Prevalence and association of malaria with ABO blood group and hemoglobin level in individuals visiting Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia: a cross-sectional study

Belaynesh Tazebew1 · Abaineh Munshea1,2 · Endalkachew Nibret1,2

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Abstract
Malaria is a serious and sometimes fatal mosquito-borne disease caused by protozoan parasite of the genus Plasmodium. ABO blood group antigens represent polymorphic traits inherited among individuals and populations. Differences in blood group antigen expression can increase or decrease host susceptibility to many infections. This study was undertaken to determine the prevalence of malaria and its possible association with ABO blood group and hemoglobin level among individuals attending Mekaneeyesus Primary Hospital, Estie District, northwestern Ethiopia. Sociodemographic variables and relevant data were collected from 390 randomly selected individuals through structured questionnaire. Then, thick and thin smears were prepared from finger pricked blood samples, stained, and examined microscopically for detection and identification of malaria parasites. ABO blood group and hemoglobin levels of the same subjects were also determined. The data generated were analyzed for descriptive and logistic regression models. Variables with \( p \) value < 0.05 in multivariable logistic regression were considered explanatory variables. The overall prevalence of malaria was 8.5%; Plasmodium vivax (5.6%) was the most predominant, followed by P. falciparum (2.3%), and mixed infection of the two species (0.5%). In our study, being male (AOR = 3.48), under-five years of age (AOR = 72.84), rural residence (AOR = 2.64), and failing to use bed net (AOR = 4.65) were significantly associated with the risk of malaria. Most (14.6%) of malaria-positive cases were among individuals with blood group "A," while the least numbers of cases were among subjects with blood group "O." Individuals with blood group "A" were about four times at risk of malaria as compared to individuals with blood group "O" (AOR = 3.74). The prevalence of anemia was 23.1% and significantly associated with malaria (\( p < 0.05 \)). Prevalence of malaria in this study is still higher compared to some of previous reports from Ethiopia. Thus, there is a need to intensify effort in malaria prevention among potentially at risk segments of population, including males, rural residents, and under-five children, and promotion of ITNs use in the community. Supplementation of iron-rich diet for iron-deficient anemia people is needed. Further in-depth investigation is also necessary to clearly establish the role that ABO blood group plays in malaria.

Keywords ABO blood group · Adjusted odds ratio · Anemia · Hemoglobin level · Malaria

Introduction
Malaria is a life-threatening disease caused by protozoan parasites that are transmitted to humans through the bites of infected female Anopheles mosquitoes. According to the latest WHO estimates, in 2018 alone, there were 228 million cases and 405,000 deaths of malaria worldwide. The burden is the heaviest in the WHO African Region accounting for 93% of all malaria cases and 94% deaths in the same year (WHO 2020).

More than 75% of the total area of Ethiopia is malarious, where it has been the major cause of illness and death for many years (Ayle et al. 2012). Plasmodium vivax and P. falciparum exist commonly in Ethiopia with P. falciparum...
prevailing all year (Alemu et al. 2012). Malaria has continued to be one of the major public health challenges in Amhara regional state of Ethiopia (Alemu et al. 2013). The region accounts for 31% of the national malaria burden (FMOH, 2014), 22% of outpatient visits, 24% of hospital admissions, and 10% of health facility deaths (ANRSHB 2012). South Gondar zone, where Estie district is located, accounts for the third greatest number of malaria cases in the region next to West Gojam and North Gondar zones (Alelign et al. 2018).

ABO blood group system is genetically controlled and proportions of various ABO groups differ significantly in different populations and ethnic groups. Blood group antigens represent polymorphic traits inherited among individuals and populations (Beardmore and Karimi-Booshehri 1983). Differences in blood group antigen expression can increase or decrease host susceptibility to many infections. Blood groups can play a direct role in infection by serving as receptors and/or co-receptors. Polymorphisms in blood groups can modify the innate immune response to infection. Several distinct phenotypes associated with increased host resistance to malaria are reported in populations living in areas where malaria is endemic, as a result of evolutionary pressures (Cooling 2015).

ABO blood group antigens are formed by terminal glycosylation of glycoprotein and glycolipid chains present on cell surfaces. Glycosylation modulates all kinds of cell-to-cell interactions and this may be relevant in malaria pathophysiology, in which adhesion has been increasingly implicated in disease severity (Kuadzi et al. 2011). The adherence of parasitized RBCs to other cells is central to the pathophysiology of severe malaria syndromes including cerebral malaria, respiratory failure, multi-organ failure, and death. Parasitized RBCs adhere to the vasculature through a process termed sequestration closely mimicking inflammatory leukocyte attachment (Otajevwo and Igoniwari 2014).

Several epidemiological studies were conducted to investigate the association between ABO blood group system and some disease conditions including malaria. Association between the phenotypes of blood groups and malaria remains controversial and was not yet fully elucidated nor explained clearly. Some studies reported significant associations, suggesting the impact of ABO blood groups on infection status of the individuals possessing a particular ABO blood group (Zerihun et al. 2011). Individuals with blood groups A and B are more susceptible to malaria infection as compared with individuals of blood group O; however, the severity of infection differs due to differential host susceptibility (Gayathri et al. 2013; Tadesse and Tadesse 2013). On the other hand, many other reports demonstrated the absence of significant association of all ABO blood groups with malaria, implying that all individuals with any type of blood group type are being equally susceptible to malaria (Otajevwo and Igoniwari 2014; Tela et al. 2015; Bamou and Sevidzem 2016).

Anemia undoubtedly is a major health problem in malarious areas. Malaria causes anemia by destruction and removal of parasitized red blood cells and shortening of the life span of non-parasitized red blood cells and decreasing the rate of erythrocyte production in bone marrow (Devitt et al. 2004). Its cause is frequently multifactorial. One of the main impacts of malaria is anemia among other mechanisms through hemolysis, increased spleen clearance of infected and uninfected red blood cells, and cytokines-induced dyserythropoiesis (White 2018). Other factors frequently contribute to anemia in many malarious areas including malnutrition and genetic factors (Machteld et al. 2010). Malaria-infected patients are at high risk to develop anemia when compared to non-infected individuals (Alemu et al. 2012).

