The Prevalence and Severity of Anemia Among Malaria Patients With and Without Soil-Transmitted Helminths in Three Health Facilities of Arba Minch Town, Southern Ethiopia, a Comparative Cross-Sectional Study

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Research Article

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Abstract

Background

Anemia is one of the severe clinical outcomes associated with concomitant infection of malaria and soil-transmitted helminths. It affects growth, physical fitness, and cognitive function, especially in children. The findings of previous studies done to assess the effect of malaria soil-transmitted helminths co-infection on anemia showed some variation. This indicates the need for further studies in different populations.

Method

An institutional-based cross-sectional study was conducted at three Governmental Health Institutions, Arba Minch, and southern Ethiopia from November 2020-February 2021 on a total of 321 malaria-positive study participants. Thick and thin blood films were prepared for microscopic examination of malaria parasites and identification of species. Malaria parasite count was done to determine the intensity of the infection. Stool wet mount was done to identify soil-transmitted helminths. Kato-Katz was done for microscopic quantitative examination of soil-transmitted helminths. Complete blood cell count was done to determine hemoglobin level and anemia prevalence. Socio-demographic data was collected using questionnaire. Data were analyzed using SPSS version 25. Independent samples t-test, one-way ANOVA, binary and multivariate logistic regression analyses were done.

Results

Anemia prevalence in this study was 38.3% and it was higher in the malaria with multiple soil-transmitted helminth co-infection groups (55.1%). Co-infection status (AOR = 3.591, CI: 1.468–8.788, P = 0.005), hookworm infection (AOR = 1.632, CI: 1.027–2.593, P = 0.038), and its intensity, A.lumbricoides infection (AOR = 2.095, CI: 1.286–3.411, P = 0.003), and its intensity were significant predictors of anemia in this study. Malaria parasite density was significantly higher in malaria with multiple STHs co-infected study participants F (2, 318) = 20.075. It increased with increasing intensity of hookworm, T.trichiura, and number of co-infecting helminth species. But it decreased with increasing intensity of A.lumbricoides and S.mansoni.

Conclusion

this finding suggests that co-infection of multiple soil-transmitted helminth and malaria are associated with anemia and low Hgb levels, this indicates the importance of integrated disease control interventions.

1 Background
Anemia is reduction in the oxygen-carrying capacity of the blood as a result of fewer circulating erythrocytes than is normal or a decrease in the concentration of hemoglobin [1]. Anemia has detrimental physical, social and economic effects. Even mild to moderate anemia affects the sense of wellbeing, resulting in fatigue, stress and decrease in work capacity. Severe anemia may cause cardiac failure and death [2, 3]. It is one of the severe clinical outcomes associated with concomitant infection of malaria and soil-transmitted helminths. Anemia due to soil-transmitted helminths is by increasing of blood and iron loss in the intestine. On the other hand, malaria causes anemia by decreasing the amount of hemoglobin, increasing the destruction of parasitized red blood cells, and shortening the lifespan of non-parasitized RBCs [4, 5]. Globally anemia affects around 2 billion people which nearly accounts for 30% of the world’s population. In Ethiopia anemia is also the most common public health problem, and according to the Ethiopia Demographic and Health Survey (EDHS) report there was an estimated 56% prevalence of anemia among children under the age of 5 years, 23% among women of reproductive age (15–49) and 18% among adult men (15–49) in the 2016 [6].

Hence, STH infections mostly share similar geographical areas with malaria, the influence of co-infections on the epidemiology and course of anemia deserves greater consideration to assess the impact of interventions, the adequacy of strategies implemented, and the progress made in the fight against anemia. It is also important to set integrated control programs and evaluating the effectiveness of control interventions. Also assessing anemia prevalence with the level of malaria parasite density and intensity of soil-transmitted helminth infection is important because it indicates the influence of co-infections on the severity and disease burden at the individual level, Also it is important for evaluating the potential severity of the infections, and identification of high-risk populations for targeting of interventions [5, 7]. So this study was done to investigate the prevalence of anemia and severe anemia among soil-transmitted helminth negative malaria patients and malaria patients co-infected with single or multiple soil transmitted helminths, to assess the association between hemoglobin concentrations and intensity of soil-transmitted helminth infection in patients with malaria, and to assess the association between malaria parasite density and intensity of soil-transmitted helminthiasis in patients with malaria in three health facilities of Arba Minch, 2020–2021.

