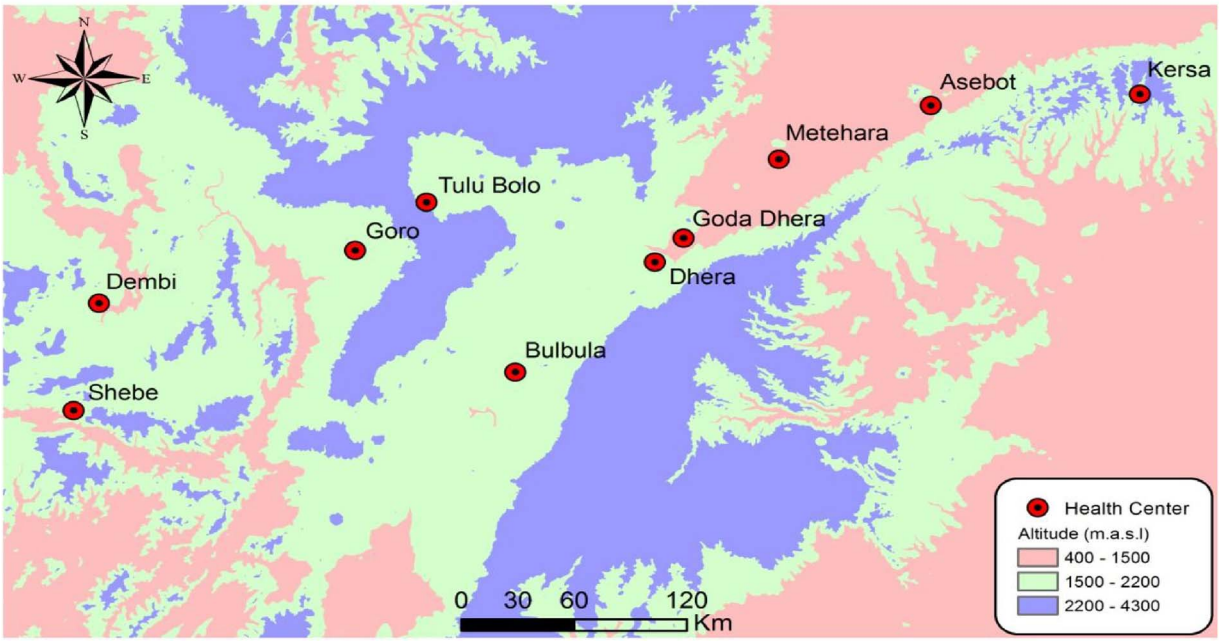

Fig. 1. Map of the study area.

(World Health Organization, 2008).

Anaemia has numerous short and long term health impacts on children: It affects their cognitive and psychomotor development and learning performance; impairs behavioral and physical growth with increased risk of morbidity and mortality (World Health Organization, 2014; WHO et al., 2001). Furthermore, anaemia impairs language coordination capacity of children and is also linked to poor intelligent quotient (WHO et al., 2001). Overall, anaemia remains one of the obstacles to national development, and it is an indicator of both poor nutrition and poor health status (World Health Organization, 2008).

Even though anaemia involves a variety of causes, the majority of *Plasmodium* infections are concomitant with some degree of anaemia among children especially in tropical areas where malaria is endemic (WHO et al., 2001; Douglas et al., 2012; Collins et al., 2003; Satpathy et al., 2004). Although its severity depends on a number of factors including individual and parasite-specific factors, malaria anaemia is capable of causing severe morbidity and mortality especially in individuals infected with *P. falciparum* (World Health Organization, 2008; Douglas et al., 2012; Collins et al., 2003). Essentially, severe form of malaria contributes up to 62% of severe malaria admissions in malaria endemic settings (Satpathy et al., 2004). Thus, malaria anaemia, particularly, severe malaria anaemia (SMA) is a complex disease leading to rapid haemoglobin reductions of 20–50% (Sowunmi et al., 2011). Studies done in Ethiopia and elsewhere widely documented that infections with malaria parasite associated with anaemia (Gutema et al., 2014; Douglas et al., 2012; Collins et al., 2003; Satpathy et al., 2004; Jamal et al., 2015; Newton et al., 1997; Carneiro et al., 2006; Rogerson, 2017; Sumbele et al., 2016; Deribew et al., 2013; Gari et al., 2017; Ketema and Bacha, 2013; Kateera et al., 2015; Alemu et al., 2012; Barreiro et al., 2017; Magalhães and Clements, 2011; Menendez et al., 2000; Safeukui et al., 2015; Erhabor et al., 2014; Crawley, 2004). Moreover, systematic reviews and meta-analysis also support that malaria is one of the major risk factors for anaemia (Kassebaum et al., 2014; McCuskee et al., 2014; Korenromp et al., 2004; World Health Organization, 2015a; Brooker et al., 2007). In addition, some evidences implied that the amount of anaemia in a population is a proxy for estimating the endemicity and burden of malaria (World Health Organization, 2015a; Brooker et al., 2007; Korenromp et al., 2004; McCuskee et al., 2014; Senn et al., 2010; Savage et al., 2007).

In tropical areas, malaria control measures have significant impacts on overall reduction of anaemia, and as a result, malaria control program is considered to be a collateral strategy for reduction of anaemia particularly among highly susceptible group (Korenromp et al., 2004; World Health Organization, 2015a; Brooker et al., 2007; Crawley, 2004). These days, malaria elimination program has received utmost global and national priorities (World Health Organization, 2015b, 2016). Likewise, Ethiopia has given a considerable attention to malaria elimination program with the aim to maintain the current gains and accelerate the progress towards elimination targets (Ministry of Health, 2014). Hence, malaria elimination program has great advantage for anaemia prevention and control efforts suggesting the need to carefully combine anaemia prevention strategies with malaria elimination initiatives. In malaria endemic settings and in areas with recently reduced malaria transmission, many children with malaria parasitaemia are asymptomatic, thus malaria remains undiagnosed (Okell et al., 2012; Okell et al., 2009; World Health Organization, 1988). This situation could also contribute to under-diagnosis of anaemia associated with malaria. Therefore, it is vital to measure the association between exposure to malaria and anaemia, particularly in the context of reduced transmission of malaria. Previous scientific inquiries mainly focused on classical metrics of malaria such as parasitaemia and spleen size to assess the association between exposure to malaria and haemoglobin level and little is known about the association between cumulative exposure (i.e. presence of anti-malarial antibodies) to malaria and its effects on haemoglobin level among children in most malaria endemic settings including Ethiopia. Therefore, this study aimed at examining the association between malariometirc indices (parasitaemia, spleen rate and anti-malarial antibody levels) and haemoglobin level/anaemia among children of 2–9 years old living in different malaria transmission settings of Ethiopia. Consequently, this study could add to a growing body of literature on effects the relationship between malaria and anaemia in the context of malaria elimination targets.