Malaria is a complex disease and its local characteristics are determined by a variety of geographical, environmental, vector, host, and parasite factors. Although several studies have been conducted on the effects of malaria and its risk factors in Ethiopia, there is still a lack of information in some localities of the country. Therefore, this study aimed to determine the prevalence of malaria and its possible association with ABO blood group and hemoglobin level among individuals attending at Mekaneeyesus Primary Hospital, Estie District, northwestern Ethiopia.

Materials and methods

Study setting, design, and population

A cross-sectional study design was conducted from September 2017 to April 2018 to determine the prevalence of malaria and its possible association with ABO blood group and hemoglobin level among people attending Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia. Mekaneeyesus is the capital town of the district and is located in South Gondar.

Estie is one of the 105 districts in the Amhara regional state of Ethiopia. Geographically, the study area lies on the coordinates of 11° 34’N, latitude and 36° 41’ E, longitude and at an altitude range of 1500–4000 meters above sea level (m a.s.l). The minimum and maximum mean annual rainfall of the area is 1307–1835 mm and the mean annual minimum and maximum temperature is 8.3–25 °C. The district exhibits four climate zones: Wurch (upper highlands above 3200 m a.s.l), Dega (highlands 2300–3200 m a.s.l), Woina dega (midlands 1500–2300 m a.s.l), and Kola (lowlands 500–1500 m a.s.l). These zones correspond to the four major eco-epidemiological strata of malaria in the country: highland areas above 2500 m altitude (malaria free), highland fringe areas between 1500 and 2500 m (with frequent epidemics), lowland areas below 1500 m (with seasonal pattern of transmission), and stable malaria areas (characterized by all year round transmission) (FMOH 2010; WHO 2020). The peak period of malaria transmission occurs between September and December following
the main rainy season (June–September). A second minor transmission period occurs from April to June, following a short rainy season of February to March.

Estie is about 676 km northwest of Addis Ababa and about 110 km north of Bahir Dar. The total area of this Woreda is 132,373.9 km². It has 42 rural kebeles and 3 urban kebeles. Based on figures published by the Central Statistical Agency (CSA) in 2005, Estie has an estimated total population of 403,956, of whom 199,325 are men and 204,631 are women; 16,014 (3.96%) of its population are urban dwellers.

The source population of this study consisted of all individuals who visited Mekaneeyesus Primary Hospital for different kinds of services during the study period while the study population included those individuals who visited the hospital during data collection.

Sample size determination and sampling techniques

The sample size of the study was determined using a single population proportion formula for cross-sectional studies (Naing et al. 2006),

\[ n = \frac{z^2 p(1-p)}{d^2} \]

where \( n \) represents the minimum sample size required; \( z \) was 1.96, which is the standard normal deviate (for a 95% confidence interval, CI); \( d \) was 0.05, the acceptable error willing to be committed; and \( p \), which is 0.5, is the estimated proportion of malaria prevalence as there was no similar study in the study area. Accordingly, the sample size of the study was

\[ = \frac{3.8416 \times 0.5 \times 0.5}{0.0025} = 384.16 \approx 384 \]

Anticipating non-response rate, 5% was added to the normal calculated sample size, making the final sample size of the study 403. Based on these assumptions, study participants were selected using simple random sampling techniques until the required sample size was obtained.

Data collection procedure

Questionnaire survey

A pretested structured questionnaire was administered to collect information on gender, age, marital status, education, religion, occupation, residence, family income, house types, presence or absence of mosquitoes breeding sites nearby residence, distance of residence from stagnant water, and bed net utilization. The questionnaire was first developed in English and translated into the local language (Amharic) back into English to check for consistency.

Laboratory examination

Parasite detection

Before commencing collection of blood samples and filling questionnaire, explanation about the study was given and signed written informed consent was obtained from every study participant to assure their willingness to take part in the research process. Then, capillary blood samples were collected by finger pricking using 70% isopropanol and sterile disposable lancet. Immediately, thin film was spread on grease free, frosted end of labeled slide using a smooth edged slide spreader. The thick smear was also prepared on the same slide by spreading larger drop of blood. The thin blood smear was allowed to air dry for 10 min and then fixed with absolute methanol for 5 s and then air-dried. The thick smears were air-dried for about 30 min, not fixed in methanol but dipped in water to dehemoglobinize. The blood films were stained with 10% Giemsa for 10 min (Cheesbrough 2005). Finally, the films were examined under a microscope using an oil immersion microscope objective \((\times 100)\) for Plasmodium species identification.

ABO blood group typing

ABO blood groups were typed by standard hemagglutination techniques using commercially available monoclonal anti-A, anti-B, and anti-D blood grouping sera (Agappe Diagnostics Ltd., India) following methods described by Cheesbrough (2005). A drop of whole blood was placed into different places of the clean slide on which a drop of antisera for blood groups A, B, and Rh was added. The blood cells and the antigen were mixed thoroughly with an applicator stick. Then, the slide was rotated to detect for agglutination. The drops of blood that showed agglutination were considered to be positive for a particular blood grouping reagent (Rosenfield 1976). The results were recorded accordingly, as blood groups A+, B+, AB+, and O+ or A-, B-, AB-, and O-.

Determination of hemoglobin (Hb) Concentration

The hemoglobin concentration was determined using HemoCue (HemoCue HB 301, Anghelom, Sweden). The hemoglobin values were used to assess the status of anemia and hemoglobin concentrations were expressed in g/dl (WHO 2011). Accordingly, the study participants were classified as anemic or non-anemic based on their hemoglobin level.

Data analysis

The data gathered were double entered into Microsoft Excel datasheets and were crosschecked and imported into SPSS version 21 for analysis. Descriptive statistics were carried
out to measure relative frequencies and percentages of the variables. Logistic regression analysis was performed to examine associations between variables by using an odds ratio. Odds ratios (OR) were calculated with 95% confidence interval (CI). Variable having $p$ values less than 0.25 in univariate test was selected and entered for multivariate logistic regression analysis to identify the most important predictors of malaria risk factors based on the test from logistic regression (Bursac et al. 2008). Allelic frequencies were determined using the Bernstein method and genotypes were calculated by Hardy-Weinberg model (Kumar et al. 2014; Ndoula et al. 2014). The Hardy-Weinberg equilibrium (HWE) was tested using the goodness-of-fit chi-square test. The calculation for allelic frequencies and HWE was done using S2 ABO estimator software. The values were considered to be statistically significant when $p$ values are less than 0.05.