2. Methods And Materials

2.1. Study Area and Period

The study was conducted at three governmental health facilities in Arba Minch; the city located about 454 kilometers south of Addis Ababa, at an elevation of 1278 meters above sea level with an average annual temperature of 29.7°C and receiving an average annual rainfall of about 2818.1 mm. It is the largest town in Gamo Zone. Based on the 2007 census conducted by the CSA, the town has a total population of 74,879. There are three governmental health institutions in the city, one is Arba Minch General Hospital, and the others are Secha and Sikela Health centers. The area is one of the endemic
areas of malaria in the Gamo Zone; even if previous studies show different results soil-transmitted helminths are also prevalent in the area.

The study was conducted from November 2020 – February 2021.

2.2. Study Design

An institution based cross-sectional study was conducted

2.3. Population

2.3.1. Source Population

All malaria suspected patients attending three governmental health facilities in Arba Minch town during the study period

2.3.2. Study Population

All malaria positive patients attending at three governmental health facilities in Arba Minch town during the study period and fulfilling the inclusion criteria of the study.

2.4. Inclusion and Exclusion Criteria

2.4.1. Inclusion Criteria

Malaria patients with permanent residence in the study area for at least 6 months before enrolment and did not take the antimalarial anthelmintic drugs for the last 4 weeks were included.

2.4.2. Exclusion Criteria

Malaria patients who had any parasitic infection other than STHs during stool met mount and kato katz examinations, pregnant mothers and children aged less than 6 months, malaria patients with any other known chronic infections, and malaria patients with unsuitable stool samples for kato katz egg counts were excluded.

2.5. Sample Size Determination and Sampling Procedures

2.5.1. Sample Size Determination

This study had 3 groups; only malaria-infected, malaria with single STH infected and, malaria with multiple STHs infected patients. The sample size was calculated by assuming malaria patients with no STH co-infection and malaria patients with single STH co-infection have a similar risk of anemia [8, 9]. Using the prevalence of anemia among malaria patients, \( p_1 = 40\% \) (from the study conducted in Azezo, northern Ethiopia [7], and prevalence of anemia among malaria-multiple-SThS co-infected patient, \( p_2 = 60\% \) (from the study conducted in Tanzania) [10]. with 95% confidence level, 5% of marginal error, and
80% power $(1 - \beta)$. After the calculation and addition of 10% for non-respondent rate, the final sample size was 321 (107 for each group).

### 2.5.2. Sampling Procedures

A list of the individuals within the center who fulfill the eligibility criteria was obtained from the institution. After generating the frame, the sample was selected in a non-random manner using convenience sampling. Screening for STHs was used in the convenient sampling frame; i.e. Sequential recruitment by stool examination.

### 2.6. Data Collection and Procedures

#### 2.6.1. Data Collection Instruments

**A. Socio-Demographic Variables**

Most indicative variables of the socio-demographic status of the study participants were collected using a pre-tested structured questionnaire. Other data related to the patient's medical conditions were collected from the patient's registration card.

**B. Blood specimen collection**

Two ml EDTA anti-coagulated whole blood was collected from each study participants for hematological tests and to prepare thick and thin blood film smears using vacutainer

**C. Stool specimen collection**

A fresh fecal specimen was collected using a labeled, clean, dry, leak-proof container. The study participants were carefully oriented on how to provide his/her stool sample without mixing water and urine and with a sufficient amount.

#### 2.6.2. Laboratory investigation

##### 2.6.2.1 Blood slide microscopy

Thick and thin blood films were prepared, the thin blood films were fixed in methanol for 30 seconds. Then smears were stained with 10% Giemsa solution. A hundred fields were examined before the negative result being reported. Plasmodium parasite species identification was done on the thin smears [2].

##### 2.6.2.2 Counting parasite density in thick blood film

Blood film was air-dried at room temperature and parasite density was estimated in a thick blood film by counting the number of asexual parasites along with 200 white blood cells (WBC) or 500 WBC when the parasite count was less than 10 parasites per 200 WBC. A total of $8,000/\mu l$ white blood cell count was considered for the determination of parasitemia [11].
2.6.2.3. Stool sample processing and examination

Stool samples were examined for the presence of ova of soil transmitted helminths within 20 minutes for both macroscopic and microscopic examination, using normal saline (8.5g NaCl/liter of water) as a wet mount [12].

2.6.2.4. Kato-Katz thick smears

Form each stool sample two Kato-Katz slides were prepared for microscopic quantitative examination of STHs. To obtain egg counts per gram, the average egg counts of the two Kato Katz slides were multiplied by 24. Egg counts were classified into light, moderate, or heavy infection intensities according to respective reference ranges for each STH parasite.


2.6.2.5. Hematological analysis

For measurement of hemoglobin (Hgb) and hematocrit (HCT), the blood sample was analyzed using automated people republic of china made Sysmex KX-21N hematology analyzer.

