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

Balaynesh Tazebew
Bahir Dar University Department of Biology
Abaineh Munshea Abitew (abitew2003@yahoo.com)
Bahir Dar University College of Science
Endalkachew Nibret
Bahir Dar University Department of Biology

Research

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Abstract

Background Malaria continues to be a major health problem in developing sub-Saharan countries including Ethiopia. Malaria is a complex disease and its local characteristics are determined by a variety of geographical, environmental, insect vector, host, and parasite factors.

Methods A hospital based cross-sectional study was conducted to determine the prevalence of malaria and its possible association with hemoglobin level and ABO blood group among individuals attending Mekaneeyesus Primary Hospital, Estie District, northwestern Ethiopia. Socio-demographic 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 entered into a computer and analyzed for descriptive and logistic regression models using SPSS version 23.0. Variables with p-value < 0.05 in multivariable logistic regression were considered as explanatory variables.

Results 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 multivariate logistic regression analysis, 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 infection. Most (14.6%) of malaria positives cases were among individuals with blood group “A”, while the least number of cases were among subjects with blood group “O”. Individuals with blood group “A” were about four times more likelihood of getting malaria as compared to individuals with blood group “O” (AOR= 3.74). The prevalence of anemia was 23% and significantly associated with malaria (p<0.05).

Conclusions Malaria in the study area is still higher than the average prevalence at country level. Therefore special attention should be given to the prevention and control strategies with the objective of increasing the awareness of local community towards malaria.

Background

Malaria is one of the most severe public health problems worldwide. Malaria is a mosquito borne infectious disease caused by an obligate intracellular protozoan parasite of the genus *Plasmodium*. The malaria parasites invade and multiply in the liver and red blood cells (RBCs) during their life cycle in human being [1]. The treatment of malaria has been a great task causing both economic and social stress on the patients. Susceptibility to malaria infection varies with individuals and location [2]. 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% and 94% of all malaria cases and deaths in the same year, respectively. Malaria is not only the cause of suffering and death, but also the cause of many socioeconomic problems in this region [3-5].

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 [6]. However, according to Deribew *et al.* [7], Ethiopia has achieved a 50% reduction target of malaria of the millennium development goals. *Plasmodium vivax* and *P. falciparum* exist commonly in Ethiopia with P*. falciparum* prevailing all year round [8]. The transmission of malaria in Ethiopia depends on environmental factors, which influence the availability of vectors and also altitude and rainfall with a lag time varying from a few weeks before the beginning of the rainy season to more than a month after the end of the rainy season [9]. The three components in the malaria transmission triangle are the host, the parasite and the vector. All interact within the influence of environmental factors. The contribution of the host has a distinct role in the transmission process, based on its capacity to affect the reservoir base of parasites [10]. Prevention and control activities of malaria in Ethiopia are implemented as guided by the National Strategic Plan (NSP) with four major intervention strategies-early diagnosis and prompt treatment with the highly efficacious artemisinin-based combination therapies (ACTs), selective vector control that involves use of insecticide-treated mosquito nets (ITNs), indoor residual spraying (IRS) and environmental management to ultimately reduce the burden of malaria to a level where it is no longer a public health problem [11].

Most clinical manifestations of malaria are the host inflammatory response, which produces the characteristic chills and fever as well as other related phenomena, and anemia, arising from the enormous destruction of red blood cells [12]. Major clinical complications which include cerebral malaria, severe anemia, jaundice, renal failure, metabolic acidosis, hypoglycemia, and acute respiratory distress syndrome occur mainly in severe *P. falciparum* malaria [13].

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 [14]. 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 [15].

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 [16]. 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 [17].

Several studies were conducted to investigate the association between ABO blood group system and some disease conditions including malaria. Some of these studies reported significant associations,

suggesting the impact of ABO blood groups on infection status of the individuals possessing a particular ABO blood group [18]. Individuals with blood group 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 [19-20]. On the other hand, many other reports demonstrated 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 [2,17, 21].

Anemia resulting from iron deficiency adversely affects cognitive and motor development, causes fatigue and low productivity [22]. 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 [23]. 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 dyserythoropoeis [24]. Other factors frequently contribute to anemia in many malarious areas including malnutrition and genetic factors [25]. Malaria infected patients are at high risk to develop anemia when compared to non-infected individuals [8].