**Inclusion and exclusion criteria**

Individuals who consented to fill the questionnaire and give blood sample were included in the study, while individuals, who cannot communicate due to impairment or sever sickness like coma, and mentally sick people and those who were currently on anti-malaria drugs were exclude from the study. People who did not provide consent were also excluded from the study.

**Data quality control**

In order to make this study more reliable and valid, a questionnaire was carefully prepared using questionnaires used in formerly conducted similar studies after minor amendment for data collection in this study. Five percent of this questionnaire was pretested on individuals who visited another health institution in the district prior to the actual data collection. Uncertainty and problem expressed by participants in understanding of any of the items in the questionnaire and their comments and feedback were noted and considered in the revision and modification of the final questionnaire. Besides, the questionnaire was translated into local Amharic language to make it more understandable to the subjects and the trained data collectors.

All the necessary reagents, chemicals, and instruments were checked by known positive and negative samples before processing and examination of samples of the study subjects. Known ABO blood group types were used to check the reliability of the anti-A, anti-B, and anti-D antisera. Positive samples were reexamined by another laboratory technologist at the hospital, which was blinded for the first examination results. The results of the laboratory examination were recorded in a well-prepared format carefully.

**Result**

**Sociodemographic characteristics**

Of the total of 403 individuals invited to participate, 390 (96.8%) filled questionnaire and provided blood samples and the remaining 13 (3.2%) who declined to participate at sample collection stage were excluded from the study. Two hundred eleven (54.1%) of the participants were males and 179 (45.9%) were females. The mean age of the sampled population was 31.48 ± 15.62 years and the highest 108 (27.7%) number of participants were found to be within the age range of 25–34 years. Participants in the urban setting had much income than those in rural settings. Most 242 (62.1%) of the subjects were urban residents and the involvement of married 186 (47.7%) and unmarried 176 (45.1%) participants was relatively similar. The educational background of the study participants varied from those who were illiterate to those who attended college and above (Table 1).

**The overall prevalence of malaria**

Out of the 390 microscopically examined blood samples, 33 samples were found positive for malaria infection with the overall prevalence rate of 8.5%. Two *Plasmodium* species were detected in this investigation; *P. vivax* (5.6%) was more dominant species than *P. falciparum* (2.3%) and mixed infection of the two species accounted for (0.5%) (Fig. 1). Varied prevalence rates of malaria infection were detected across all age categories and male and female subjects. The prevalence was higher among males 25 (11.8%) than among females 8 (4.5%). A threefold (16.9%) of malaria infection was observed among rural residents compared to their urban counterparts (3.3%). Regarding the educational status of the participants, higher rate of the disease was detected among those who attended elementary school education (grade levels 1–8) (19.0%) and subjects who were uneducated (15.6%) while the least was in those who had attained college and above educational levels (Table 2).

Of the total sociodemographic, practice and related variables, male sex (AOR = 3.48, 95% CI: 0.86–14.06, $p=0.02$), being under-five years old (AOR = 72.84, 95% CI: 2.68–1979.83, $p=0.01$), rural residence (AOR = 2.64, 95% CI: 0.570–12.22, $p=0.05$), and failing to use bed net (AOR =4.65, 95% CI: 1.14–18.99, $p=0.01$) were found to be the significant independent predictors of *Plasmodium* infection in the multivariate analysis (Table 3). While, no significant associations between the infection and other variables were found.

In our study, 3.5 times elevated risk of malaria infection was observed in males than in females (AOR = 3.48, 95% CI 0.86–14.06, $p=0.02$). Under-five age category was positively associated with malaria and the likelihood of malaria infection
| Variable                        | Frequency ($n$) | Percentage (%) |
|--------------------------------|-----------------|----------------|
| Sex                            |                 |                |
| Male                           | 211             | 54.1           |
| Female                         | 179             | 45.9           |
| Age (year)                     |                 |                |
| Under 5                        | 12              | 3.1            |
| 5–14                           | 26              | 6.7            |
| 15–24                          | 94              | 24.1           |
| 25–34                          | 108             | 27.7           |
| 35–44                          | 72              | 18.5           |
| 45–54                          | 40              | 10.3           |
| ≥55                            | 38              | 9.7            |
| Marital status                 |                 |                |
| Unmarried                      | 176             | 45.1           |
| Married                        | 186             | 47.7           |
| Widow/widower                  | 15              | 3.8            |
| Divorced                       | 13              | 3.3            |
| Educational status             |                 |                |
| Uneducated                     | 109             | 27.9           |
| 1–8                            | 42              | 10.8           |
| 9–12                           | 41              | 10.5           |
| College and above              | 198             | 50.8           |
| Religion                       |                 |                |
| Orthodox                       | 273             | 70.0           |
| Muslims                        | 95              | 24.4           |
| Protestant                     | 20              | 5.1            |
| Catholic                       | 2               | 0.5            |
| Occupational status            |                 |                |
| Unemployed                     | 45              | 11.5           |
| Daily laborer                  | 30              | 7.7            |
| Student                        | 73              | 18.7           |
| House wife                     | 20              | 5.1            |
| Farmer                         | 71              | 18.2           |
| Merchant                       | 74              | 19.0           |
| Government employee            | 77              | 19.7           |
| Residence                      |                 |                |
| Rural                          | 148             | 37.9           |
| Urban                          | 242             | 62.1           |
| Family income (in Ethiopian Birr/month) |         |                |
| Less than 500                  | 74              | 19.0           |
| 500–1000                       | 63              | 16.2           |
| 1001–1500                      | 43              | 11.0           |
| 1501–2000                      | 31              | 7.90           |
| Above 2000                     | 179             | 45.9           |
| House type                     |                 |                |
| Thatched roof and local dung plaster | 2         | 0.50           |
| Corrugated iron sheet and stick/mud plaster | 324 | 83.1 |
| Brick                          | 64              | 16.4           |
among under-five children was about 73 times higher (AOR = 72.84, 95% CI 2.68–1979.83, p=0.01). Besides, rural residence was significantly associated with risk of malaria; the dwellers in this setting had 2.6 times higher risk of malaria than urban residents (AOR = 2.64, 95% CI 0.57–12.22, p= 0.05). The odds of malaria was also about five times significantly higher in individuals who did not use ITNs than those who used ITNs (AOR =4.65, 95% CI 1.14–18.99, p=0.01) (Table 3).