2.7. Data Quality Control

Detail orientation was given for clinical data and specimen collectors, Standard operating procedures and manufacturers’ instructions were strictly followed for all laboratory activities. Socio-demographic data, Malaria blood film and parasite load count results were also entered into a separate spreadsheet at the time of diagnosis and double entered in Excel. In addition, blood film and Kato Katz slides were read by two experts who were blinded to each other’s and discrepant results were confirmed by third technician. Malaria parasite density count and kato katz egg counts were counted by two experts using a multiple type tally counter and the average was taken. All specimens were checked for their label and quality. Questionnaires were checked daily for completeness and consistency of the responses. Each laboratory test result was recorded, reported and specimens were managed properly.

2.8. Data Processing and Analysis

Data were first entered into Epi-data version 3.1, then the analysis was done using SPSS version 25.0. Infection intensities were calculated as the mean of eggs per gram of feces and parasites per micro liter of blood for STHs and malaria respectively. Independent samples t-test and one-way analysis of variance (ANOVA) were used when mean comparisons for two or more than two groups were performed respectively. Descriptive statistics of means, standard deviations, and a percentage were calculated. Binary logistic regression and multivariate logistic regression analyses were done to determine the presence of associations between variables, an odd ratio of 95% confidence intervals was used to
determine the degree of association between dependent and independent variables. P-values less than 0.05 were considered statistically significant.

### 2.9. Ethical considerations

To obtain the informed consent of the respondent, every subject on an individual basis was communicated with brief information concerning the research i.e. the research objectives, benefits, and the importance of the study.

The choice to participate in the study was completely voluntary. It was clarified that the collection of a venous blood sample to laboratory analysis had some discomfort to the subject. The study was conducted after approval by DERC, DMIP, and SoM. A legal letter was also submitted to three governmental health facilities in Arba Minch town, where the study was carried out. Written informed consent was obtained from all selected patients and the parents or guardians of the children after full explanations of the goals and procedures of the study. Also, assent was obtained from the children before starting the study. After taking permission from the health institutions and study participants the data collection was conducted. *Plasmodium falciparum* positive study participants were treated with artemether, *plasmodium vivax* positive study participants were treated with chloroquine, and soil-transmitted helminths positive study participants were treated with albendazole and praziquantel. Confidentiality of study findings, as well as patient identity was maintained.

### 3. Results

#### 3.1. Socio-demographic characteristics of study subjects

A total of 2311 malaria suspected patients visited the three governmental health facilities in Arba Minch from November 2020 to February 2021. Among those cases, 321 malaria-positive patients who fulfilled the inclusion criteria were included in the study with a 100% response rate. From a total of 321 malaria-positive patients included in the study, 192(59.8%) were males and 129(40.2%) were females. The majority 194(60.4%) of study participants were between 15 and 49 years, followed by 6–14 years (29.0%; n = 93) and 6–59 months (10.6%; n = 34). The majority 186(57.9%) of study participants were students and about 176(54.8%) of study participants were form urban areas (Table 1).
Table 1
Socio-demographic characteristics of the study participants, Arba Minch, 2020–2021

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>Malaria mono infected patients N (%)</th>
<th>Malaria-single STH co-infected patients N (%)</th>
<th>Malaria–multiple STHs co-infected patients N (%)</th>
<th>Totals N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>68(63.6)</td>
<td>63(58.9)</td>
<td>61(57.0)</td>
<td>192(59.8)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>39(36.4)</td>
<td>44(41.1)</td>
<td>46(43.0)</td>
<td>129(40.2)</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 6 years</td>
<td>13(12.1)</td>
<td>10(9.3)</td>
<td>11(10.3)</td>
<td>34(10.6)</td>
</tr>
<tr>
<td></td>
<td>6–14 years</td>
<td>27(25.2)</td>
<td>27(25.2)</td>
<td>39(36.4)</td>
<td>93(29.0)</td>
</tr>
<tr>
<td></td>
<td>&gt; 15 years</td>
<td>67(62.6)</td>
<td>70(65.4)</td>
<td>57(53.3)</td>
<td>194(60.4)</td>
</tr>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>72(67.3)</td>
<td>46(43.0)</td>
<td>58(54.2)</td>
<td>176(54.8)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>35(32.7)</td>
<td>61(57.0)</td>
<td>49(45.8)</td>
<td>145(45.2)</td>
</tr>
<tr>
<td>Educational level</td>
<td>Unable to read and write</td>
<td>33(30.8)</td>
<td>30(28.0)</td>
<td>14(13.1)</td>
<td>77(24.0)</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>55(51.4)</td>
<td>58(54.2)</td>
<td>49(45.8)</td>
<td>162(50.5)</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>14(13.1)</td>
<td>18(16.8)</td>
<td>24(22.4)</td>
<td>56(17.4)</td>
</tr>
<tr>
<td></td>
<td>college and above</td>
<td>5(4.7)</td>
<td>1(0.9)</td>
<td>20(18.7)</td>
<td>26(8.1)</td>
</tr>
<tr>
<td>Occupation</td>
<td>Farmer</td>
<td>14(13.1)</td>
<td>8(7.5)</td>
<td>11(10.3)</td>
<td>33(10.3)</td>
</tr>
<tr>
<td></td>
<td>Merchant</td>
<td>16(15.0)</td>
<td>21(19.6)</td>
<td>17(15.9)</td>
<td>54(16.8)</td>
</tr>
<tr>
<td></td>
<td>Government employee</td>
<td>5(4.7)</td>
<td>1(0.9)</td>
<td>10(9.3)</td>
<td>16(5.0)</td>
</tr>
<tr>
<td></td>
<td>Student</td>
<td>59(55.1)</td>
<td>59(55.1)</td>
<td>68(63.6)</td>
<td>186(57.9)</td>
</tr>
<tr>
<td></td>
<td>House wife</td>
<td>13(12.1)</td>
<td>18(16.8)</td>
<td>1(0.9)</td>
<td>32(10.0)</td>
</tr>
</tbody>
</table>