2. Material and methods

2.1. Study setting

The study was conducted in June 2016 as part of a larger

community based cross sectional study aimed at quantifying malaria endemicity in Ethiopia. The data were collected mainly from sentinel sites established for malaria surveillance in Ethiopia (Fig. 1). The surveillance sites consisted of ten Primary Health Care Units (PHCUs) covering a different eco-epidemiologic settings with low to high malaria transmission in Ethiopia (Yukich et al., 2014). Eight of the ten sentinel sites (i.e. Kersa, Goro, Metehara, Bulbula, Tulu Bollo, Asebot, Dhera, and Dembi) were included in the study and two sentinel sites were replaced by similar primary health care units (i.e Goda Dhera and Shebe Sombo) due to local priorities and some problems associated with accessing the study community (Yukich et al., 2014).

2.2. Sample size and sampling techniques

For the main study, samples were collected from children 2–9 years of age as per the WHO's recommendation for malaria endemicity study (World Health Organization, 1968). The sample size was determined using single population formula, $n = (z_{1-\alpha/2})^2\, p\,(1-p)/d^2$, based on the following assumptions: Prevalence of malaria (parasite rate) among children (p = 10.5%) (Deribew et al., 2012), 2% marginal error, 95% confidence interval, and 15% non-response rate. This yields a sample size of 1038 children.

2.3. Sampling procedure

Children were sampled from ten PHCUs (eight malaria sentinel sites, and two non-malaria sentinel sites) in Oromia Regional state, Ethiopia. In brief, sampling process was implemented as follows: First, the sample size (1038 children) was equally allocated to each PHCU. Second, considering resource available for the study, two satellite health posts/villages, which are parts of the PHCU were selected randomly. Equal numbers of children were considered from each of the selected health posts. Within the selected health posts, list of households with eligible children (age 2–9 years) was obtained from family register which was employed to select eligible households using simple random sample. Then, parents were invited to the nearest health facility through community based health workers. Up on arrival, parents were given detail information about the study purpose and signed written consent for children that participated in the study. Parents were interviewed to collect children's demographic data, history of malaria attack, fever, ownership, access to and use of Long-Lasting Insecticidal Net (LLIN) (Roll Back Malaria Partnership, 2011; Roll Back Malaria and WHO, 2009).

2.4. Determination of haemoglobin concentration

Haemoglobin concentration was determined using HemoCue analyzer in the field (HemoCue Hb 301, Sweden). Anaemia was classified according to WHO's guideline (INACG, 2002) using altitude and age adjusted haemoglobin (Hb) values: non-anaemia (Hb ≥ 11.0 g/dL), mild anaemia (10.0–10.9 g/dL), moderate (7.0–9.9 g/dL), severe (< 7.0 g/dL) for children 2–5 years. For children 5–9 years of age Hb ≥ 11.5 was considered as non-anaemic and mild anaemia; moderate and severe anaemia was defined as Hb 11.0–11.4 g/dL, 8.0–10.9 g/dL, and Hb < 8.0 g/dL respectively (INACG, 2002; World Health Organization, 2011).

2.5. Malariometric indices

2.5.1. Splenomegaly rate (SR)

Spleen palpation was conducted by clinician at the 'standing position' technique as suggested by WHO (World Health Organization, 1968). For the determination of the degree of enlarged spleens, Hackett's method of classification was used. Consequently, the size of enlarged spleen was graded as '0' normal spleen (not palpable even on deep inspiration); "1" spleen palpable below the costal margin; '2' spleen palpable below the costal margin, but not projected beyond a horizontal line half way between the costal margin and the umbilicus, measured along a line dropped vertically from the left nipple; '3' spleen with lowest palpable point projected more than half way to the umbilicus but not below a line drawn horizontally through it; '4' spleen with lowest palpable point below the umbilical level but not projected beyond a horizontal line situated half way between the umbilicus and the symphysis pubis; '5' spleen with lowest point palpable beyond the lower limit of class. Spleen rate was estimated as proportion of children with palpable spleens; individuals of with classes 1–5 of the size of palpated spleen.

2.5.2. Malaria parasitaemia

For detection of malaria parasitaemia and antibody, 3–5 ml venous blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes from each child who participated in the study. For children who refused to give venous blood or venous blood was insufficient, finger prick was used to get the blood sample for Rapid Diagnostic Test (RDT), blood film examinations and Haemoglobin determination. For RDT, CareStart malaria Pf/Pv (HRP2/pLDH) Ag Combo RDT was used. For microscopic examination, thick and thin films were prepared and air-dried in the field, and the thin film was fixed with methanol; both smears were stained with 10% Giemsa stain. Each slide was read by an experienced laboratory technologist at Jimma University Specialized Hospital. Absence of malaria parasite in 100x oil immersion objective in thick blood film was considered as negative. A slide was considered as negative after 100 fields were examined and no parasite seen (World Health Organization, 2010).

