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Malaria helminth co-infections and their contribution for aneamia in febrile patients attending Azzezo health center, Gondar, Northwest Ethiopia: a cross sectional study

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ABSTRACT

Objective: To assess the prevalence of malaria helminth co-infections and their contribution for aneamia in febrile patients attending Azzezo health center, Gondar, Northwest Ethiopia. **Methods:** A cross section study was conducted among febrile patients attending Azezo health center from February-March 30, 2011. Convenient sampling technique was used to select 384 individuals. Both capillary blood and stool were collected. Giemsa stained thick and thin blood film were prepared for identification of *Plasmodium* species and stool sample was examined by direct wet mount and formalin-ether concentration technique for detection of intestinal helminthes parasites. Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer. **Results:** Out of 384 febrile patients examined for malaria parasites, 44 (11.5%) individuals were positive for malaria parasites, of which *Plasmodium vivax* accounted for 75.0% (33), *Plasmodium falciparum* for 20.5% (9) infectious, whereas two person (4.5%) had mixed species infection. Prevalence of malaria was higher in males (28) when compared with prevalence in females (16). More than half (207, 53.9%) of study participants had one or more infection. Prevalence was slightly higher in females (109, 52.7%) than in males (98, 47.3%). About helminths, *Ascaris lumbricoides* was the predominant isolate (62.1%) followed by hookworms (18.4%). Only 22 participants were co-infected with malaria parasite and helminths and co-infection with *Ascaris lumbricoides* was predominant (45.0%). The prevalence of anemia was 10.9% and co-infection with *Plasmodium* and helminth parasites was significantly associated with ($P<0.0001$) higher aneamia prevalence compared to individuals without any infection. **Conclusions:** Prevalence of malaria and soil transmitted helminths is high and the disease is still major health problem in the study area. Hence, simultaneous combat against the two parasitic infections is very crucial to improve health of the affected communities in economically developing countries.

1. Introduction

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus *Plasmodium*. It is one of the leading causes of illness and death in the world. Nine out of ten of these deaths occur in Africa and the rest occur in Asia and Latin America, being the world's most prevalent vector-borne disease. It is the fourth leading cause of death of children under the age of five years and pregnant women in developing countries[1,2]. The disease remains one of the most important causes of human morbidity and mortality with enormous medical, economic and emotional impact in the world. More than half of the world's population is at risk of acquiring malaria, and the proportion increases each year because of deteriorating health systems, growing drug and insecticide resistance, climate change and natural disasters[3,4].

Also 2 billion individuals are infected with helminths in global, out of these majorities live in resource-poor settings[5,6]. World health organization (WHO) estimated the common soil transmitted helmnths infections (STHIs) in world as: 250 million cases for ascariasis, 151 million cases of hookworm diseases, 100 million cases of strongyloidiasis and 45.5 million cases of trichuriasis[7]. The occurrence of helminthic infections is associated with socioeconomic,

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environmental and other factors like, ignorance of simple health promoting factors and overcrowding, limited access to clean water, tropical climate and low altitude[5-7].

Human co-infection with *Plasmodium falciparum* (*P. falciparum*) and helminths are ubiquitous throughout Africa, although its public health significance remains a topic for which there are many unknowns[8]. Therefore, overlapping distribution of intestinal helminths and malaria parasites result in a high rate of aneamia. This may result both the synergism and additive interaction between helminths and malaria parasite. One of the main impacts of malaria and helminths infection is aneamia among other mechanisms through hemolysis, increased spleen clearance of infected and uninfected red blood cells and cytokines induced dyserythoropoeis, similarly intestinal helminths are significant causes of aneamia as a result of direct blood loss, nutritional theft and impairment of appetite due to immunological factors. Based on the distinct mechanism by which malaria and helminths reduce hemoglobin level, it can be speculated that their combined presence might interact to enhance the risk of aneamia[8-10].