3.2. Plasmodium species and parasite density of malaria infection

The majority of malaria infection was due to *Plasmodium falciparum* 231(72.0%) followed by *Plasmodium vivax* 80(24.9%) and mixed infection of the *Plasmodium falciparum* and *Plasmodium vivax* 10(3.1%); the mean malaria parasite density of study participants was 6648.66 ± 356.388/µl (95%CI = 5850.76, 7259.65).
3.3 Prevalence and intensity of soil transmitted helminths

Hookworm was the most prevalent STH parasite (47.4%; n = 152) followed by *A. lumbricoides* (31.2%; n = 100) and *S. mansoni* (12.1%; n = 39) *T. trichiura* was the least prevalent (8.7%; n = 28). With regards to the intensity of infections, all STHs infections were generally light to moderate infections. Out of them, 102(67.1%), 73(73.0%), 16(57.1%), 17(43.6%) of hookworm, *A. lumbricoides*, *T. trichiura*, *S. mansoni* infections were light infections; and 50(32.9%), 27(27.0%), 12(42.9%), 22(56.4%) were moderate infections respectively.

3.4 Association between malaria parasite density and STH infection intensities

The one-way ANOVA analysis of variance showed a statistically significant in average malaria parasite density for the three study infection groups. Post hoc comparisons using Tukey HSD indicated that the mean malaria parasite density for malaria with multiple STHs co-infection study participants was significantly higher from malaria-mono infected, and malaria with single STHs–co-infection study participants (Table 2).

### Table 2
One way ANOVA for significant difference in mean parasite density between infection groups of study participants, Arba Minch, 2020–2021

<table>
<thead>
<tr>
<th>Co-infection status</th>
<th>Mean</th>
<th>95% CI</th>
<th>One way ANOVA</th>
<th>Tukey HSD multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono malaria</td>
<td>3688.41</td>
<td>2880.94, 4495.88</td>
<td><em>F</em> = 20.075</td>
<td><em>P</em> = 0.022 <em>a</em>, &lt; 0.001<em>b</em></td>
</tr>
<tr>
<td>Malaria with single STHs</td>
<td>6746.92</td>
<td>5555.21, 7938.62</td>
<td><em>P</em> &lt; 0.001*</td>
<td><em>P</em> = 0.001<em>b</em></td>
</tr>
<tr>
<td>Malaria with multiple STHs</td>
<td>8982.43</td>
<td>7541.38, 10423.48</td>
<td><em>df</em> (2,318)</td>
<td></td>
</tr>
</tbody>
</table>

*Note: CI: confidence interval; *statistically significant At 0.05 levels. a = Malaria with single STHs; b = as compared to Malaria with multiple STHs*

