FULL-LENGTH ARTICLE

Magnitude of Anemia in patients infected with *Plasmodium vivax* malaria at Showa Robit Health Center, Central Ethiopia

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ABSTRACT
Malaria is still a major public health concern in some parts of the world. *Plasmodium vivax* is common parasite in Ethiopia. Although there were reports about its association with severe anemia in malaria patients, an in-depth study that targeted assessing the magnitude of this pathology is not reported from Ethiopia. Therefore, this study was aimed to assess the frequency of *P.vivax* associated anemia and risk factors in clinical *P. vivax* malaria patients. Accordingly, a descriptive cross-sectional study was conducted in malaria suspected febrile patients seeking medication at Showa Robit Health Center between 2018 and 2019. *P. vivax* diagnosis was carried out following a standard parasitological procedure. Haemoglobin (Hb) level was measured using Hemcue™; socio-demographic and clinical data were collected by health professionals. Data were analyzed using R-software. Among the 10,459 malaria suspected febrile cases tested, 1,113 (10.64%) were found positive for *P.vivax*. Among the positive cases, only 227 cases were fulfilled the inclusion criteria and recruited for the study. The prevalence of anemia was 40.52%. Patients aged between 5 to 14 years have significantly (AOR: 0.19, 95%CI: 0.067-0.544) lower chance of developing anemia compared to younger children, aged <5years. Also, some severe pathologies such as being febrile (body temperature >37.5^{o}C) and having high parasite load didn't show an association with higher risk of anemia due to *P. vivax*. This study provides evidences for the fact that *P. vivax* is a risk factor for the incidence of anemia, which makes it an important public health concern in vivax malaria endemic regions.

Keywords: Anemia, Haemoglobin, *P.vivax*, malaria, Showa Robit, Ethiopia

INTRODUCTION
Malaria is a vector born disease mainly found in tropical and subtropical regions of the world. There are five Plasmodium species that cause malaria in humans. Among them, *P. falciparum* is the dominant parasite in Africa, while *P. vivax* is minor in terms of distribution, affected population, and only confined in the Horn of Africa (WHO, 2019). It is widely accepted that *P. falciparum* is a deadly parasite, which is accountable for the majority of malaria-associated severe life-threatening symptoms and death, whereas *P. vivax* was considered as a mild parasite (WHO, 1986; 2000). However, in recent years, the benign *P. vivax* parasite has become virulent and some severe symptoms such as severe anemia have been reported for vivax malaria despite its lower level of parasitemia (Douglas et al., 2012; White, 2018). According to the World Health Organization (WHO) estimate, about 216 million illness and 445,000 deaths due to malaria were recorded in 2016 (WHO, 2018). A significant portion of these deaths resulted directly or indirectly from anemia (WHO, 2018). However, as the main cause

of anemia, *P. vivax* did not get the right attention yet by concerned health authorities compared to *P. falciparum* (Golassa and White, 2017).

Comparatively, *P. vivax* is the most widely distributed species of Plasmodium globally, and causes an estimated infection of around 14 million people world-wide annually (Battle et al., 2019). Overall, there are about 3 billion people who are at risk of *vivax* malaria across the world (Amott et al., 2012). In Ethiopia, *P. falciparum* and *P. vivax* co-exist and they account for about 60% and 40% of all malaria cases, respectively (Deressa et al., 2003). Ethiopia together with India, Indonesia, and Pakistan accounts for more than 80% of the global *P. vivax* burden (WHO, 2013), and the country alone contributed about 12% of the global cases and deaths due to *P. vivax* (WHO, 2012).
Although some life-threatening severe malaria symptoms due to *P. vivax* have been widely reported, detailed and comprehensive information on some of the commonly encountered severe malaria symptoms were not fully studied yet. Therefore, the aim of this study is to assess the magnitude of anemia and its associated risk factors among clinical *P. vivax* malaria mono-infected patients in Showa Robit, one of the malaria endemic areas in Ethiopia.