2.5.3. Enzyme immuno assay (EIA) malaria antibody detection

Malaria EIA test (BioRad) that uses four recombinant antigens in a sandwich form was used to detect antibodies to malaria during all stages of infections. The test detects specific antibodies (i.e. IgG, IgM and IgA) to the four species of *Plasmodium* parasites; *P. falciparum, P. vivax, P. ovale and P. malariae*. Whole blood collected into EDTA was centrifuged to get plasma for malaria antibody detection. The test was based on the binding of specific antibodies present in the plasma to antigens immobilized on a 96-well EIA plate. The assay was performed as recommended by the manufacturer (Malaria EIA AbAssay and Clinical Diagnostics, 2016). The plate was read at absorbance wavelength of 450 nm using automated EIA plate reader. The cut-off value was calculated as the mean of the optical density (OD) of value of the three negatives control plus 0.100. Samples with OD values greater than cut-off value were considered as positive for specific antibody to *Plasmodium* species. The test does not distinguish between *Plasmodium* species, nor IgG and IgM and between an acute and chronic infection.

2.6. Data processing and analysis

The data were analyzed using stata version 12.0. In this study, anaemia status was the main outcome variable and independent variables included background characteristics (age, sex, place of residence, family size), LLIN indicators (ownership, previous night use, and access). Ownership of LLIN was defined as the proportion of sampled households with at least one LLIN. On the other hand, access to LLIN was defined as 1) sufficient LLIN access-a situation in which at least two nets were available for every two people in the household and 2) no access to LLIN means households who had some nets or no nets at all (Roll Back Malaria Partnership, 2011; Roll Back Malaria and WHO, 2009). In addition, life time history of malaria attack, two weeks history of fever, fever (axillary body temperature ≥37.5 °C), malariometric indices (parasitaemia, reactive antibody, and splenomegaly) was considered as independent variables. Analyses were done segregated by study sites and with demographic factors like age and sex. One-way ANOVA was used to compare mean optical density by anaemia status. Logistic regression was used to assess association of some background

factors with anaemia. In addition, the relationship between malariometric indices and anaemia was explored using logistic regression. A 95% confidence interval and level of significance less than 0.05 were used to declare statistically significant association.

2.7. Ethical consideration

The study was reviewed and approved by Jimma University Institutional Review Board (Ref. No. RPGC/112/2015) and the World Health Organization Ethics Review Committee (Protocol ID: B40082, approval date: 15/06/2015). In addition, the study was reviewed and approved by Oromia regional health bureau (Ref. No. BEFO/AHITQFTF050/83). Permission to undertake the study was obtained at all levels. Parents were given detailed information about the purpose of the study, specimen collection procedures and possible risks/discomforts and benefits of participating in the study through consent process. Informed written consent was obtained from all parents/caretakers whose children participated in the study. In this case, caretakers were mostly parents (either mother or father), and in few cases, sisters, brothers or other close relatives were presented as caretakers. A child was included in the study only if the child the caretakers both agreed. Despite parental consent, a child's decision not to give blood was respected. Children diagnosed with malaria parasitaemia (using RDT) received anti-malarial treatment, according to the national malaria treatment guideline. Parents' whose children had mild and moderate anaemia were counseled on nutritional management of anaemia. Children with severe anaemia were referred directly to the nearest health facility for further evaluation and treatment.

3. Results

3.1. Demographic characteristics of the participants

Seven hundred sixty-three children participated in the study, making an overall response rate of 73.1%. Haemoglobin analysis, spleen rate and examination of malaria parasitaemia was based on 763 samples. However, due to inadequate blood sample, malaria EIA antibody test was done only for 672 samples. Males represent more than half, 53.1% (405), of the participants, and the mean age was 5.2 ± 1.9 years. In terms of place of residence, 72.7% (555) were recruited from rural areas.

3.2. Haemoglobin levels among children

The overall mean haemoglobin level of the children was 12.6 g/dL ± 1.7 (min = 3.3 g/dL, max = 16.3 g/dL). The mean haemoglobin level significantly varied by study sites (F test = 17.5, p = 0.001). Fig. 2 shows the distribution of haemoglobin level by study sites. The lowest mean haemoglobin level was seen in Asebot (10.6 g/dL) which was significantly lower compared to all other study sites (p = 0.001). On the other hand, highest mean haemoglobin level was recorded in Tullu Bollo (13.4 g/dL) and it was significantly different as compared to mean haemoglobin level from Asebot (p = 0.001), Kersa (p = 0.005), Dhera (p = 0.004), and Metehara (p = 0.041). Altitude has a significant effect on haemoglobin concentration; a unit increase in altitude (in meter) was associated with 0.01 g/L increment of haemoglobin concentrations (β = 0.01 g/L, 95% CI: 0.00–0.02, p = 0.001).

3.3. Prevalence of anaemia among children

The overall prevalence of anaemia was 17.3% (132/763, 95% CI: 14.6–19.9%). Mild, moderate and severe anaemia accounted for 7.3% (56/763), 7.2% (55/763) and 2.8% (21/763) respectively. The prevalence of anaemia was significantly different by study sites (x^2 = 107.75, p = 0.001). Fig. 3 shows the distribution of anaemia by study sites. Accordingly, in Asebot site, more than half, 56.1% (41/73), of the studied children had anaemia. In this community, 13.7% (10/73) of the children had severe anaemia. A large proportion of children in Kersa (24.2%, 12/50) and Dhera (24.0%, 23/95) also suffered from Anaemia. In contrast, the prevalence of anaemia was very low in Shebe (5.0%, 4/80) and Metehara (6.0%, 5/84).

3.4. Demographic factors associated with anaemia

Table 1 presents results of multivariate analyses of selected demographic characteristics associated with anaemia among children aged 2–9 years old. Consequently, only age of the child and ownership of LLIN significantly associated with anaemia. Anaemia was significantly higher among children of 2–4 years (22.2%) and households without LLIN (34.0%). Accordingly, children aged 2–4 years old were 2.24 times more likely to be anaemic compared to children in the age group of 7–9 years. Likewise, prevalence of anaemia was lower by 55.0% among children living in households with at least one LLIN.