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 hemoglobin level and ABO blood grouping among individuals attending at Mekaneeyesus Primary Hospital, Estie District, northwestern Ethiopia.

Methods

Study setting and design

A cross-sectional study design was conducted from September 2017 to April 2018 to determine the prevalence of malaria and its possible association with hemoglobin level and ABO blood group amomg 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 1307-1500 mm and the mean annual minimum and maximum temperature is 8.3^0C -25^0C. The district exhibits four climate zones: *Wurch* (upper highlands above 3200 m a.s.l), *Dega* ((highlands 2,300 – 3,200 m a.s.l), *Woina dega* (midlands 1,500 – 2,300 m a.s.l), and *Kola* (lowlands 500 – 1,500 m a.s.l). The peak times of malaria transmission occur between September and December following the main rainy season from June to August and from April to June.

Estie is about 676 km northwest of Addis Ababa and about 110 km north of Bahr Dar. The total area of this Woreda is 132,373.9 km^2. 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.

Sample size determination and sampling techniques

The sample size of the study was determined using a single population proportion formula for cross-sectional studies [26],

$$n = \frac{z^2 p\,(1-p)}{d^2}$$

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

$$= \frac{3.8416\times0.5\times0.5}{0.0025} = 384.16 \approx 384$$

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 simple random sampling techniques was employed until the required data 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, 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 minutes and then fixed with absolute methanol for 5 seconds and then air dried. The thick smears were air-dried for about 30 minutes, not fixed in methanol but dipped in water to dehaemoglobinize. The blood films were stained with 10% Giemsa for 10 minutes [27]. Finally, the films were examined under the microscope using an oil immersion microscope objective (100×) for *Plasmodium s*pecies 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 [27]. 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 were 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 [28]. The results were recorded accordingly, as blood group A+, B+, AB+, 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 [29]. 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 explanatory variables of malaria risk factors based on the test from logistic regression [30]. Allelic frequencies were determined using the Bernstein method and genotypes were calculated by Hardy-Weinberg model [31, 32]. 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.

Data quality control

Before commencing the actual data collection, a pretest on 5% of the total sample size was conducted to ensure the validity of the data collection tools. 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 re-examined 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.

Results

Sociodemographic characteristics

Of the total of 403 individuals invited to participate, 390 (96.8%) filled questionnaires 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 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 was detected across all age categories and male and female subjects. The prevalence was higher among males 25 (11.8%) than 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, the highest prevalence of the disease was found among those who attended elementary school education (grade levels 1-8) (19.0%), followed by those who were uneducated (15.6%), while the lowest prevalence was found in those who attained college and above educational levels (Table 2).

Of the total sociodemographic, practice and related variables, being male (AOR = 3.48, 95% CI: 0.86-14.06, p=0.017), being under five years old (AOR = 72.84, 95% CI: 2.68 -1979.83, p= 0.011), rural residence (AOR = 2.64, 95% CI: 0.570-12.22, p= 0.048) and failing to use bed net (AOR =4.65, 95% CI:1.14-18.99, p=0.010) were found to be the significant explanatory variables 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 females (AOR = 3.48 95% CI 0.86-14.06, p=0.017). Under-five age category was associated with malaria and the likelihood of malaria infection among under five children was about 73 times higher (AOR = 72.84 95% CI 2.68-1979.83, p=0.011). Besides, rural residence was significanty 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= 0.048). 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=0.010) (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

The highest (14.63%) prevalence of *Plasmodium* 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 degree of distribution of *Plasmodium* infection in ABO blood group was in the descending order of A > B > AB > O. Overall, there was statistical significant association between ABO blood group and *Plasmodium* infection ($\chi 2=$11.039, p=0.012) (Table 2).

Adjusted* (adjusted odds ratios from multivariate logistic regression models = adjusted for the effects of age, sex and anemia).

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 *Plasmodium* 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= 3.7495%CI 1.14 - 12.29, p= 0.030) 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^O, I^A, and I^B of ABO blood group in malaria cases 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<0.053) (Table 5).