MATERIALS AND METHODS

Description of the Study Area

This study was carried out in Showa Robit Health Center, located in Showa Robit town, Amhara Regional State, Central Ethiopia. The town is located 225 km north of Addis Ababa on the main road to Asmera, at an altitude of about 1,280m. The town lies at longitude and latitude of 10°060N39°590E and 10.1°N39.983°E, respectively. It is divided into 9 kebeles (the lowest administrative level), with a total population of about 50,528, of which 24,638 (48.8%) were male and 25,890 (51.2%) were female. Most of the dwellers of the town were merchants, government employees, daily laborers, and urban farmers. Malaria was one of the major health problems in the town. The peak season for malaria transmission is from September to November, after the main rainy seasons (June to September). The area has been characterized by a hot climatic condition and the mean annual temperature ranges from 28 to 37oC with a mean rainfall of 1000mm.

Study design, population and sample size

In this study, a descriptive cross-sectional study design was used. All malaria suspected patients seeking medication or visiting the health center during the data collection period (September 2018 to May 2019) were considered in the study. The inclusion criteria used were; *P. vivax* mono-infection, and willingness to participate in the study. Potential confounding variables of anemia such as having chronic illness including helminthic infection, admission at Tb or ART clinic, pregnancy, prior-treatment with any drug within the last 24hr, and mentally sick or unstable individuals were excluded from the study as described in Figure 1.

Data collection methods and tools

Socio-demographic, clinical, parasitological, and hematological data of the study participants were recorded on predesigned data record forms by trained health professionals working at the health center. For parasitological and hematological data, a few drops of blood samples from the lancet pricked finger were collected on a clean glass slide. Then, a single drop of blood was used to prepare thin and thick blood smears

(in duplicates) per patient for microscopic examination. Giemsa (10%) stained thick and methanol fixed thin blood smears were examined under a microscope. Each blood smear was examined by an experienced laboratory technician in the health center. Parasite load was calculated after counting asexual parasites per 200 white blood cells (WBC), assuming the mean WBC count is 8,000/μL.

$$\text{Parasite count}/\mu\text{L} = \frac{\text{Number of observed asexual parasites} \times \text{mean 8000 WBC count}/\mu\text{L}}{200 \text{ WBCs}}$$

In addition, haemoglobin level for each patient was measured using a handheld Hemocue™ machine. Anemia was categorized as per WHO classification, where Hb level <11g/dl in children <5 years, <11.5g/dl in old children between 5 and 12 years, <12g/dl in teens aged 12 to 14 years and non-pregnant women, and >13g/dl for men aged >15 years were considered as anemic (WHO, 2011).

Data analysis

Data were checked for completeness, entered into Excel sheets, and then exported to R-software (version 4.0.0) for analysis. Descriptive statistical analysis was used to compute the proportions and percentages of clinical, demographic, and parasitological data. Association between variables was computed using Pearson Chi-square test. Binary logistic regression model was used to identify determinant variables for anemia. Significance level was considered at 95%.

Ethical consideration

The study was ethically approved by the Research and Ethical Review Board of College of Natural Sciences, Jimma University. A written consent was obtained from all participants after they had been informed of the objectives, benefit, and expected outcomes of the study. The participants were assured that the collected information will be kept confidential and will not be used for any purpose other than this study.

RESULTS

Characteristics of the study participants

In this study, a total of 10,459 malaria suspected febrile patients seeking medication at the health center were examined. Among this, 1,113 were found positive for malaria out of which 378 were infected with *P. falciparum* while 723 were infected with *P. vivax*. Only 227 *P. vivax* mono-infected patients were fulfilled the inclusion criteria and recruited to the study. The remaining 496 were excluded for reasons such as comorbidity or having chronic illness such as HIV/AIDS and Tuberculosis, pregnancy, received any treatment within the past 24hr, and confirmed mentally ill (Figure 1).