3.5. Malariometric indices and anaemia

Table 2 presents malariometric indices by degree of anaemia. Overall, malaria parasitaemia was found in 2.5% (19/763) of the cases (using RDT), and infection rate was 1.6% (12/763), and 1.2% (9/763) for *P. falciparum and P. vivax* respectively. Generally, detectable parasitaemia and anaemia co-occurred in 0.9% (7/763) of the children. In other words, of the 132 children with anaemia, 5.3% (7/132) had detectable malaria parasitaemia. Only 3.6% (2/56) children with mild anaemia had detectable malaria parasitaemia. Malaria parasitaemia was relatively prevalent among children with moderate anaemia, 9.1% (5/55). However, none of the children with severe anaemia had detectable malaria parasitaemia. *P. falciparum* more frequently co-occurred with moderate anaemia; of the six *P. falciparum* infections associated with anaemia, five were found among children with moderate anaemia.

The study also indicated that malaria reactive antibody and anaemia co-occurred in 3.1% (21/672) of the samples available for EIA antibody test. The prevalence of anaemia was approximately equal among malaria EIA reactive (16.7%, 21/126) and non-reactive (88/546) children. The study found that as the severity of anaemia increased, the seropositive rate for malaria decreased. The prevalence of reactive malaria antibody was 20.4% (10/49), 19.0% (8/42) and 16.7% (3/18) among children with mild, moderate and severe anaemia, respectively. In terms of age, the prevalence of reactive malaria antibody was lower among under five children (13.1%) compared to children ≥five years old (22.1%) whereas anaemia was higher among under five children and lower among older children (data not shown). With respect to relationship between splenomegaly and anaemia, of the children with enlarged spleen (n = 13), 61.5% (8/13) had anaemia.

3.6. Association between malariometric indices and anaemia

Even though in multivariate analysis no significant association was observed, bivariate analysis indicated that children infected with *P. falciparum* were 4.96 times more likely to be anaemic as compared to children with no detectable *falciparum* (OR: 4.96, 95% CI: 1.57–15.63, p = 0.006). On the contrary, of children with *P. vivax* infections, only 2 were anaemic and infections with *P. vivax* did not show significant association with anaemia. Moreover, no significant association was observed between prolonged exposure to malaria (reactive immunity) and anaemia, (p > 0.05). However, children with enlarged spleen were 14.93 times more likely to have anaemia as compared to children with non-palpable spleen (AOR = 14.93, 95% CI: 3.57–62.40; p = 0.001) (Table 3).

The mean haemoglobin concentration also did not vary significantly with malaria serostatus (p > 0.05). The mean haemoglobin level was 12.79 ± 1.43 g/dL and 12.62 ± 1.68 g/dL among the exposed (i.e.

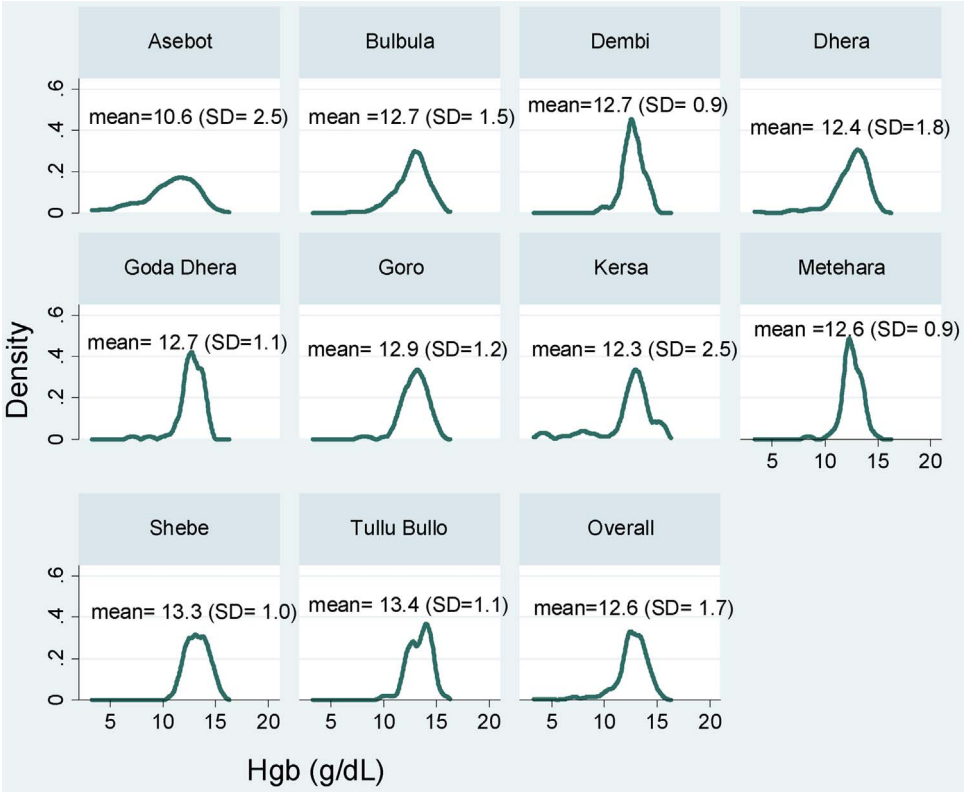

Fig. 2. Probability density function of Haemoglobin level by study sites, June 2016, Ethiopia.

reactive antibody) and the unexposed (non-reactive antibody), respectively. Fig. 4 shows the distribution of haemoglobin level by reactive malaria antibody. The distribution of haemoglobin level in both reactive and non-reactive subjects approximately follow the same distribution, with slight skewed to left for subjects' non-reactive to malaria antibody test. Haemoglobin level was regressed against malaria antibody optical density (OD) (Fig. 5) and the result indicated that the regression model explained none of the variability of haemoglobin level among children (R^2 = 0.0%). Only few children with mild (10/56), moderate (8/55) and severe (3/21) anaemia were found in reactive (OD