Frequency distribution of ABO blood group systems

The most dominant ABO blood type was O (146 (37.44%)), followed by type A, which accounted for 123 (31.54%). The prevalence of blood types B and AB was 103 (26.41%) and 18 (4.62%), respectively. Of the total study subjects, majority (360 (92.2%)) were Rh positives while the rest (30 (7.8%)) were Rh negatives (Fig. 2).

Association between ABO blood group and malaria infection

The highest (14.63%) prevalence of malaria infection was observed among subjects with blood group A followed by those with blood group B (8.74%) and AB (5.56%). While individuals with blood group O were the least (3.42%) affected. The distribution malaria infection rate in ABO blood group was in the order of A > B > AB > O. Overall, there was statistically significant association between ABO blood group and malaria infection (χ²=11.04, p=0.01) (Table 2).

The likelihood of infection was 2.23-fold higher in individuals with blood group A compared to individual with other blood types. Likewise, the magnitude of the risk of malaria infection of each blood group in contrast to “O” type was estimated using COR and AOR at 95% CI with the help of logistic regression model. Individuals with A blood type were 3.74 times more increased risk of malaria infection (AOR= 3.74, 95% CI 1.14–12.29, p= 0.03) compared to those having “O” type. However, in the other blood types, the associations were not statistically significant (Table 4).

The allelic frequencies of I(A), I(B), and I(O) of ABO blood group in malaria infected were 0.459, 0.365, and 0.173, respectively, while these proportions were 0.189, 0.170, and 0.639, respectively, among malaria-negative participants. There was statistically significant difference in the distribution of ABO allele frequencies among malaria-positive subjects (p<0.05) (Table 5).

The frequencies of allele A and genotype AA were substantially higher among malaria-infected individuals as compared to malaria-uninfected ones and there was also a twofold representation of AB genotypes among malaria-infected individuals, while the proportions of allele O and genotype OO were considerably higher among malaria-negative than among malaria-positive subjects.

Hemoglobin (Hb) level and prevalence of anemia

The mean hemoglobin level of the study participants was 13.3 g/dl ± 1.87 and ranged from 5.6 to 18.60g/dl. The overall prevalence of anemia was 23.1%. Out of the total participants, 77 (19.7%) had mild anemia, 9 (2.30%) had moderate anemia, and only 4 (1%) had severe anemia (Table 6).

Chi-square analyses indicated statistically significant association between status of anemia of the participants and malaria infection (χ²= 128.45, p= 0.00). All of the individuals with severe anemia were infected with malaria. This is
Table 2 Cross tabulation of chi-square analysis of association of malaria with sociodemographic and environmental risk factors of the study participants

| Variables                           | Number examined | Malaria positive | Malaria negative | Chi-square, p value ($\chi^2$, p) |
|-------------------------------------|----------------|------------------|------------------|----------------------------------|
| **Sex**                             |                |                  |                  |                                  |
| Male                                | 211 (54.1)     | 25 (11.8)        | 186 (88.2)       | 6.80, 0.01                       |
| Female                              | 179 (45.9)     | 8 (4.46)         | 171 (95.5)       |                                  |
| **Age categories**                  |                |                  |                  |                                  |
| Under 5                             | 12 (3.1)       | 5 (41.6)         | 7 (58.3)         | 32.32, 0.00                      |
| 5–14                                | 26 (6.7)       | 7 (26.9)         | 19 (73.1)        |                                  |
| 15–24                               | 94 (24.1)      | 7 (7.4)          | 87 (92.6)        |                                  |
| 25–34                               | 108 (27.7)     | 7 (6.5)          | 101 (93.5)       |                                  |
| 35–44                               | 72 (18.5)      | 4 (5.6)          | 68 (94.4)        |                                  |
| 45–54                               | 40 (10.3)      | 1 (2.5)          | 39 (97.5)        |                                  |
| ≥ 55                                | 38 (9.7)       | 2 (5.3)          | 36 (94.7)        |                                  |
| **Marital status**                  |                |                  |                  |                                  |
| Unmarried                           | 176 (45.1)     | 22 (12.5)        | 154 (87.5)       | 10.27, 0.02                      |
| Married                             | 186 (47.7)     | 7 (3.8)          | 179 (92.2)       |                                  |
| Divorced                            | 13 (3.3)       | 2 (15.4)         | 11 (84.6)        |                                  |
| Widowed/widower                     | 15 (3.8)       | 2 (13.3)         | 13 (86.7)        |                                  |
| **Education status**                |                |                  |                  |                                  |
| Uneducated                          | 109 (27.9)     | 17 (15.6)        | 92 (84.4)        | 22.32, 0.00                      |
| 1–8                                 | 42 (10.8)      | 8 (19.0)         | 34 (81.0)        |                                  |
| 9–12                                | 41 (10.5)      | 3 (7.3)          | 38 (92.7)        |                                  |
| College and above                   | 198 (50.8)     | 5 (2.5)          | 193 (97.5)       |                                  |
| **Occupation**                      |                |                  |                  |                                  |
| Student                             | 73 (18.7)      | 8 (11.0)         | 65 (89.0)        | 11.80, 0.07                      |
| Daily laborer                       | 30 (7.7)       | 4 (13.3)         | 26 (86.7)        |                                  |
| Unemployed                          | 45 (11.5)      | 7 (15.6)         | 38 (84.4)        |                                  |
| House wife                          | 20 (5.1)       | 2 (10.0)         | 18 (90.0)        |                                  |
| Farmer                              | 71 (18.2)      | 8 (11.3)         | 63 (88.7)        |                                  |
| Merchant                            | 74 (19.0)      | 2 (2.7)          | 72 (97.3)        |                                  |
| Government employee                 | 77 (19.7)      | 2 (2.6)          | 75 (97.4)        |                                  |
| **Family income (in Birr/month)**   |                |                  |                  |                                  |
| Less than 500                       | 74 (19.0)      | 18 (24.3)        | 56 (75.7)        | 46.22, 0.00                      |
| 501–1000                            | 63 (16.2)      | 11 (17.5)        | 52 (82.5)        |                                  |
| 1001–1500                           | 43 (11.0)      | 1 (2.3)          | 42 (97.7)        |                                  |
| 1501–2000                           | 31 (7.9)       | 0 (0.0)          | 31 (100.0)       |                                  |
| Above 2000                          | 179 (45.9)     | 3 (1.7)          | 176 (98.3)       |                                  |
| **Residence**                       |                |                  |                  |                                  |
| Rural                               | 148 (37.9)     | 25 (16.9)        | 123 (83.1)       | 21.89, 0.00                      |
| Urban                               | 242 (62.1)     | 8 (3.3)          | 234 (96.7)       |                                  |
| **House type**                      |                |                  |                  |                                  |
| Thatched roof and local dung plaster| 2 (0.5)        | 2 (100.0)        | 0 (0.0)          | 28.06, 0.00                      |
| Corrugated iron sheet and stick/mud plaster | 324 (83.1)   | 31 (9.6)         | 293 (90.4)       |                                  |
| Brick                               | 64 (16.4)      | 0 (0.0)          | 64 (100.0)       |                                  |
| **Availability of mosquitoes breeding sites nearby home** |    |                  |                  |                                  |
| Yes                                 | 334 (85.6)     | 31 (9.3)         | 303 (90.7)       | 2.02, 0.16                       |
| No                                  | 56 (14.4)      | 2 (3.6)          | 54 (96.4)        |                                  |
| **Distance of stagnant water from homes** |           |                  |                  |                                  |
| Below 1km                           | 78 (20.0)      | 26 (33.3)        | 52 (66.7)        | 78.06, 0.00                      |
| 1 and above                         | 312 (80.0)     | 7 (2.24)         | 254 (98.1)       |                                  |
| **Blood group**                     |                |                  |                  |                                  |
followed by those with moderate (66.7%) and mild (24.7%) anemia. The least (1.3%) infection was observed among non-anemic subjects (Table 6).