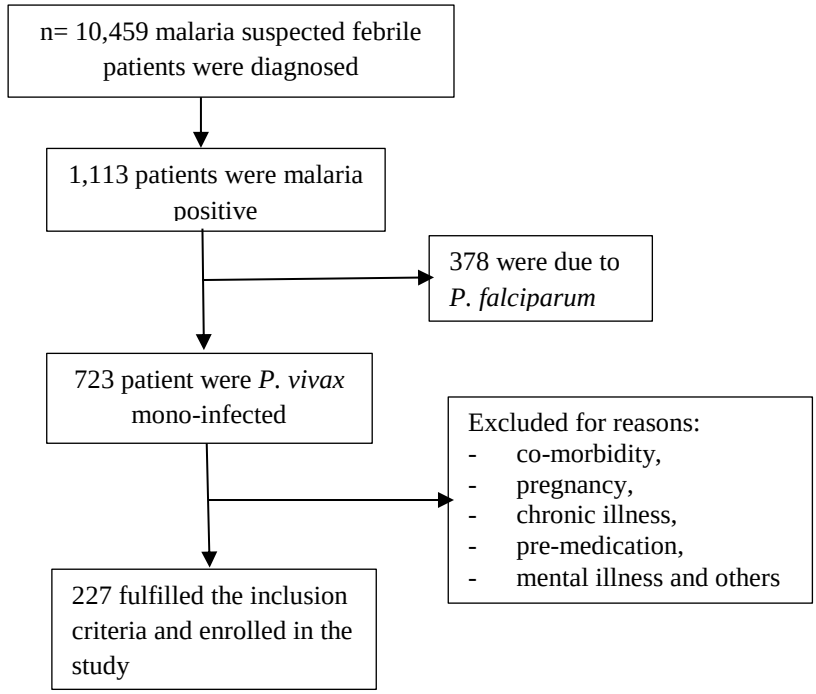

Figure 1. Study flow diagram

Among the 227 recruited patients, 56.4% (n= 128) were male and most of them [n=151 (66.51%)] were adults aged >15 years. The median age of the study participants was 23 years (ranging from 4 months to 65 years). The proportion of patients with severe complication symptoms (including vomiting and diarrhea) were 52% and 16.7%, respectively. Common malaria symptoms such as chills, headache, loss of appetite, nausea, and muscle pain were presented by the majority of the participants (Table 1).

Table 1: Proportion of common malaria symptoms in clinical *P. vivax* mono-infected patients at Showa Robit Health Center, Central Ethiopia

Variables		**Proportion (%)**
Median age (range)		23 years (4months to 65 years)
<5		25(11.01)
5-14		51(22.47)
+14		151(66.51)
Gender	Male	128 (56.4)
	Female	99 (43.6)
Chill		210 (92.5)
Febrile patients		222 (97.8)
Headache		224 (98.7)
Loss of appetite		206 (90.7)
Nausea		117 (51.5)
Muscle pain		111 (48.9)
Diarrhea		38 (16.7)
Vomiting		109 (48)
Pallor (whiteness)		45 (19.8)

Among the 227 *P. vivax* mono-infected patients, 92 (40.52%) were anemic. In fact anemia documented in this study was mild (Hb level of the participants ranged from 9 g/dl to 17.6g/dl). Proportions of anemic patients were higher among patients aged >14 years, those with vomiting, diarrhea, underweight, parasite load between 999-9999 and body temperature ≥37.5^{o}C, but there was no significant differences among the patients considering their gender (Table 2).