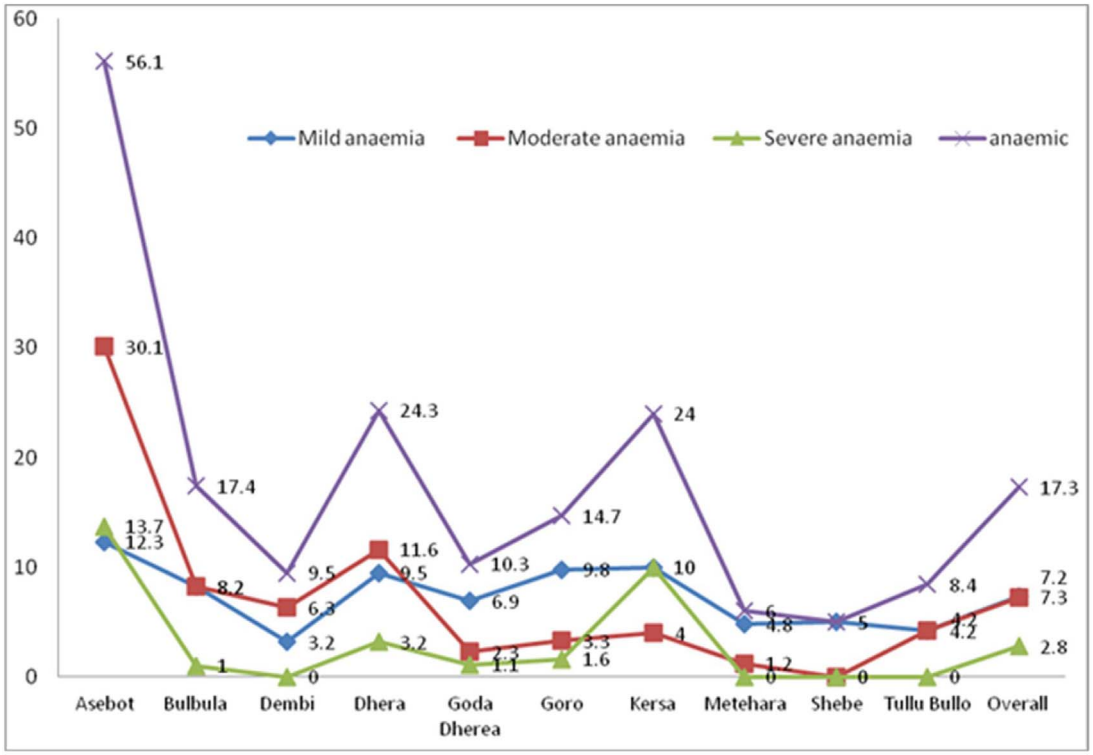

Fig. 3. Prevalence of anaemia by study sites, June 2016, Ethiopia.

Table 1
Selected background factors associated with anaemia, Ethiopia, June 2016.

Variables		Anaemia		AOR(95%CI)
		No	Yes	
Sex	Male	338 (83.5)	67 (16.5)	0.92 (0.62–1.37)
	Female	293 (81.8)	65 (18.2)	1
Place of residence	Rural	440 (79.3)	115 (20.7)	1.67 (0.79–3.52)
	Urban	102 (93.6)	7 (6.4)	0.44 (0.14–1.36)
	semi-urban	89 (89.9)	10 (10.1)	1
Age of child	2–4	242 (77.8)	69 (22.2)	2.24 (1.33–3.77)*
	5–6	186 (83.4)	37 (16.6)	1.48 (0.84–2.63)
	7–9	203 (88.6)	26 (11.4)	
Family size	1–4	228 (86.0)	37 (14.0)	0.67 (0.40–1.09)
	≥5	340 (79.1)	90 (20.9)	1
Ownership of LLIN	Yes	535 (82.9)	110 (17.1)	0.45 (0.21–0.96)**
	No	33 (66.0)	17 (34.0)	1
Previous night LLIN use	Yes	401 (83.7)	78 (16.3)	0.88 (0.55–1.42)
	No	167 (77.3)	49 (22.7)	1
Access to LLIN	No	319 (79.6)	82 (20.4)	0.89 (0.55–1.46)
	Yes	249 (84.7)	45 (15.3)	1
Altitude above seas level	< 1500m	263 (84.0)	50 (16.0)	2.22 (0.89–5.52)
	1500–2000 m	302 (79.9)	76 (20.1)	2.20 (0.88–5.46)
	> 2000 m	66 (91.7)	6 (8.3)	1

* Significant at 0.01.
** Significant at 0.05.

cut-off value > 0.149) region of malaria antibody.

3.7. Intensity of malaria antibody and anaemia

Strength of malaria antibody (i.e. as measured mean optical density) did not significantly vary with degree of anaemia (P > 0.05). Nevertheless, highest mean OD value was recorded among children with mild anaemia (mean OD = 0.561) followed by moderate anaemia (mean OD = 0.430), but it decreased as severity of anaemia increased. Lowest mean OD value was noted for children with severe anaemia (mean OD = 0.327), even compared to children without anaemia (mean OD = 0.414).

Table 3
Association of malaria indices with anaemia among children 2–9 years old, Ethiopia.

Malaria indices and related variables	p-value	AOR (95%IC)
Two weeks history of fever (Yes, No[a])	0.375	0.75 (0.39–1.42)
Fever (Yes, No[a])	0.087	0.13 (0.01–1.35)
History of malaria (reported) (Yes, No[a])	0.309	0.77 (0.46–1.28)
Splenomegaly (Yes, No[a])	0.001	14.93 (3.57–62.40)
Malaria parasitaemia (RDT) (Yes, No*)	0.462	5.22 (0.06–426.79)
P. falciparum (Yes, No[a])	0.784	1.79 (0.03–115.12)
P. vivax (Yes, No[a])	0.539	0.31 (0.01–13.20)
Reactive malaria antibody (Yes, No[a])	0.252	0.68 (0.35–1.32)

[a] Reference category.

3.8. Levels of anaemia and seropositive rate by study sites

The pattern of anaemia and reactive malaria antibody was irregular. In Asebot and Kersa where overall anaemia was very high, reactive malaria antibody was extremely low. Conversely, in areas with high reactive malaria antibody such as Goro and Goda Dhera, overall anaemia was relatively low (Fig. 6).