Severe anemia was detected only in individuals with blood group A (4 (3.3%)). Moderate anemia appeared among 7 (5.7%) and 2 (1.4%) of blood type A and O participants respectively, while mild anemia appeared in subjects with all ABO blood types with slightly varying proportions (21.2, 23.3, 16.7, and 16.4% in A, B, AB, and O, respectively). There was statistically significant association between ABO blood group and hemoglobin levels of the studied subjects ($p=0.01$) (Table 7).

The highest overall prevalence of anemia was detected among individuals with blood group A (30.1%) followed by those with type B (23.3%) and it was 16.7 and 17.8% in subjects with AB and O blood groups, respectively. However, no statistically association was found between overall anemia and ABO blood group ($p=0.10$).

Of the total 33 malaria-positive cases, majority (30 (90.9%)) were found to be anemic while the remaining (3 (9.1%)) were non-anemic. Of all malaria-positive anemic subjects, 4 (13.3%) and 26 (86.7%) had severe malarial anemia (SMA) and non-severe malarial anemia (non-SMA), respectively. There was no statistically significant association between SMA and ABO blood groups ($p=0.31$) (Table 7).

### Discussion

Decades of rigorous fight against malaria has resulted in a remarkable decline in the burden of the disease in Ethiopia.

### Table 2 (continued)

| Variables       | Number examined   | Malaria positive | Malaria negative | Chi-square, $p$ value ($\chi^2$, $p$) |
|-----------------|-------------------|------------------|------------------|---------------------------------------|
|                 | $n$ (%)           | $n$ (%)          | $n$ (%)          |                                        |
| A               | 123 (31.5)        | 18 (14.6)        | 105 (85.4)       | 11.04, 0.01                            |
| B               | 103 (26.4)        | 9 (8.7)          | 94 (91.3)        |                                        |
| AB              | 18 (4.6)          | 1 (5.5)          | 17 (94.4)        |                                        |
| O               | 146 (37.4)        | 5 (3.4)          | 141 (96.6)       |                                        |
| Bed net utilization |               |                 |                  |                                        |
| No              | 65 (16.7)         | 23 (35.4)        | 42 (64.6)        | 72.99, 0.00                            |
| Yes             | 325 (83.3)        | 10 (3.1)         | 315 (96.9)       |                                        |

Table 3  Multivariate logistic regression analysis of some selected risk factors of malaria and seemingly significant explanatory variables in Mekaneeyesus Hospital, 2018

| Variable       | $N$ (%) | $n$ (%) | Crude OR (95% CI) | $p$ value | Adjusted OR (95% CI) | $p$ value |
|----------------|---------|---------|-------------------|-----------|----------------------|-----------|
| Sex            |         |         |                   |           |                      |           |
| Male           | 211 (54.1) | 25 (11.8) | 2.87 (1.26,6.54)  | 0.01      | 3.48 (0.86, 14.06)   | 0.02      |
| Female         | 179 (45.9) | 8 (4.5) | 1.00 | | 1.00 | |
| Age category   |         |         |                   |           |                      |           |
| Under 5       | 12 (3.1) | 5 (41.6) | 12.86 (2.06, 80.05) | 0.01 | 72.84 (2.68, 1979.83) | 0.01 |
| 5–14           | 26 (6.7) | 7 (26.9) | 6.63 (1.35, 35.12) | 0.03 | 23.25 (0.69, 773.93) | 0.08 |
| 15–24          | 94 (24.1) | 7 (7.4) | 1.45 (0.29, 7.31) | 0.65 | 7.29 (0.59, 90.79) | 0.12 |
| 25–34          | 108 (27.7) | 7 (6.5) | 1.25 (0.25, 6.28) | 0.79 | 2.53 (0.22, 28.65) | 0.45 |
| 35–44          | 72 (18.5) | 4 (5.6) | 1.06 (0.18, 6.06) | 0.95 | 17.44 (0.25, 1237.86) | 0.19 |
| 45–54          | 40 (10.3) | 1 (2.5) | 0.46 (0.04, 5.31) | 0.54 | 1.55 (0.06, 37.76) | 0.79 |
| ≥ 7 (ref)      | 38 (9.7) | 2 (5.3) | 1.00 | | 1.00 | |
| Residence      |         |         |                   |           |                      |           |
| Rural          | 148 (37.9) | 25 (16.9) | 5.94 (2.60, 13.57) | 0.00 | 2.64 (0.57, 12.22) | 0.09 |
| Urban (ref)    | 242 (62.1) | 8 (3.3) | 1.00 | | 1.00 | |
| Bed net utilization |       |         |                   |           |                      |           |
| No             | 65 (16.7) | 23 (35.4) | 17.25 (7.68, 38.75) | 0.00 | 4.65 (1.14, 18.99) | 0.01 |
| Yes (ref)      | 325 (83.3) | 10 (3.07) | 1.00 | | 1.00 | |

*N* total number of study participants, *n* number of malaria cases

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However, malaria is still continued to be reported as one of the top three leading causes of outpatient visits, admissions, and deaths among all age group in country (USAID 2015).