Table 2: Descriptive summary of anemic status of clinical *P. vivax* malaria patients at Showa Robit Health Center, Central Ethiopia

Covariates	Category	Anemia Status		
		Non-Anemic No. (%)	Anemic No. (%)	Total No. (%)
Age	0-5 years	28 (12.3)	5 (2.2)	33(14.5)
	5-14 years	23 (10.1)	20(8.8)	43(18.9)
	>14 years	84 (37.0)	67(29.5)	151(66.5)
Gender	Female	57 (25.1)	43 (18.9)	100 (44.1)
	Male	78 (34.4)	49 (21.6)	127 (55.9)
Fever	No	5(2.2)	0 (0.0)	5(2.2)
	Yes	130 (57.3)	92(10.5)	222(97.8)
Vomiting	No	76 (33.5)	42(18.5)	118(52.0)
	Yes	59 (26.0)	50(22.0)	109(48.0)
Diarrhea	No	115 (50.7)	74(32.6)	189(83.3)
	Yes	20 (8.8)	18(7.9)	38(16.7)
Pallor	No	110 (48.5)	73(32.2)	183(80.6)
	Yes	25 (11.0)	19(8.4)	44(19.4)
Body Temperature(°C)	<37.5	72 (31.7)	40(17.6)	112(49.3)
	≥37.5	63 (27.8)	52(22.9)	115(50.7)
BMI(kg/m2)	Underweight	70 (30.8)	54(23.8)	124(54.6)
	Normal	56 (24.7)	35(15.4)	91(40.1)
	Overweight	9 (4.0)	3(1.3)	12(5.3)
Parasite load (count/µl)	<999	64 (28.2)	27(11.9)	91(40.1)
	999-9999	65 (28.6)	64(28.2)	129(56.8)
	≥10000	6 (2.6)	1(0.4)	7(3.1)

Mean age of anemic patients was 22.28 years. All anemic patients had a history of fever for the last three days. Geometric parasite load, mean Hb level, and mean body temperature of anemic patients were 2942.93 parasite count/µl, 10.9 g/dl, and 37.62^{o}C, respectively. Among anemic cases, 54.3% and 19.6% had vomiting and diarrhea, respectively (Table 3).

Table 3: Socio-demographic and clinical characteristics of *P. vivax* associated anemic patients at Showa Robit Health Center, Central Ethiopia

Characteristics	Proportion (%) Anemic (n=92)
Age: mean± SD	22.28±13.54
Median(Range)	19 (4-65)
Sex: Female	43 (46.7)
Male	49 (53.3)
Fever	92 (100)
Vomiting	50 (54.3)
Diarrhea	18 (19.6)
Pallor	19 (20.7)
Mean Body Temperature (^{o}C)	37.62±1.06
Mean BMI (kg/m^2)	17.60±3.33
Mean Hb level (g/dl)	10.90±0.72
Mean Parasite load(count/µl)	2942.93±2572.66

Predictors of anemia associated with *P. vivax*

Some socio-demographic and severe clinical symptoms of *P. vivax* malaria patients were evaluated for their determinants of anemia. The findings have shown that there were an association between age, body temperature (°C), and parasite load (count/µl) with anemic conditions (Table 4).

Table 4: The Association between characteristics and Anemia in clinical *P. vivax* malaria patients at Showa Robit Health Center, Central Ethiopia

Covariates	Pearson chi-square value	df	Sig.
Age	10.381	2	**0.006***
Sex	0.453	1	0.501
Fever	3.484	1	0.061
Vomiting	2.483	1	0.115
Diarrhea	0.886	1	0.347
Pallor	0.159	1	0.690
Body Temperature (°C)	2.126	1	**0.014***
BMI(kg/m2)	1.931	2	0.400
Parasite load(count/µl)	10.868	2	**0.004***

*significant at 5%

The binary logistic regression analysis has shown that the odds of having anemia pathology was 0.191(95% CI: 0.067-0.544) times lower for children in the age range of 5 to14 years as opposed to young children aged from 0-5 years. Moreover, the odds of having anemia was 0.561(95% CI: 0.313-1.005) times lower for those with body temperature ≥37.5 °C as opposed to those with body temperature < 37.5 °C. A significant difference was not observed among patients of different parasite loads with regards to magnitude of anemia (Table 5).