4. Discussion

This study measured haemoglobin level and magnitude of anaemia among children aged 2 − 9 years old in malaria endemic settings of Ethiopia and explored its relationship with malariometric indices and selected demographic factors. Haemoglobin concentration is the most reliable indicator of anaemia at the population level (World Health Organization, 2008). The mean haemoglobin level (12.6 g/dL ± 1.7) obtained in the present study was somewhat higher as compared to earlier finding in Ethiopia (Assefa et al., 2014). Of course, there was also a study that reported a similar level of haemoglobin concentration from Ethiopia (Mesfin et al., 2015). Moreover, magnitude of anaemia was low as compared to many earlier studies (Assefa et al., 2014; Ketema and Bacha, 2013; World Health Organization, 2011; Mesfin et al., 2015; Choge et al., 2014; Noland et al., 2014). Correspondingly, the prevalence of mild, moderate and severe anaemia was also low

Table 2
Malariometric indices and anaemia among children 2–9 years old, Ethiopia.

Malariometric indices and related variables	Category	Anaemia status		Degree of anaemia		
		Non-anaemia	Anaemia	Mild anaemia	Moderate anaemia	Severe anaemia
Malaria Parasitaemia (RDT)	Negative	619 (98.1)	125 (94.7)	54 (96.4)	50 (90.9)	21 (100)
	Positive	12 (1.9)	7 (5.3)	2 (3.6)	5 (9.1)	0 (0)
Malaria Parasitaemia (Microscopy)	Negative	620 (98.3)	125 (94.7)	54 (96.4)	50 (90.1)	21 (100.0)
	Positive	11 (1.7)	7 (5.3)	2 (3.6)	5 (9.9)	0 (0.0)
P. falciparum	Negative	625 (99.0)	126 (95.5)	55 (98.2)	50 (90.9)	21 (100)
	Positive	6 (1.0)	6 (4.5)	1 (1.8)	5 (1.9)	0 (0)
P. vivax	Negative	624 (98.9)	130 (98.5)	55 (98.2)	54 (98.2)	21 (100)
	Positive	7 (1.1)	2 (1.5)	1 (1.8)	1 (1.8)	0 (0)
Spleen size	Normal	626 (99.2)	124 (93.9)	52 (92.9)	53 (96.4)	19 (90.5)
	Enlarged	5 (0.8)	8 (6.1)	4 (7.1)	2 (3.6)	2 (9.5)
Malaria antibody	Negative	458 (81.3)	88 (80.7)	39 (79.6)	34 (81.0)	15 (83.3)
	Positive	105 (18.7)	21 (19.3)	10 (20.4)	8 (19.0)	3 (16.7)
Reported history of malaria	No	466 (82.0)	102 (18.0)	37 (68.5)	38 (73.1)	17 (81.0)
	Yes	109 (85.8)	18 (14.2)	17 (31.5)	14 (26.9)	4 (19.0)
Fever	No	373 (65.7)	195 34.3)	55 (98.2)	54 (98.2)	21 (100)
	Yes	92 (72.4)	35 (27.6)	1 (1.8)	1 (1.8)	0 (0)
Two weeks history of fever	No	466 (82.0)	102 (18.0)	44 (81.5)	45 (86.5	20 (95.2)
	Yes	109 (85.8)	18 (14.2)	10 (18.5)	7 (13.5)	1 (4.8)

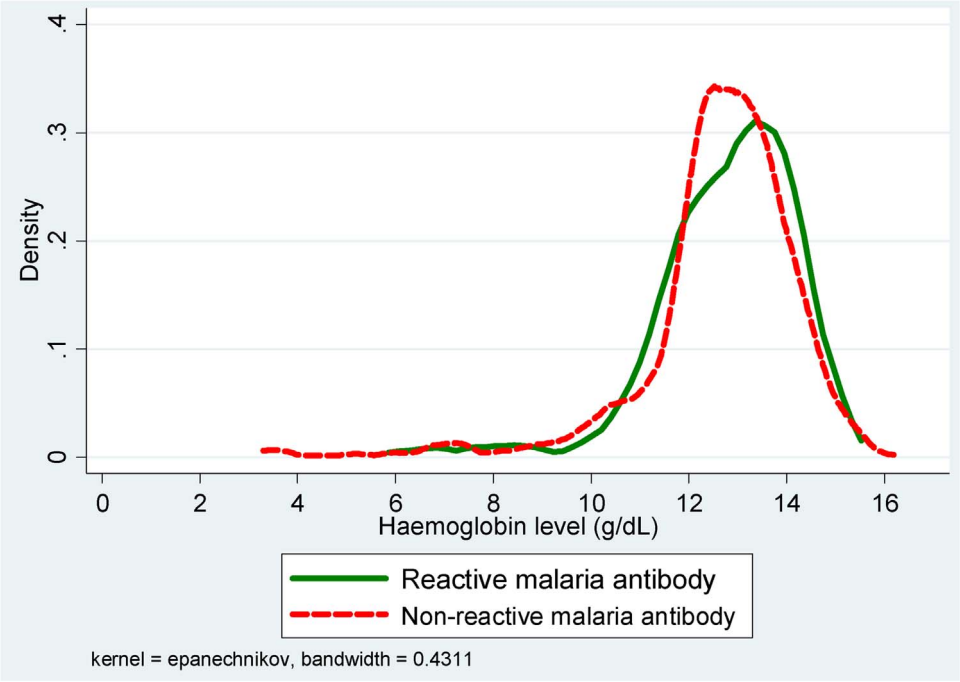

Fig. 4. Distribution of haemoglobin level among children 2–9 years old, by reactive malaria antibody status, Ethiopia (June 2016).