Results from the independent samples t-test also revealed that there was significant difference in mean parasite density of malaria with respect to hookworm infection intensity (*MD* = -3233.435, 95% CI = -5683.264, -783.607, *t* (150) = -2.608, *p* = 0.010), with respect to *A. lumbricoides* infection intensity (*MD* = 3290.675, 95% CI = 591.836, 5989.513, *t* (80) = 2.426, *p* = 0.017) and with respect to *T. trichiura* infection intensity groups (*MD* = -4560.000, 95% CI = -9011.707, -108.293, *t* (26) = -2.106, *p* = 0.045). On the other hand there was no significant difference in the mean parasite density of malaria among individuals with different intensities of *S. mansoni* infection (*MD* = 2959.840, 95% CI = -706.753, 6626.432, *t* (22) = 1.673, *p* = 0.108) (Table 3).
### Table 3
Independent samples t-test for significant difference in mean parasite density between intensity groups of soil transmitted helminths, Arba Minch, 2020–2021

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Mean malaria parasite density</th>
<th>95% CI for mean</th>
<th>t(df)</th>
<th>Sig. (2tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hook worm infection intensity</td>
<td>Light infection</td>
<td>7581.76</td>
<td>6264.94, 8898.59</td>
<td>-2.608 (150)</td>
<td>0.010*</td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>10815.20</td>
<td>8519.55, 13110.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. lumbricoides infection intensity</td>
<td>Light infection</td>
<td>9938.08</td>
<td>7976.25, 11899.91</td>
<td>2.426 (80)</td>
<td>0.017*</td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>6647.41</td>
<td>4729.28, 8565.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. trichiura infection intensity</td>
<td>Light infection</td>
<td>4320.00</td>
<td>2192.44, 6447.56</td>
<td>-2.106 (26)</td>
<td>0.045*</td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>8880.00</td>
<td>4198.85, 13561.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mansoni infection intensity</td>
<td>Light infection</td>
<td>7955.29</td>
<td>4530.25, 11380.34</td>
<td>1.673 (22)</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>4995.45</td>
<td>4594.06, 7977.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: CI: confidence interval; t: t-test value; df: degree of freedom *statistically significant at 0.05 levels.

### 3.5 Prevalence of anemia

The overall anemia prevalence was (38.3%; n = 123) with only (0.9%; n = 3) severe anemia cases. The prevalence was (55.1%) in malaria with multiple STHs co-infection study participants followed by malaria with single STHs co-infection study participants (35.5%), and mono malaria-infected study participants (24.3%). The entire severe anemia cases were from malaria with multiple STHs co-infection study participants.

The one-way ANOVA analysis of variances showed that the co-infection status of study participants significantly influenced the mean hemoglobin concentration. Post hoc analyses conducted using Tukey’s post hoc test showed that the mean hemoglobin concentration of malaria with multiple STHs co-infection study participants was significantly lower than mono malaria-infected and malaria with single STHs co-infection study participants. Also, malaria with single STHs co-infection study participants had lower mean hemoglobin concentration than mono malaria-infected study participants (Table 4).
Table 4
One way ANOVA for significant difference in hemoglobin concentration between the study participants’ co-infection status groups, Arba Minch, 2020–2021

<table>
<thead>
<tr>
<th>Co-infection status</th>
<th>Mean Hgb</th>
<th>95% CI</th>
<th>One way ANOVA</th>
<th>Tukey HSD multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono malaria</td>
<td>14.074</td>
<td>13.686, 14.462</td>
<td>F = 16.685</td>
<td>P = 0.044 *&lt;sup&gt;a&lt;/sup&gt;, &lt; 0.001</td>
</tr>
<tr>
<td>Malaria with single STHs</td>
<td>13.333</td>
<td>12.912, 13.754</td>
<td>P &lt; 0.001&lt;sup&gt;*&lt;/sup&gt;</td>
<td>P = 0.004 *&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malaria with multiple STHs</td>
<td>12.373</td>
<td>11.941, 12.804</td>
<td>df (2,318)</td>
<td></td>
</tr>
</tbody>
</table>

Note: CI: confidence interval; *statistically significant at 0.05 levels. a = Malaria with single STHs; b = as compared to Malaria with multiple STHs

The multivariate logistic regression test showed that the prevalence of anemia and severe anemia was associated with the co-infection status of study participants. Malaria with multiple STHs co-infection study participants were 3.829 times more likely to be anemic than mono malaria-infected study participants (AOR = 3.591, CI: 1.468–8.788, P = 0.005). Besides malaria with single STHs co-infection study participants were 1.68 times more anemic than malaria-infected study participants who are not co-infected by STH even though the association was not statistically significant (AOR = 1.683, CI: 0.649–4.367, P = 0.285) (Table 5).
### Table 5
Important predictors of anaemia among study participants, Arba Minch, 2020–2021