DISCUSSION

In the current study, the magnitude of anemia due to *P. vivax* was 40.52%. Among patients of different age groups, the lower prevalence of anemia was observed among children found in the age range of 5-14 years than younger children, aged less than 5 years. This finding was in line with reports from southern Papua, where *P. vivax* was reported to be a major determinant of anemia in infants and young children (Douglas et al., 2012; Kenangalem et al., 2016). The higher anemia prevalence detected in younger children might be due to frequent recurring illness caused by repeated relapses of *P. vivax* (WHO, 1986; Kenangalem et al., 2016)]. Moreover, vivax malaria has a strong preference to reticulocytes, which are most abundant in the first months of life (Kling et al., 1996). As the age of the children increase, the possibility of developing protective malaria immunity will increase as a consequence of which the parasite burden will decrease (Kling et al., 1996).

Similar to the earlier reports on the association of parasite burden and fever to the prevalence of anemia (Douglas et al., 2012; Kling et al., 1996), there was no significant differences between parasite load and anemia. Furthermore, the prevalence of anemia was significantly lower among febrile patients, whose body temperature was >37.5^{o}C, than non-febrile patients. Largely, findings of the current study are supportive evidence for the fact that *P. vivax* is one of the major causes of malaria-associated anemia. This

Table 5: Binary logistic regression results on characteristics of clinical *P. vivax* malaria patients at Showa Robit Health Center, Central Ethiopia

Characteristics	Category	COR (95% CI)	AOR(95% CI)	P-value
Age	0-5 years **(ref)**	1	1	
	5-14 years	0.135 (0.043-0.418)	0.191(0.067-0.544)	**0.001***
	>14 years	0.703 (0.319-1.549)	0.904(0.446-1.834)	0.382
Sex	Female **(ref)**	1	1	
	Male	1.163(0.631-2.142)	-	0.629
Vomiting	No **(ref)**	1	1	
	Yes	0.773 (0.409-1.462)	-	0.428
Diarrhea	No **(ref)**	1	1	
	Yes	1.099 (0.474-2.551)		0.826
Pallor	No**(ref)**	1	1	
	Yes	0.903 (0.425-1.917)		0.790
Body Temperature(°C)	<37.5**(ref)**			
	≥ 37.5	0.507(0.270-0.951)	0.561(0.313-1.005)	**0.034***
BMI(kg/m^2)	Underweight **(ref)**	1	1	
	Normal	3.128(0.730-13.397)		0.124
	Overweight	1.602(0.381-6.743)		0.520
Parasite load (count/µl)	<999 **(ref)**	1	1	
	999-9999	1.698(0.176-16.371)	2.052(0.225-18.68)	0.647
	>=10,000	3.988(0.424-37.547)	4.465(0.523-41.28)	0.227

*significant at 5%, COR = Crude Odds Ratio, C.I = Confidence Interval, AOR = Adjusted Odds Ratio

might happens mainly during its intra-erythrocytic cycle, where *P.vivax* promotes extensive changes in the host reticulocyte leading to its rupture (Brabin, 1992). Early death of infected young RBCs due to *P. vivax* infection is enough to lead to extreme anemia over a period of time by disturbing the supply of mature red blood cells into the system (White et al., 2014). In addition, parasite antigens and other toxic products released into the circulation could stimulate the immune system (McQueen, 2010). The activation of immune system in response to parasite antigens or toxins, in turn, enhances the detection and removal of infected and abnormal but uninfected red blood cells (Lamikanra et al., 2007; Wickramasinghe and Abdalla, 2000).