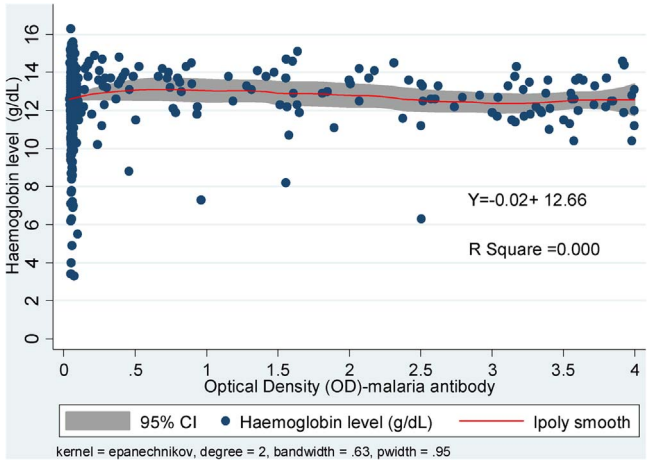

Fig. 5. Regression of haemoglobin levels (g/dL) against malaria antibody optical density (OD) among children aged 2–9 years, in the study area.

compared to the findings of previous studies (Central Statistical Agency of Ethiopia, 2011; Assefa et al., 2014; Ketema and Bacha, 2013; Choge et al., 2014; Noland et al., 2014; EHNRI, 2012). One recent study in Ethiopia reported zero prevalence of severe anaemia among children (Assefa et al., 2014). There was also a study that documented comparable results on prevalence of severe anaemia (Deribew et al., 2010). In contrast with previous studies which cited anaemia as a severe public health problem in Ethiopia (Ethiopian Health and Nutrition Research Institute, 2011; Central Statistical Agency of Ethiopia, 2011), the present study found that a 'mild' public health problem in the study community (World Health Organization, 2008). This may indicate that the burden of childhood anaemia was declining in Ethiopia. In fact, it is somewhat difficult to compare the results of this study with the findings of many previous studies since there were some methodological and measurement variations. Most studies on anaemia among children were focusing on either pre-school or school age children (WHO et al., 2001) which is somewhat different from the specific study population addressed in the present study (i.e. children 2–9 years of old). Thus, these variations could account for the observed

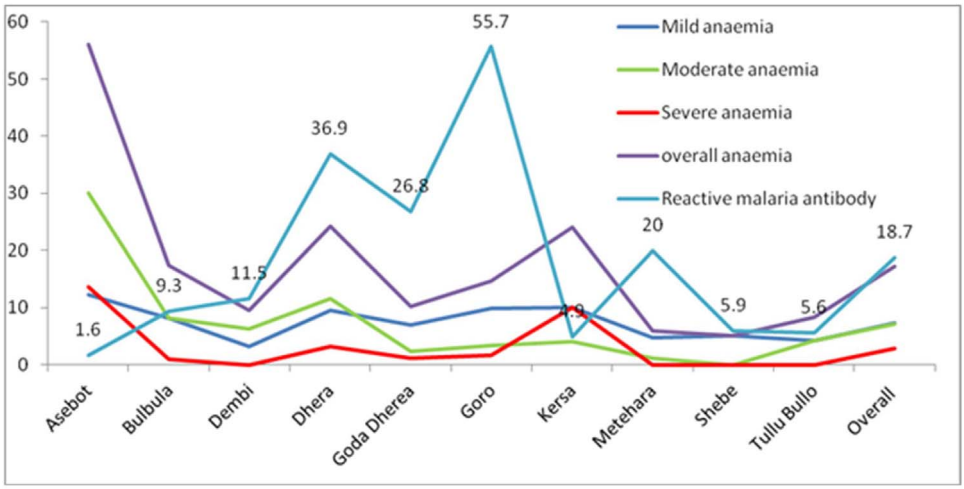

Fig. 6. sualization of degree of anaemia and reactive malaria antibody by study sites, June 2016.

differences in the magnitude of anaemia.

Consistent with existing evidences, this study indicated that younger children (pre-school children) were at increased risk of developing anaemia (Mesfin et al., 2015; Noland et al., 2014; Menon and Yoon, 2015). Global data have also shown that the risk and burden of anaemia is highest among pre-school children (American society of hematology, 2016) suggesting that anaemia prevention and control efforts need to preferentially address most vulnerable age groups of children, especially children under the age of five. Unlike in some earlier studies (Central Statistical Agency of Ethiopia, 2011; Ketema and Bacha, 2013; Menon and Yoon, 2015), demographic factors such as sex, place of residence and family size did not show significant association with anaemia. Nevertheless, the magnitude and severity of anaemia vary considerably by localities and altitude calling for designing and implementing tailored and location specific interventions strategies that fit into the local burden of anaemia.

In malaria endemic settings, the prevalence of anaemia decreases with age since children gradually build up their acquired immunity against malaria and its outcome, anaemia (Centers for Disease Control and Prevention, 2010). In line with this assumption, the present finding has shown that younger children (2–4 years old) were more likely to be affected by anaemia. Since degree of cumulative exposure to malaria increased with age (i.e. as measured through reactive malaria antibody), this repeated exposure might have helped older children to develop protective immunity against malaria and its consequence such as anaemia. In fact, this is not a plausible argument since a large proportion (80.7%) of children with anaemia was non-reactive to malaria antibody test. This finding is in contrast with historical evidence which widely cited that repeated exposure to malaria is one of the major risk factor for anaemia, especially in malaria endemic areas (Sowunmi et al., 2011; Ketema and Bacha, 2013; Safeukui et al., 2015; Crawley, 2004; Kassebaum et al., 2014; McCuskee et al., 2014; Selvam and Baskaran, 1996; Price et al., 2001; Miller et al., 2002; Koukounari et al., 2008; Taylor et al., 2012). Probably, other causes of anaemia such as nutritional deficiencies, infections with intestinal parasites, feeding habits and availability of food might be largely contributed to anaemia in this setting.

In this study, *P. vivax* (detectable parasitaemia) infection was less likely to be associated with anaemia which is less congruent with existing evidences (Douglas et al., 2012; Ketema and Bacha, 2013). Evidence has shown that repeated exposure to the same strain of *P. vivax* causes little hematological disturbances (Boyd and Kitchen, 1936) whereas repeated exposure to different strains of *P. vivax* usually causes more prominent hematological effects that lead to anaemia (Collins et al., 2003). Specially, if individuals are repeatedly exposed to different and/or homologous strains of *P. vivax* before haemoglobin concentration has returned to normal state, even with the infection could have significant hematologic effect (Douglas et al., 2012; Selvam and Baskaran, 1996). Possibly, the lack of association between infections with *P. vivax* and anaemia, in the present study suggests children might have been infected with the same strain of *P. vivax* with adequate recovery time for haemoglobin level. On the other hand, it may also show that the *P. vivax* strain being circulating in the study community is less virulent to cause significant hematologic insult that led to anaemia. Further studies are required to better understand the hematologic impacts of infections with homologous and heterologous strains of *P. vivax*, particularly in areas with reduced transmissions of malaria. Even though the association was insignificant, this demonstrated that children were more likely to develop moderate anaemia during infections with *P. falciparum* which is supported by previous studies (Price et al., 2001; Miller et al., 2002).