In the present study, the prevalence of microscopically confirmed malaria parasite was 8.5%. The prevalence rate of malaria parasite identified in this study is lower than 25% and 16% which were reported from eastern (Tadesse et al. 2018) and southern parts of Ethiopia (Molla and Ayele 2015) and those of 38% and 27.3% reported from India (Chaubey et al. 2017) and Nigeria (Gomerep et al. 2017), respectively. In contrary, the present result is higher than 5.2, 4.1, and 0.93% prevalence rates reported from south (Alemu and Mama 2016), southwest (Alemu et al. 2011a), and south central (Woyessa et al. 2012) parts of Ethiopia, respectively. The observed disparities in the prevalence rates might be due to the differences in the abundance of vectors, seasonality of malaria, control measures, study population, and sample size.

The predominant Plasmodium species detected in this investigation was P. vivax (5.6%) followed by P. falciparum (2.3%) and mixed malaria infection of both species accounted for (0.5%). This agrees with several other previous studies conducted in different parts of Ethiopia that also reported predominance of P. vivax followed by P. falciparum and mixed infection of the two, respectively (Woyessa et al. 2012; Tefera 2014). The current

![Fig. 2] Distribution of ABO blood groups and Rh factors among study participants

### Table 4

| Outcome                  | Blood type | Logistic regression models | Odds ratio  | 95% CI     | p value |
|--------------------------|------------|----------------------------|-------------|------------|---------|
| Malaria positive         | A vs non A | Crude                      | 2.88        | 1.40–5.93  | 0.01    |
|                          |            | Adjusted*                  | 2.23        | 0.93–5.34  | 0.07    |
|                          | B vs non B | Crude                      | 1.05        | 0.47–2.34  | 0.91    |
|                          |            | Adjusted*                  | 1.09        | 0.42–2.89  | 0.85    |
|                          | AB vs non AB| Crude                     | 0.63        | 0.08–4.85  | 0.65    |
|                          |            | Adjusted*                  | 0.80        | 0.07–8.90  | 0.86    |
|                          | O vs non O | Crude                      | 0.27        | 0.10–0.73  | 0.01    |
|                          |            | Adjusted*                  | 0.32        | 0.10–0.98  | 0.05    |
|                          | A vs O     | Crude                      | 4.83        | 1.74–13.44 | 0.00    |
|                          |            | Adjusted*                  | 3.74        | 1.14–12.29 | 0.03    |
|                          | B vs O     | Crude                      | 2.70        | 0.877–8.31 | 0.08    |
|                          |            | Adjusted*                  | 2.58        | 0.698–9.50 | 0.16    |
|                          | AB vs O    | Crude                      | 1.66        | 0.18–15.05 | 0.65    |
|                          |            | Adjusted*                  | 1.85        | 0.14–23.95 | 0.64    |

Adjusted* (adjusted odds ratios from multivariate logistic regression models = adjusted for the effects of age, sex, and anemia)
result is also consistent with findings reported outside Ethiopia (Chaubey et al. 2017; Vajda and Webb 2017).

The dominance of *P. vivax* over *P. falciparum* attributed to the ambient temperature of the study area might have affected *P. falciparum* within the vector, active case detection and early treatment of *P. falciparum* cases by artemisinin-based therapy. The predominance of *P. vivax* over *P. falciparum* could also be due to the spreading of chloroquine resistance of *P. vivax* strains, its peculiarity in relapsing and decrease in the efficacy of standard dose of primaquine as antirelapse for *P. vivax* (Rajgor et al. 2014). However, the present finding contradicts the reports of Alelign and Dejene (2016) from south and Deressa (2017) from south central parts of Ethiopia that reported predominance of the *P. falciparum* species over *P. vivax* species. It also disagrees with the findings by other investigators (Gayathri et al. 2013; Worku et al. 2016).

Gender-wise, a significant 3.5 times higher risk of malaria infection was observed in males than in females. The increased risk of malaria infection in male subjects in our study is supported by Muntaka and Opoku-Okrah (2013), who reported about twice higher odds of developing malaria in males as compared to females. The finding of our study also agrees with observations of Moise and Robert (2017) (Nigeria), Muawia and Abdalla (2017) (Sudan), and Deressa (2017) (Ethiopia). The reason behind this might be involvement of males in irrigation and other outdoor activities which might increase males’ exposure to the bites of malaria vector and subsequent development of the disease than their female counterparts (Uneke 2007; Worku et al. 2016). However, reports contradicting the present study were reported from Nigeria (Otajevwo 2013) and Ethiopia (Tadesse and Tadesse 2013). On the other hand, other studies did not find any significant disparity in the risk of malaria infection between male and female participants (Fischer and Boone 1998; Olasunfunmi et al. 2013).

With regard to association between age category of the subjects and malaria infection, it was observed that under-five children were about 73 times more likely to contract the disease. This finding is in accord with other studies that reported relatively higher rates of malaria among under-five children (Tadesse and Tadesse 2013; Olawumi et al. 2014). Contrary to our finding, Tadesse and his colleagues (2018) reported statistically significant 3.7 to 6.7 times more likelihood of malaria infection among 16 to 44 years old (Tadesse et al. 2018). Likewise, Molla and Ayele (2015) in southern Ethiopia and Sultana et al. (2017) in Kenya found increased odds of malaria in children 10 to 24 years old compared to those under-five years of age.

Several studies have demonstrated that the use of ITNs is effective in reducing malaria-related morbidity and mortality (D’Alessandro et al. 1995). In the present study, the odd of malaria was significantly five times higher in individuals who did not use ITNs than in those who used ITNs. Likewise, similar investigations were also confirmed that individuals who did not use ITN were much more likely to suffer from malaria than those who used bed nets (Sultana et al. 2017).