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>COR (95% CI)</th>
<th>P</th>
<th>AOR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.100 (1.325, 3.329)</td>
<td>0.002</td>
<td>1.939 (0.930, 4.046)</td>
<td>0.077</td>
</tr>
<tr>
<td>Co-infection status</td>
<td>Mono malaria</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaria with single STHs</td>
<td>1.716 (0.948, 3.105)</td>
<td>0.075</td>
<td>1.683 (0.649, 4.367)</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>Malaria with multiple STHs</td>
<td>3.829 (2.137, 6.862)</td>
<td>0.001</td>
<td>3.591 (1.468, 8.788)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Hook worm infection</td>
<td>Not-infected</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>1.770 (1.124, 2.789)</td>
<td>0.014</td>
<td>1.632 (1.027, 2.593)</td>
<td>0.038*</td>
</tr>
<tr>
<td>Hook worm infection intensity</td>
<td>Light infection</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>2.526 (1.262, 5.055)</td>
<td>0.009</td>
<td>2.225 (1.084, 4.564)</td>
<td>0.029*</td>
</tr>
<tr>
<td>A. lumbricoides infection</td>
<td>Not-infected</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>2.227 (1.376, 3.606)</td>
<td>0.001</td>
<td>2.095 (1.286, 3.411)</td>
<td>0.003*</td>
</tr>
<tr>
<td>A. lumbricoides infection intensity</td>
<td>Light infection</td>
<td>4.592 (1.721, 12.254)</td>
<td>0.002</td>
<td>4.292 (1.571, 11.729)</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>T. trichiura infection</td>
<td>Not-infected</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>3.435 (1.540, 7.662)</td>
<td>0.003</td>
<td>2.328 (0.949, 5.714)</td>
<td>0.065</td>
</tr>
</tbody>
</table>

#### 3.6 Association between anemia and STHs infections

The results of multivariate logistic regression showed that the prevalence of anemia was associated with the prevalence of hookworm infection (AOR = 1.632, CI: 1.027–2.593, P = 0.038), and with the prevalence of *A. lumbricoides* infection (AOR = 2.095, CI: 1.286–3.411, P = 0.003). On the other hand, no association was observed between prevalence of anemia with *S. mansoni* infection prevalence (AOR = 1.138, CI: 0.575–2.250, P = 0.711), and *T. trichiura* infection prevalence (AOR = 2.328, CI: 0.949–5.741, P = 0.065). With regards to infection intensities; The results indicated that the prevalence of anemia was associated with the infection intensities of hookworm (AOR = 2.225, CI: 1.084–4.564, P = 0.029), and *A.
lumbricoides (AOR = 4.292, CI: 1.571–11.729, P = 0.005). But not with T. trichiura (AOR = 3.889, CI: 0.636–23.792, P = 0.142) and S. mansoni infection intensities (AOR = 2.400, CI: 0.630–9.136, P = 0.199).

An independent samples t-test also was conducted to assess significant difference in mean hemoglobin concentration with respect to infection intensities of each of identified STHs and S. mansoni infections. The results indicated that there was significant difference in mean hemoglobin concentration with respect to hookworm infection intensities (\(MD = 1.4128, 95\% \text{ CI} = 0.6287, 2.1969, t(150) = 3.560, p < 0.001\)), and A. lumbricoides infection intensities (\(MD = -0.9249, 95\% \text{ CI} = -1.7360, -0.1138, t(62) = -2.280, p = 0.026\)). On the other hand there was no significant difference in average hemoglobin concentration level between study participants co-infected with moderate and light infection intensities of T. trichiura (\(MD = 1.3521, 95\% \text{ CI} = -0.3268, 3.0309, t(26) = 1.655, p = 0.110\)), and S. mansoni (\(MD = 1.2666, 95\% \text{ CI} = -0.2412, 2.7743, t(36) = 1.702, p = 0.097\)) (Table 6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>t-test for Equality of Means</th>
<th>(\text{Mean Hgb})</th>
<th>95% CI of the mean</th>
<th>(t(\text{df}))</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hook worm infection intensity</td>
<td>Light infection</td>
<td>13.261</td>
<td>12.806, 13.716</td>
<td>3.560(150)</td>
<td>0.000*</td>
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</tr>
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<td>Moderate infection</td>
<td>11.848</td>
<td>11..205, 12.491</td>
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<tr>
<td>A. lumbricoides infection intensity</td>
<td>Light infection</td>
<td>12.260</td>
<td>11.752, 12.768</td>
<td>-2.280(62)</td>
<td>0.026*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>13.185</td>
<td>12.536, 13.834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. trichiura infection intensity</td>
<td>Light infection</td>
<td>13.144</td>
<td>11.972, 14.316</td>
<td>1.655(26)</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>11.792</td>
<td>10.487, 13.096</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mansoni infection intensity</td>
<td>Light infection</td>
<td>13.553</td>
<td>12.507, 14.599</td>
<td>1.702(36)</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate infection</td>
<td>12.838</td>
<td>11.128, 13.444</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 6**

Independent samples t-test for significant difference in mean hemoglobin between intensity groups of soil transmitted helminths, Arba Minch, 2020–2021

Note: CI: confidence interval; \(t\): t-test value; df: degree of freedom *statistically significant at 0.05 levels.