In this study, neither *P. vivax* nor *P. falciparum* was associated with severe anaemia. None of the children with severe anaemia had detectable malaria parasitaemia, which is against existing evidences (Ketema and Bacha, 2013; Kassebaum et al., 2014; McCuskee et al., 2014; Taylor et al., 2012; Fendel et al., 2010; Gosling and Hsiang, 2013; Perkins et al., 2011; Castro-Gomes et al., 2014; Imam, 2009). Some studies (Fendel et al., 2010; Gosling and Hsiang, 2013; Perkins et al., 2011; Castro-Gomes et al., 2014; Imam, 2009) documented that severe malaria anaemia is common in settings with high malaria transmission. However, the context of this study was characterized by reduced or low malaria transmission in which the impact of malaria would be insignificant (Schellenberg et al., 2003; Menendez et al., 2000). Certainly, in the context of reduced transmission of malaria, it is appropriate to conduct further study on etiology of anaemia to generate better evidence on the relative contributions of malaria to the overall burden of anaemia using sufficient sample sizes of malaria cases. Interestingly, despite lack of association between anaemia and malaria, the overall prevalence of both indices is nearly the same (18.7% reactive antibody, vs 17.3% anaemia). This conveys important messages to malaria and anaemia prevention program. Some evidence suggests that iron supplementation in malaria endemic regions may increase risk of severe malaria (Sazawal et al., 2006; Goheen et al., 2016; Consultation WHOS, 2007), and efforts to integrate iron supplementation with malaria control programs should be made with caution.

In this study, splenomegaly was the only malariometric indice that significantly associated with increased risk of anaemia. Some evidence suggest that enlarged spleen may also filter normal red blood cells as well as abnormal ones, which actually increases risk of anaemia (Safeukui et al., 2015; Price et al., 2001; Del Portillo et al., 2012; Chen et al., 2016). Nevertheless, since the proportions of children with enlarged spleen were so few in the study, the association of splenomegaly with anaemia maybe accepted as plausible or reasonable. Yet, this evidence could be useful as it suggests the need to carefully screen anaemia among children with enlarged spleen during routine child care service provisions. Some earlier studies attempted to predict malaria transmission levels and burden from haemoglobin level of the population and results were mixed (Korenromp et al., 2004; McCuskee et al., 2014; Savage et al., 2007; Senn et al., 2010; Muwonge et al., 2013; Philipose and Umashankar, 2016; World Health Organization, 2017). In the present study, even though spleen rate was strongly correlated with anaemia, other indices of malaria, namely parasite rate and serostatus were not associated with anaemia. Thus, in the present settings, haemoglobin levels are not useful marker for estimating malaria transmission.

In this study, ownership of LLIN had a significant positive effect on children's haemoglobin level: owning at least one LLIN significantly reduced anaemia among children. Earlier evidences also documented that ownership and use of LLIN improved anaemia-related outcomes in young children (McCuskee et al., 2014; Menon and Yoon, 2015; Carneiro et al., 2006; Casals-Pascual et al., 2006; Kuile et al., 2003; Deribew et al., 2012; Smithson et al., 2015). However, in this study, LLIN use did not have association with anaemia. In fact, there was a study which reported similar findings (WHO, 2016). It is important to explore in depth how the presence of LLIN in the household contributes to reduction of anaemia since utilization did not have impact on anaemia. Yet, malaria prevention programs are beneficial to anaemia prevention among children.

5. Strength and limitations of the study

This study assessed the association between exposure to malaria and haemoglobin level of children, using combination of classical and immunological technique through community based approach involving multiple sites with diverse eco-epidemiologic areas in Ethiopia. Thus, we anticipate that the findings of the study are generalized to settings with different malaria transmission spectra in Ethiopia and other similar malaria endemic settings in tropical areas. Furthermore, the study is the first of its kind in Ethiopia in assessing the association between exposures to malaria (as measured using serological marker) and anaemia. Thus, it can serve as first-hand information on the relationship between exposure to malaria and anaemia on among

children in Ethiopia. However, our study has some limitations. We did not collect data on nutritional status and other potential risk factors for anaemia. Moreover, EIA test was not done for some children due to inadequate blood sample. This might have affected the estimates. In addition, we relied on existing lists for selection of children which might be introduced selection bias.

6. Conclusions

Despite these limitations, the following conclusions can be made from the findings of this study. Compared to the findings of previous studies, the magnitude and degree of anaemia was somewhat low, making anaemia a mild public health in the study community. This study revealed that infection with detectable parasitaemia and malaria serostatus did not have significant effect on haemoglobin levels of children. Thus, malaria attributable anaemia was less likely in present context; instead other risk factors for anaemia might have played more prominent roles. This suggests that the use of haemoglobin concentration as a useful metric of malaria burden and transmission is not worthwhile in low transmission settings. Thus, malaria prevention program has little benefit to anaemia prevention efforts among children aged 2–9 years even though focusing on *P. falciparum* might help to reduce moderate anaemia. In fact, ownership of LLINs plays some positive roles in the prevention of anaemia among children and could be emphasized in malaria prevention program Given that anaemia due to malaria was very minimal in this study, interventions aimed at anaemia prevention and management must be carefully designed considering ground level risk factors and the etiology of anaemia. Therefore, in settings with low malaria transmission, it is imperative to review the historical evidence and quantify the relative contribution of malaria to anaemia for development of effective and evidence based anaemia prevention and control strategies.

Authors' contributions

ZB and DY conceived the study and were involved in the design and conduct of the study. DY and YY were involved in the design and conduct of the study. DE, DF, EK, SK and KG involved in the design, conduct of the study and sample analysis. ZB drafted the manuscript while DY, YY, DE, DF, EK, SK and KG critically reviewed it for intellectual content. All authors read and approved the final version of the manuscript.

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