If *P. vivax* parasite develop resistance to anti-malarial drug, it could also delay parasite clearance and contribute to anemia (Wickramasinghe and Abdalla, 2000). Thus, the earlier report on the emergence of chloroquine resistant *P. vivax* reported from the same health center or study area (Seifu et al., 2017) might have contributed to the delaying of parasite clearance and consequently resulted in the observed anemia. Parasitaemia is typically lower in cases of *P. vivax* infection as compared to *P. falciparum* infection. However, the absolute number of red blood cells removed from the circulation is higher during *P. vivax* infection, hence the degree of anemia resulting from infection by the two species was different (Douglas et al., 2012). According to results of the logistic regression analysis, the parasite load did not show a significant association with the prevalence of anemia in case of *P.vivax* infection, as opposed to that of *P. falciparum*. During *P. vivax* malaria infection, approximately 34 non-infected cells were cleared for every one infected cell, whereas in *P. falciparum* malaria, this ratio is closer to 8 to 1 (Thiago et al., 2014). Even, the ratio of un-parasitized red cells to parasitized red cells lost in acute malaria is higher in *P.vivax* than in *P. falciparum* (Thiago et al., 2014).

The other possible factor that could exert an influence on the erythropoiesis process is the production of immune mediators by the host cells in response to parasite products when released. These mediators could damage the surrounding haematopoietic cells, altering their morphology and functions (Wickramasinghe and Abdalla, 2000). In this regard, it has been demonstrated that the presence of hemozoin, a metabolic product generated during haemoglobin digestion, in plasma, WBCs, or erytroid precursors, can be able to inhibit the erythropoiesis process (Collins et al., 2003). Reports from studies conducted on bone marrow sections of children died due to severe malaria showed an association between the amount of hemozoin and the percentage of abnormal erythrocytes (Casals-Pascual et al., 2006).

The negative effects of hemozoin on the erythroid expansion seem to be related to its ability to stimulate the release of different molecules that could prevent the process of erythropoiesis in bone marrow (Lamikanra et al., 2006). High proportions of peripheral blood monocytes containing malaria pigment (hemozoin) reflect higher parasite burden and are associated with anemia in African children (Van den Eede et al., 2016). Some of these molecules perhaps influence other severe patho-physiologies associated with malaria, including iron trafficking or shortage of iron (Van den Eede et al., 2016; CDC, 2019). Moreover, *P. vivax*-infected RBCs seem to be able to attach to endothelial cells; but. Unlike falciparum-infected cells, however, they have a limited tendency to adhere to endothelial cells, hence, adherence to the deep microvasculature might not be the major factor in the pathogenesis of *P. vivax* malaria (McQueen et al., 2004).

Another possible route of RBCs damage is via rosetting (adherence of infected RBCs to uninfected RBCs). Accordingly, *P. vivax*-infected RBCs adhere to uninfected red blood cells and this might interfere with the processes of erythropoiesis in the bone marrow (Anstey et al., 2007). However, the mechanisms that link cyto-adherence/rosetting to anemia remain unknown. *P. vivax*-infected cells become more deformable as the parasite matures and are thought to retain the ability to squeeze through splenic slits (Chotivanich et al., 1998). Increased deformability of infected RBCs due to *P. vivax* malaria may limit the proportion of red blood cells that are removed during passage through the splenic microcirculation. In addition, a cryptic infection in bone marrow, spleen, and deep tissues might contribute to the hidden burden of anemia associated with *P. vivax* (Monteiro et al., 2020).

Limitations of the study

The study included a small sample sizes (n=227) and data were collected only from those symptomatic patients who visited the health center during the study period. These could potentially underestimated the true prevalence of anemia due to *P. vivax* and may not reflect the real magnitude of anemia in the study area. Moreover, the lack of in depth immunological assay in the study has limited depth of discussion in line with the assumptions made.

CONCLUSION

Prevalence of anemia amongst clinical *P. vivax* malaria mono-infected individuals at Showa Robit Health Center was 40.52%. The commonest form of anemia documented in this study was mild anemia. This study suggested that *P. vivax*-associated anemia is an important public health concern that might underscore the importance of reducing global transmission of *P. vivax.* In addition, to clearly understand the causes of anemia associated with *P. vivax*, a comprehensive and in-depth study using larger sample size, haemoglobin of diverse groups of population, and different geographical locations is crucial.

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