With the reference to the relationship between place of residence and malaria risk, the finding of the present study is consistent with recently reported research findings from Ethiopia (Tadesse et al. 2018) and Kenya (Sultana et al. 2017) that demonstrated a significantly increased risk of malaria infection among rural residents as compared to urban residents. According to Hay and his colleagues (2005), urban areas are considered to be at lower risk of malaria compared to rural areas because of improved housing, higher socioeconomic status, and limited number of breeding sites (Hay et al. 2005). With increasing distance from breeding sites, the number of *Anopheles* and the risk of receiving infective bites are decreasing. In support of this, Alemu et al. (2011b)

| Table 5 Allelic and genotypic frequency of ABO blood group |
|---------------------------------|-----------------|-----------------|
| Variables | Allelic frequency | Genotype frequency |
| Alleles | Plasmodium infected | Plasmodium non-infected |
| p(A) | 0.365 | 0.189 |
| q(B) | 0.173 | 0.170 |
| r(O) | 0.459 | 0.639 |
| Hardy-Weinberg Log likelihood | -38.3199 | -438.0141 |
| x(Adikwu et al. 2017) | 4.5901 | 2.4560 |
| p value | 0.0322 | 0.1171 |
| Genotype | Genetic frequency | Genetic frequency |
| AA | 0.605 | 0.036 |
| AO | 0.366 | 0.242 |
| BB | 0.038 | 0.038 |
| BO | 0.159 | 0.220 |
| AB | 0.127 | 0.065 |
| OO | 0.211 | 0.409 |
and Olasunkanmi et al. (2013) reported strong association between the prevalence of malaria and the proximity of residence to potential mosquito breeding sites.

Reports of several investigations about the potential association between ABO blood group and the risk of malaria in diverse populations are contradictory. The present study revealed significantly varied degrees of malaria infection among the four blood types ($p = 0.01$). The highest (14.63%) malaria infection was observed among individuals with blood group A followed by those with blood group B (8.74%) and AB (5.56%) phenotypes, while individuals with blood group O phenotype were the least (3.56%) affected. This result is in agreement with the findings of Tekeste and Petros (2010), Zerihun et al. (2011), and Tadesse and Tadesse (2013).

In accordance with the distribution of blood group phenotypes, the frequencies of allele A and genotype AA were substantially higher among malaria-infected individuals as compared to malaria-uninfected ones and there was also a twofold representation of AB genotype among malaria-infected individuals, while proportions of allele O and genotype OO were considerably higher among malaria-negative as compared to malaria-positive subjects. Besides, there was statistically significant difference in the distribution of ABO allele among malaria-positive subjects ($p < 0.03$). This agrees with reports of Kumar et al. (2014).

The current study found about 3.7 times increased risk of malaria infection in individual with blood type “A” ($p = 0.03$). This finding is comparable with the result of a study by Kuadzi et al. (2011), who recorded nearly three times odds of the disease in individuals with blood group “A.” The result of the present study is also in line with other studies conducted in Ethiopia (Tekeste and Petros 2010), India (Panda et al. 2011), Ghana (Afokwah et al. 2016), and Sudan (Muawia and Abdalla 2017), all revealed that individuals with blood group O were less prone to severe malaria as compared to individuals with other blood groups. The difference in susceptibility to malaria infection and severity might be attributed to the difference in rosetting ability among red blood cells of different “ABO” blood groups with a diminished rosetting potential in individuals with blood group “O” red blood cells (Deepa et al. 2011; Gupta et al. 2012). On the other hand, the study of Alemu and Mama (2016) showed about seven times more susceptibility of individuals with blood group O to Plasmodium infection than those with other ABO blood groups.

The prevalence of anemia in our study was 23.1%. Of the total participants, 19.70% had mild, 2.30% had moderate anemia, and only 1% of the participants had severe anemia. The overall prevalence of anemia in this study is much lower than the findings of Okafor et al. (2012) (61.1%) in Nigeria and Njunda et al. (2015) (44.8%) in Cameroon. The current result

### Table 6 Association of anemia status (hemoglobin levels) with prevalence of malaria

| Anemia status | Examined, $n$ (%) | Prevalence of malaria |
|---------------|------------------|-----------------------|
|               | Positive, $n$ (%) | Negative, $n$ (%)     |
| Severe        | 4 (1.0)          | 4 (100.0)             | 0 (0.0)               |
| Moderate      | 9 (2.3)          | 6 (66.7)              | 3 (33.3)              |
| Mild          | 77 (19.7)        | 19 (24.7)             | 58 (75.3)             |
| Non-anemic    | 300 (76.9)       | 4 (1.3)               | 296 (98.7)            |
| Total         | 390              | 33 (8.5)              | 357 (91.5)            |

$\chi^2 (p) = 128.452 (0.00)$

Key: non-anemic $\geq 12.0$, mild 11–11.9, moderate 8–10.9, severe < 8.0 (WHO 2011)

### Table 7 Association between ABO blood type with hemoglobin levels (anemia), overall anemia, and severe malarial anemia (SMA)

| Variable | ABO blood type | Total | $\chi^2 (p)$ |
|----------|----------------|-------|--------------|
|          | A              | B     | AB           | O             |       |
| Hemoglobin levels of participants | 4 (3.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (1.0) | 21.217 (0.01) |
| Severe   | 26 (21.2) | 24 (23.3) | 3 (16.7) | 24 (16.4) | 77 (19.7) |         |
| Moderate | 86 (69.9) | 79 (76.7) | 15 (83.3) | 120 (82.2) | 300 (76.9) |         |
| Non-anemic | 123 (31.5) | 103 (26.4) | 18 (4.6) | 146 (37.4) | 360 |         |
| Overall anemia status of participants | 37 (30.1) | 24 (23.3) | 3 (16.7) | 26 (17.8) | 90 (23.1) | 6.102 (0.10) |
| Anemic   | 86 (69.9) | 79 (76.7) | 15 (83.3) | 120 (82.2) | 300 (76.9) |         |
| Non-anemic | 123 | 103 | 18 | 146 | 390 |         |
| Severe malarial anemia (SMA) status of participants | 4 (23.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (13.3) | 3.529 (0.31) |
| SMA      | 13 (76.5) | 7 (100.0) | 1 (100.0) | 5 (100.0) | 26 (86.7) |         |
| Non-SMA | 17 (56.7) | 7 (23.3) | 1 (3.3) | 5 (16.7) | 30 |         |
is still very much lower than 86% reports of anemia prevalence (Mbah et al. 2015; Adikwu et al. 2017). Enormous cultural and economic differences may account for this variation. Socioeconomic status may affect the risk of anemia by affecting nutritional status, family size, and birth interval, as well as intensifying problems of affordability and accessibility of preventive and curative measures (Erhabor et al. 2014).