The frequencies of allele A and genotype AA were substantially higher among individuals with malaria compared to individuals without malaria and there was also a two-fold representation of AB genotypes among individuals with malaria. 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 g/dl to 18.60g/dl. The overall prevalence of anemia was 23%. Out of the total participants, 77(19.7%) had mild anaemia, 9 (2.30%) had moderate anaemia, and only 4 (1%) had severe anaemia (Table 6).

Chi-square analyses indicated statistically significant association between status of anemia of the participants and malaria infection ($\chi 2 =$ 128.452, p= 0.000). All of the individuals with severe anemia were infected with malaria. This is 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).

Discussion

Decades of rigorous fight against malaria has resulted in a remarkable decline in the burden of the disease in Ethiopia. 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 [33].

In present study, the prevalence of microscopically confirmed malaria parasite was 8.5%. The prevalence rate of malaria parasite identfied in this study is lower than 25% and 16% which were reported from eastern [34] and southern parts of Ethiopia [35] and those of 38% and 27.3 % reported from India [36] and Nigeria [37], repectively. In contrary, the present result is higher than a 5.2% and 4.1% prevalence rates reported from south [38] and southwest parts of Ethiopia [39], 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 [40, 41]. The current result is also consistent with findings reported outside Ethiopia [36, 42].

The dominance of *P. vivax* over *P. falciparum* attributed to one or more of the following: the ambient temperature of the study area that might have affected *P. falciparum* within the vector, active case detection, and early treatment of *P. falciparum* cases by artimisinin 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* [43]. However, the present finding contradicts the reports of Alelign and Dejene [44] from south and Deressa [45] 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 [19, 46].

Gender-wise, a significant 3.5 times higher risk of malaria was observed in males than in females. The increased risk of malaria in male subjects in our study is supported by Muntaka and Opoku-Okrah [47],

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 [48] (Nigeria), Muawia and Abdalla [49] (Sudan) and Deressa [45] (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 [50, 46]. However, reports contradicting the present study were reported from Nigeria [51] and Ethiopia [20]. On the other hand, other studies did not find any significant disparity in the risk of malaria infection between male and female participants [52, 53].

With regards 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 [20.54]. Contrary to our finding, Tadesse and his colleagues reported statistically significant 3.7 to 6.7 times more likelihood of malaria infection among 16 to 44 years old [34]. Likewise, Molla and Ayele [35] in southern Ethiopia and Sultana *et al*. [55] 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 [56]. In the present study, the odd of malaria was significantly five times higher in individuals who did not use ITNs than 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 bednets [55].

With the reference to the relationship between place of residence and malaria risk, the finding of present study is consistent with recently reported research findings from Ethiopia [34] and Kenya [55] that demonstrated a significantly increased risk of malaria among rural residents as compared to urban residents. According to Hay and his colleages, 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 [57]. 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*. [58], and Olasunkanmi *et al*. [53] reported a 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 degree of malaria among the four blood types ($p<0.01$). The highest (14.63%) *Plasmodium* 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 [59], Zerihun *et al*. [18] and Tadesse and Tadesse [20].

In accordance with the distribution of blood group phenotypes, the frequencies of allele A and genotype AA were substantially higher among *Plasmodium* infected individuals as compared to *Plasmodium* uninfected ones and there was also a two-fold representation of AB genotype among malaria infected individuals. While, proportions of allele O and genotype OO were considerablly 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 is agrees with reports of Kumar *et al*. [31].

The current study found about 3.7 times increased risk of *Plasmodium* infection in individuals with blood type 'A' ($p= 0.030$). This finding is comparable with the result of a study by Kuadzi *et al*. [16], 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 several studies conducted in Ethiopia [59], India [60], Ghana [61], and Sudan [49], all revealed that individuals with blood group O were being less prone to severe malaria as compared to individuals with other blood groups. The diference in susceptibility to *Plasmodium* infection and severity of the disease might be attributed to the difference in rosetting ability among red blood cells of different 'ABO' blood groups compared with a diminished rosetting potential in individuals with blood group 'O' [62, 63]. On the other hand, the study of Alemu and Mama [38], 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 this 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*. [64], (61.1%) in Nigeria and Njunda *et al*. [65] (44.8 %) in Cameroon. The current result is still very much lower than 86% reports of anemia prevalence in Nigeria [66, 67]. Enormous cultural and economic differences may account for this variation. Socioeconomic status may affect the risk of anaemia by affecting nutritional status, family size, and birth interval, as well as intensifying problems of affordability and accessibility of preventive and curative measures [68].