### 3.7 Predictors of anemia

Variables with a \(p\)-value < 0.25 in binary logistic regression were a candidate for multivariable logistic regression to identify predictors of anemia. The results indicated that co-infection status (malaria with multiple STHs co-infection), hookworm infection, hookworm infection intensity, A. lumbricoides infection, and A. lumbricoides infection intensity were significant predictors of anemia (Table 5).
4. Discussion

The results from multivariate logistic regression analysis and one way ANOVA also indicated that the parasite density of malaria was significantly associated with the co-infection status of study participants, and, the mean parasite density of malaria was higher in malaria-multiple STHs–co-infected patients as compared with patients infected with malaria with single STHs and Plasmodium parasite alone. Previous studies conducted in Nigeria, Cameroon, southern and northern Ethiopia also found similar findings with the current study [11, 13–15]. The reason might be due to the fact that helminths modulate the host immune response both to themselves and to coexisting infections or to the down regulation of the immune system by helminths. Consequently, the Plasmodium parasite could enter into the host and multiply at a faster rate in patients co-infected with STHs [10, 11]. The present findings also support the hypothesis that co-infection of malaria and STHs could aggravate clinical malaria [11].

On the other hand, the results from logistic regression analysis and the independent samples t-test demonstrated that the malaria parasite density had a variable association with different species of soil-transmitted helminths infection intensities in malaria-STHs–co-infected study participants in the present study. In the present study, malaria parasite density increased with increasing parasite intensities of hookworm and *T. trichiura* infections. Study participants with moderate infection intensities of hookworm and *T. trichiura* had significantly higher mean malaria parasite density than study participants with light co-infection intensities. In this regard, the results of the present study were in agreement with the findings of studies from Ghana, Tanzania, and southern Ethiopia [10, 12, and 16]. The reason could be due to the fact that a strong cytokine production in response to helminths high infection makes individuals more susceptible to more replication of plasmodium parasites and advanced stages of malaria [14, 17]. The results also appeared in support of the hypothesis that co-infection of malaria and helminth infections render co-infected individuals more vulnerable to increased incidence of malaria and severe diseases associated with malaria [17, 18].

On the other hand, the parasite density of malaria decreased with the increasing parasite intensities of *Ascaris lumbricoides*, and study participants with light infection intensity of *Ascaris lumbricoides* had higher mean malaria parasite density than study participants with moderate *Ascaris lumbricoides* infection intensity.

This finding was in agreement with the findings of studies conducted in Thailand, Madagascar, northern Ethiopia, and Gilgel Gibe Dam area in Ethiopia [19, 20, 11, and 8]. The reason might be due to the fact that there is a mixed Th1/Th2 immune response during moderate infection intensity of *Ascaris lumbricoides* which leads to induced low level of malaria parasite density [20]. The results also were in agreement with the hypothesis of the protective effect of *Ascaris lumbricoides* against malaria parasite density and severe forms [11, 19]. Similarly, the study also showed that study participants infected moderately with *S. mansoni* had lower Plasmodium densities compared to lightly infected patients but the association was not statistically significant. This finding was in agreement with studies carried out in Senegal, and Tanzania [10, 21]. The reason might be due to down regulation of Th1 response which could leads to an
exacerbation of Th2 dependent anti-body response. This would accelerate the process of parasite clearance and favors plasmodium parasite elimination with a better control of malaria parasite density [21, 22].

In the present study, the overall anemia prevalence was found 38.3% with only 0.9% of severe anemia prevalence. In this study anemia prevalence among malaria patients with multiple STHs co-infection group was found significantly higher than malaria mono-infected and malaria with single STHs co-infection group. The mean hemoglobin level significantly decreased with increasing co-infecting parasites. This finding is in agreement with findings of studies carried out in Cameroon, Tanzania, northern Ethiopia, and Alaba Kulito (southern Ethiopia) from where higher anemia prevalence among malaria and multiple soil-transmitted helminths co-infected study participants was reported[2, 7, 23, 24 22]. The reason for increased prevalence of anemia in co-infected study participants may be attributed to chronic blood and iron loss due to worm infections in addition to the loss due to malaria [23, 25].