Anemia is characterized by a decrease in hemoglobin concentration which may result due to acute or chronic blood loss, reduced or impaired formation of erythrocytes, nutritional deficiencies, infectious diseases, and genetic disorders (Abbaspour et al. 2014). Malaria is a major cause of anemia in malaria endemic areas. Malaria infection causes hemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis which compromises rapid recovery from anemia. In areas of high malaria transmission malaria, nearly all infants and young children and many older children and adults have a reduced hemoglobin concentration as a result (White 2018).

The present study indicated a statistically significant association between anemia status of the participants and malaria infection. The risk of malaria was shown to increase with increasing severity of anemia. All of the individuals with severe anemia were infected with malaria followed by those with moderate (66.7%) and mild (24.7%) anemia and non-anemic (1.3%) subjects. This result agrees with some studies, which demonstrated significant association between the statuses of anemia and risk of malaria (Erhabor et al. 2014; Kunihya et al. 2016; Adikwu et al. 2017). Contrary to this, a study conducted in Cameroon did not find significant association between malaria and anemia (Njunda et al. 2015). The differences in malaria prevalence with anemia status could be attributed to the difference in the levels of malaria endemicity, examination technique variation, study subject difference, season’s difference, and ecological factors.

The findings pertaining to the association between ABO blood groups and hemoglobin levels in literature are inconclusive. In the current study, we observed a statistically significant association between ABO blood group and hemoglobin levels of the studied subjects. Severe anemia (Hb level < 8.0 g/dl) was detected only among individuals with blood group A but not in other blood groups. Participants with blood type A were also more subjected to moderate anemia than those with type O blood group. Contrary to our finding, studies by Burhan et al. (2016) and Hoque et al. (2016) reported no significant variations in Hb levels among different ABO phenotypes with the lowest Hb levels among individuals with blood groups O and AB, respectively. The finding of our study disagrees with the finding of Herrera and his colleagues who reported more frequent occurrence of severe anemia among individuals with blood group O (Herrera et al. 2009). Surprisingly, another study conducted among rural population of Kanchipuram district of Tamil Nadu, India, showed significantly higher hemoglobin values in the O blood group than in the A and B blood groups (Ramalingam and Raghavan 2020).

With regard to the association between overall anemia and ABO blood group, we detected the highest prevalence of anemia among individuals with blood group A (30.1%) followed by those with type B (23.3%) while its distribution was lower but relatively similar in subjects with AB and O blood groups. However, the association was not statistically significant (p=0.10). Reshmarani et al. (2019) reported a higher burden of anemia in individuals with blood groups B and AB as compared to subjects with the other blood groups (p>0.05) (Reshmarani et al. 2019). Nevertheless, Kweni and Kweni (2016) demonstrated a significant association between anemia and ABO blood group. In this case, individuals with type AB were the most anemia affected (Kweni and Kweni 2016). This inconsistency might be attributed to variations in sample sizes, proportion of male and female participants, age, ethnicity, and socioeconomic and health conditions across the studied populations.

According to Sypniewska and his colleagues (2017), severe malaria (SM) is defined by the detection of P. falciparum by microscopy and at least one criterion for severe diseases like severe anemia (Sypniewska et al. 2017). This has been clearly demonstrated in our study, in that all of the severe malaria cases were found to be P. falciparum infected and had severe anemia. Our finding is also supported by the reports of Herrera et al. (2009) who revealed significant risk of developing severe anemia among P. falciparum-infected patients (Herrera et al. 2009).

P. falciparum is the major cause of severe malaria and severe anemia is one of the manifestations of severe malaria (World Health Organization (WHO) 2014). Furthermore, there are still reports implicating the other species of Plasmodium, P. vivax, and P. knowlesi as the causes of severe malaria (Svenson et al. 1995; Douglas et al. 2012; World Health Organization (WHO) 2014). However, we did not find P. vivax-associated severe anemia cases in our study.

Severe malarial anemia (SMA) is the major clinical presentation of severe malaria (SM). It is defined as a hemoglobin less than 5 g/dl (hematocrit less than 15%) in parasitemic children <12 years of age and a hemoglobin less than 7 g/dl (hematocrit less than 20%) in parasitemic adults (World Health Organization (WHO) 2014; Mavondo and Mzingwane 2017). Accordingly, the prevalence of SMA in our study was 4 (13.3%), all of these cases were identified among adult participants with blood group A. However, we did not find any significant association between ABO blood groups and SMA. This is line with the reports of Herrera et al. (2009) who also failed to show any association between ABO blood groups and severe anemia.
Conclusion

In conclusion, the overall prevalence of microscopically confirmed malaria parasites was 8.5%. Two species of Plasmodium, P. vivax, and P. falciparum were identified, of which, P. vivax was the dominant one. In this study, all of the severe malaria (SM) cases were found to be P. falciparum infected and had severe anemia. The prevalence of SMA in our study was 4 (13.3%), all of these cases were identified among adult participants with blood group A. In the study area, being male, under-five years of age, rural residence, and failing to use bed net were found to be the significant independent predictors of malaria. Moreover, the likelihood of malaria was substantially higher in anemic and in individuals with blood group "A," while no significant associations between the infection and other variables were found. Thus, there is need to intensify effort in malaria prevention, particularly to rural areas, under-five children, including a system to support the provision of ITNs, and other preventive measures. Further in-depth investigation is also necessary to clearly establish the role that ABO blood group plays in malaria.

Abbreviations AOR, Adjusted odds ratio; COR, Crude odds ratio; ITNs, Insecticide-treated mosquito nets; IRS, Indoor residual spraying; SMA, Severe malarial anemia; WHO, World Health Organization; FMOH, Federal Ministry of Health; USAID, United States Agency for International Development

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Authors’ contributions BT and AM designed the study; BT conducted the laboratory works, and performed data collection and wrote the first draft of the manuscript. AM and EN supervised laboratory work and data collection and critically revised the manuscript; EN performed data cleaning, provided critical review of the manuscript. All authors commented on previous versions of the manuscript and read and approved the final one.

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Availability of data and materials All data generated or analyzed during this study are not publicly available due individual privacy concerns, however, are available from the corresponding author on reasonable request.

Declarations

Ethical approval and volunteer protection The study protocol of the research was reviewed and approved by the Ethical Review Committee under Postgraduate, Research and Community service coordinating office of the College of Science, Bahir Dar University. Written informed consent was obtained from every study participant and guardians in case of children. Participants who tested positive for malaria were treated with antimalarial drugs by medical doctors based on the current national treatment guidelines of Ethiopia.

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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