This study indicated a statistically significant association between anemia status of the participants and malaria. All of the individuals with severe anemia were infected with *Plasmodium* followed by those with moderate (66.7%), mild (24.7%) anemia and non-anemic (1.3%) subjects. Our results agree with some other studies, which demonstrated significant association between the statuses of anemia and risk of malaria [67-69]. Contrary to this, a study conducted in Cameroon did not find significant association between malaria and anaemia [65]. The differences in malaria prevalence with anemia status could be attributed to the difference in the levels of malaria endemicity, variation in examination technique, study subject difference, season's difference, and ecological factors.

Study limitations

As our study was a cross-sectional one, we cannot tell about causal relationships between dependent and independent variables and there might also be selection bias.

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; *P. vivax was* dominant over *P. falciparum*. In the study area, being male, under-five years of age, rural residence and failing to use bed net were found to be statistical significant explanatory variables of the occurrence of malaria in studied subjects. Moreover, the likelihood of getting malaria was substantially higher in anemic and in individuals with blood group 'A'. However, no significant associations were found between the infection and other variables studied. Thus, there is a need to intensify efforts in malaria prevention in the study area, particularly focusing on rural areas, under-five children, and provision of insecticide-treated bed nets (ITNs).

Abbreviations

AOR: Adjusted Odds Ratio; COR: Crude Odds Ratio; ITNs: Insecticide-treated mosquito nets ; IRS: Indoor residual spraying ; WHO:World Health Organization; FMOH: Federal Ministry of Health; USAID: United States Agency for International Development

Declarations

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Authors' contributions

BT conceived the study idea with AM, designed and conducted the laboratory works, performed data collection and analysis and wrote the first draft of the manuscript; AM supervised laboratory works , data collection and analysis and critically revised the manuscript; EN performed data cleaning, provided critical review of the manuscript. All authors read and approved the final manuscript.

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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.

Ethical approval and consent to participate

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.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Department of Biology, College of Science, Bahir Dar University, P.O. Box-79, Bahir Dar, Ethiopia

2 and 3 Department of Biology, College of Science and Biotechnology Research Institute, Bahir Dar University, P.O. Box-79, Bahir Dar, Ethiopia

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Tables

'able 1: Socio-demographic characteristics of respondents at Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia, 2018.

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	7.9
Above 2000	179	45.9
House type		
Thatched roof and local dung plaster	2	0.5
Corrugated Iron Sheet and stick/mud plaster	324	83.1
Brick	64	16.4

Table 2 Cross tabulation of Chi-square analysis of association of malaria with socio demographic and environmental risk factors of the study participants

Variables	Number examined n (%)	Malaria positive n(%)	Malaria negative n(%)	Chi-square, P-value (χ2, p)
Sex				
Male	211(54.1)	25(11.8)	186(88.2)	6.808, 0.009
Female	179(45.9)	8(4.46)	171(95.5)	
Age categories				
Under 5	12(3.1)	5(41.6)	7(58.3)	32.317, 0.000
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				
Un married	176(45.1)	22(12.5)	154(87.5)	10.270, 0.016
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)	22.318, 0.000
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)	11.801, 0.067
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)				46.223, 0.000
Less than 500	74(19.0)	18(24.3)	56(75.7)	
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)	21.885, 0.000
Urban	242(62.1)	8(3.3)	234(96.7)	
House type				
Thatched roof and local dung plaster Corrugated Iron Sheet and	2(0.5)	2(100.0)	0(0.0)	28.064, 0.000
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				
No	334(85.6)	31(9.3)	303(90.7)	2.019, 0.155
	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)	78.062, 0.000
1 and above	312(80.0)	7(2.24)	254(98.1)	
Blood group				
A	123(31.5)	18(14.6)	105(85.4)	11.039, 0.012
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)	72.995, 0.000
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	Adjusted OR 95% CI	P-value
Sex					
Male	211(54.1)	25(11.8)	2.87(1.26,6.54)	3.48(0.86, 14.06)	0.017
Female(ref)	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)	72.84(2.68, 1979.83)	0.011
5-14	26(6.7)	7(26.9)	6.63(1.35, 35.12)	23.25(0.69, 773.93)	0.079
15-24	94(24.1)	7(7.4)	1.45(0.29, 7.31)	7.29(0.59, 90.79)	0.122
25-34	108(27.7)	7(6.5)	1.25(0.25, 6.28)	2.53(0.22, 28.65)	0.454
35-44	72(18.5)	4(5.6)	1.06(0.18, 6.06)	17.44(0.25, 1237.86)	0.189
45-54	40(10.3)	1(2.5)	0.46(0.04, 5.31)	1.55(0.06, 37.76)	0.787
≥55(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)	2.64(0.57, 12.22)	0.048
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)	4.65(1.14, 18.99)	0.010
Yes(ref)	325(83.3)	10(3.07)	1.00	1.00	