The present study also demonstrates that the prevalence of anemia and the mean hemoglobin concentration had a variable association with STHs infection intensities depending on parasite species. Study participants with high infection intensity of hookworm and T. trichiura were found significantly more anemic and had lower mean hemoglobin concentration than their counterpart participants having low infection intensities. This finding was in line with findings of studies carried out in Peru, Brazil, Malaysia, Uganda, Alaba Kulito and Gilgel Gibe dam area (Southwest Ethiopia) [2, 15, 26–29]. The reason might be because of T. trichiura has been shown to be associated with the anemia through erythrocyte loss from the gut.

Also high hook worm infections lead to decreased erythropoiesis through Nitric oxide (NO) release Since NO can reduce erythrocyte deformability; it could lead to increased red blood cell destruction. On the other hand, contrary findings were reported from studies carried out in Côte d’Ivoire, Tanzania, and Uganda [10, 30, and 31]. These differences could be due to high number of hook worm and T. Trichiura light infections reported in previous studies and given, the young age, the infection may have been recently acquired and did not progress to chronicity to be associated with anemia. In addition the hookworm species involved may have been the less virulent [27, 32].

Interestingly, study participants with light infection intensity of Ascaris lumbricoides were found to be significantly less anemic and had high mean hemoglobin concentration than their counterpart participants with moderate infection intensities. This finding is in agreement with findings of studies carried out in Peru, Brazil, and the Gilgel Gibe dam area (southern Ethiopia) [15, 26, 33]. The reason may be due to the fact that increased titers of IgE and high level of eosinophilia during light infection of Ascaris lumbricoides infection can cause Th1 hypersensitivity which can cause anemia. On the other hand, the findings of studies carried out in Malaysia, Nigeria, and Alaba Kulito (southern Ethiopia) showed contrary results [2, 25, and 27]. Another study carried out in Cameroon found no effect of Ascaris lumbricoides on hemoglobin level [32].
These variations may be due to differences in study participants because some of the studies included pregnant women. In most of addition in the present most of light *Ascaris lumbricoides* light infections were co-infected with other STH infections and previous studies suggest that the effect of *Ascaris lumbricoides* was not significant, unless there were two or more infecting species of intestinal helminths. Although intestinal helminth infection is mutually correlated with other factors to some degree, this infection does contribute a portion of risk for the development of anemia conditioned on the presence of other risk factors. For this reason, when analyzed alone or when independent of other risk factors, *Ascaris lumbricoides* may not seem to be a major factor for anemia [26, 27, and 33].

5. Conclusion

From this study, it could be concluded that anemia was moderately prevalent in the three governmental health institutions of Arba Minch town. The prevalence was higher in female study participants and children under the age of 18. The prevalence was associated with factors such as co-infection status, hookworm infection, hookworm infection intensity, *A. lumbricoides* infection, and *A. lumbricoides* infection intensity. Malaria and STHs co-infections were also common among study participants. Co-infection of malaria with multiple STHs could increase the risk of anemia and lower hemoglobin levels. This study found heterogeneity with regards to the association between malaria parasite density, STHs infection intensities, and anemia prevalence, and mean hemoglobin concentrations depending on different STHs species.

Declarations

The authors, undersigned, declare that this thesis is our original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been fully acknowledged.

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Conflict of interest: The authors declare no competing interests.
Data Availability: The datasets used and/or analyzed during the current study are available from the author on reasonable request (birhanufasil13@gmail.com).

Authors’ Contributions: BG and AH conceived and designed the study. BG and AH prepared the research instruments. BG collected all necessary data and analyzed and drafted the manuscript. BG led the study. The study was guided and supervised by AH. BG and AH wrote the manuscript. All authors reviewed and discussed the results, helped in the interpretation of the results, and contributed to the draft and final manuscript. All authors read and approved the final manuscript.

Ethical approval: The study was ethically approved by Research Ethical Committee of Addis Ababa University, department of medical microbiology, immunology and parasitology, Ethiopia (No.DRERC/006/2020). Prior to the sample collection, an informed written consent was obtained from all the participants in the study after a clear explanation of the research objectives.

Competing interests: The authors declare no competing interests.

Consent to participate: The patients agreed to participate in this study.

Consent for publication: All authors agreed to the publication of the manuscript.

Disclosure

The authors report no conflicts of interest for this work and declare that there is no conflict of interest regarding the publication of this manuscript. This manuscript is a part of a research thesis submitted to the Department of Medical Microbiology, Immunology and Parasitology (DMIP), College of Health Sciences, Addis Ababa University, in partial fulfillment of the requirements for M.Sc. in Medical Parasitology by BG and the thesis is unpublished.

References