Note: N = total number of study participants, n = number of malaria cases.

Table 4 The degree of association between ABO blood group and prevalence of *Plasmodium* infection among study participants, Mekaneeyesus Hospital, 2018.

Adjusted* (adjusted odds ratios from multivariate logistic regression models = adjusted for the effects of age, sex and anemia).

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	0.004
		Adjusted *	2.23	0.93- 5.34	0.074
	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	0.860
	O vs non O	Crude	0.27	0.10 - 0.73	0.009
		Adjusted *	0.32	0.10 - 0.98	0.045
	A vs O	Crude	4.83	1.74- 13.44	0.003
		Adjusted *	3.74	1.14 - 12.29	0.030
	B vs O	Crude	2.70	0.877-8.31	0.083
		Adjusted*	2.58	0.698 - 9.504	0.156
	AB vs O	Crude	1.66	0.18-15.05	0.653
		Adjusted*	1.85	0.14 - 23.95	0.640

Table 5 Allelic and genotypic frequency of ABO blood group

Variables	*Plasmodium* infected	*Plasmodium* non-infected
Alleles	Allelic frequency	
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
$\chi 2$	4.5901	2.4560
P value	0.0322	0.1171
Genotype	Genotype 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

Table 6: Prevalence of malaria and anaemia status

Anaemia status	Total examined n(%)	Malaria positives n(%)	Malaria negatives n(%)
Non-anemic	300(76.9)	4(1.3)	296(98.7)
Mild anemic	77(19.7)	19(24.7)	58(75.3)
Moderate anemic	9(2.3)	6(66.7)	3(33.3)
Severe anemic	4(1.0)	4(100.0)	0(0.0)
Total	390	33(8.5)	357(91.5)
$\chi 2$, P	128.452, 0.00		

Key non-anemic ≥ 12.0, Mild - 11-11.9, Moderate-8-10.9, Servere ≤ 8.0 (WHO, 2011)

Figures

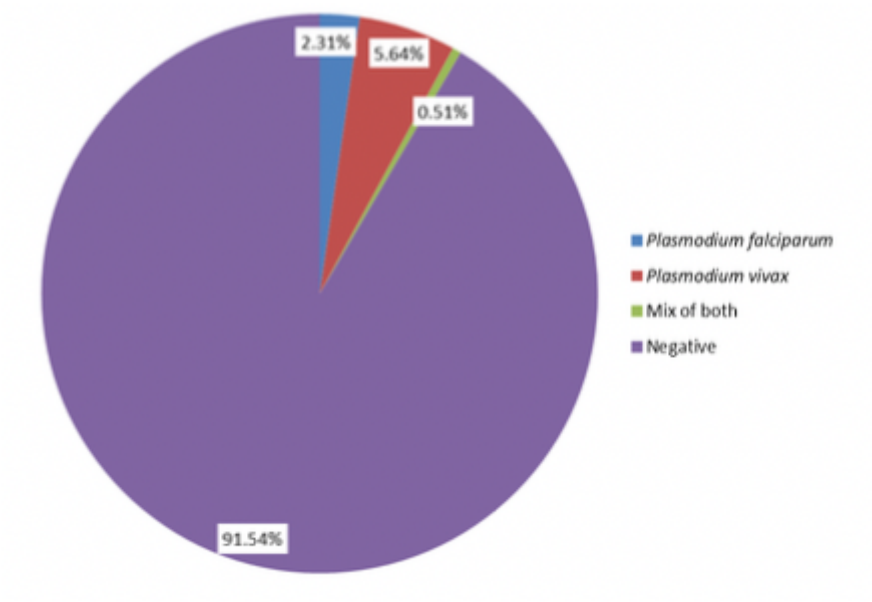

Figure 1

Prevalence of Plasmodium species among the study participants at Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia, 2018.

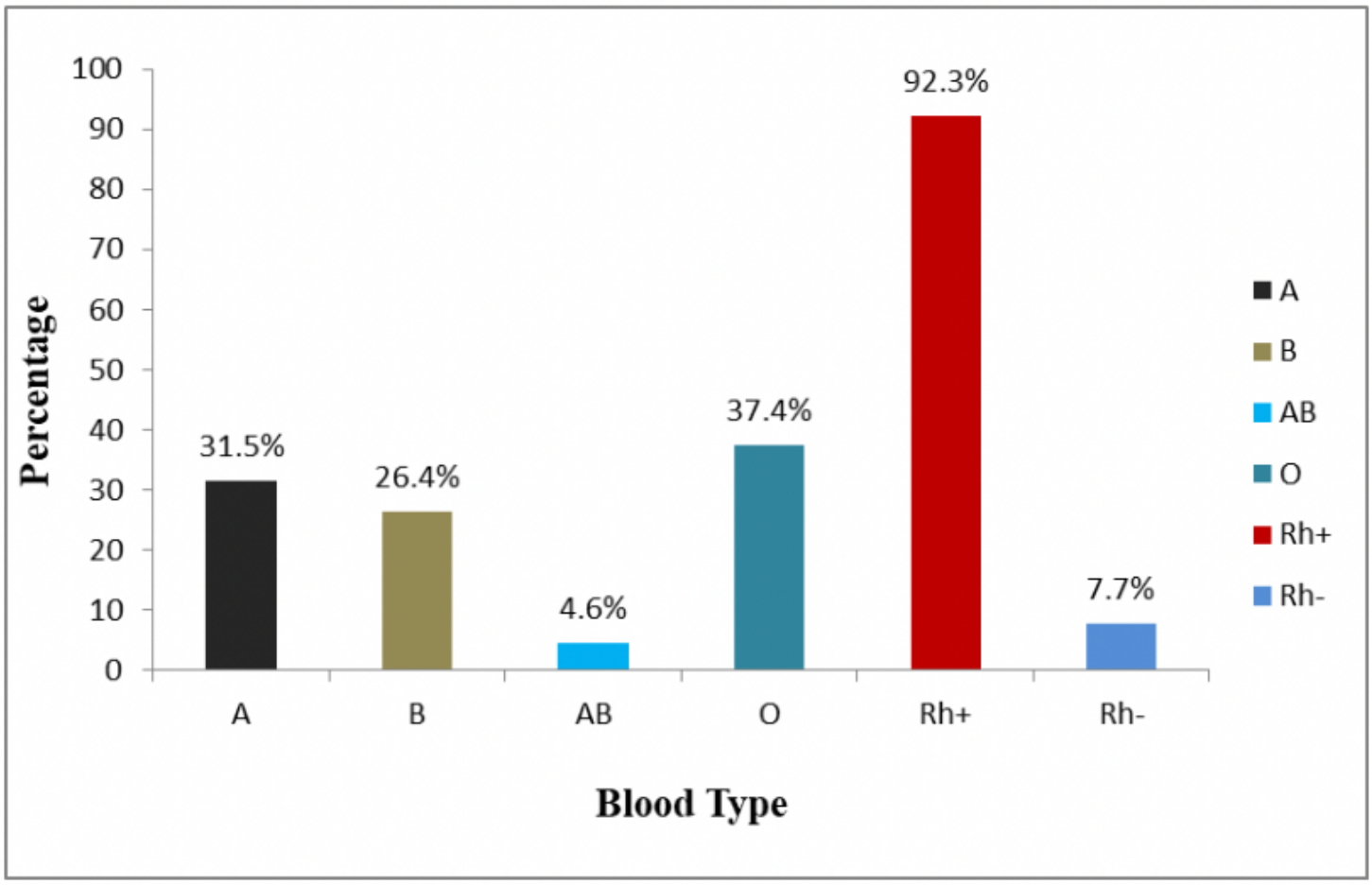

Figure 2

Distribution of ABO blood groups and Rh factors among study participants