Aneamia undoubtedly is a major health problem in malarious areas of tropical Africa and its cause is frequently multifactorial. In many regions of Sub-Saharan Africa, intestinal helminth infections particularly hookworm disease overlaps geographically with falciparum malaria where much of the morbidity associated with both disease results from aneamia[10,11]. Because of other factors that frequently contribute to aneamia in many malarious areas including malnutrition and genetic factors; it is difficult to estimate the percentage of aneamia in a particular population that can be attributed to intestinal helminth infections. However, there were attempts to determine the contribution of malaria and hookworm for the development of aneamia in malaria endemic areas of Africa.

In developing countries, parasitic infection especial helminths and malaria infection contribute for most cases of aneamia. Hookworm infection also strongly correlated with aneamia among children and pregnant women in malaria-endemic areas[12-20]. However, the extent of association of intestinal helminths infections with aneamia among all age groups in malaria endemic areas is largely unreported.

The occurrence of intestinal helminths infections alone or coinfection with is high and a number of surveys on intestinal helminths parasites in Ethiopia including north part of Ethiopia have been carried out previously[21-28]. Some authors also reported the association of STH infection and aneamia in both malaria endemic and non-endemic areas of the country Ethiopia[28-35]. However, the contribution of intestinal helminths infections as well as malaria in the development of aneamia, the concomitant occurrence of malaria and intestinal helminths infections and their clinical manifestations in malarias areas of the country have not been dealt in any detail in Ethiopia, especially in the study area. Therefore, the aim of this study was to assess the extent of intestinal helminths infections and malaria in the study area and their contribution for the development of aneamia.

2. Materials and methods

2.1. Study area and study design

The study was conducted in Azzezo, a small town located on the outskirt of Gondar, a provincial town some 470 miles (760 km) north of Addis Ababa, the capital of Ethiopia. It is surrounded by chains of mountains and lies at altitude of 4 600 feet (1 400 m) above sea level. The average temperature ranges from 50 to 80 degrees Fahrenheit (10 ℃ to 26 ℃). Demaza River flows through the heart of Azzezo which demarcats the former military camp from the town. Shinta is the other river that bounds Azzezo on the east side. The town is located along the main highway that links the provincial town Gondar with such cities like Bahr Dar and Addis Abeba and towns like Gorgora and Chelega. Azzezo comprises three small boroughs commonly called Kebeles. Total population is about 35 000 and that accounts for 15% of the Gondar population. Malaria transmission in Azzezo is unstable, seasonal and depends on altitude and rainfall like other parts in Ethiopia. There are two main seasons for transmission of the disease: September to December, after the heavy summer rains, and March to May, after the light rains.

The study was conducted on acute malaria patients that attended Azzezo Health Center in February to March 2011. Cases positive for *Plasmodium* species and older than one year, had no history of anti-malarial drug administration in the two weeks prior to screening and absence of any other serious chronic infection were included in the study. However, pregnant women, children younger than one year and individuals with known concomitant chronic infection were excluded from the study.

2.2. Sampling size determination

The sample was estimated to determine prevalence of malaria using the formula for estimating single proportion at 95% confidence interval (*CI*) level [$Z(1-\alpha/2)=1.96$] by taking the proportion of malaria helminths co-infection prevalence in study area but the prevalence of co-infection is unknown in the study area, so we have used 0.50, to have the largest sample size. The expected margin of error to be 5% (d=0.05). Based on these entities 384 study subjects were included in our study.

2.3. Microscopic examination of malaria parasite

Socio-demographic survey and other necessary data were collected by trained data collectors. The staining techniques and blood film examination for malaria parasite detection was conducted according to a standard operating procedure in Gondar University specialized hospital laboratory. In brief, peripheral blood was collect from finger by disposable blood lancet and thick and thin films were made on the same

slide. After being air-dried in a horizontal position, the thin blood films were fixed in methanol for 30 s. Then smears were stained with 10% Giemsa solution for 20 min. Each slide was examined under oil immersion microscopic objective by experienced laboratory technicians who were certified on malaria diagnosis and species identification from Ethiopia Ministry of Health. Hundred fields were examined before negative result was reported. The thin smear was used to identify the type of *Plasmodium* species. The second round confirmatory microscopic examination done by experienced laboratory technician who was blind for the first result.

2.4. Determination of haemoglobin concentration

Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially designed microcuvette (the Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden). Then, the haemoglobin values were used to assess the status of aneamia. For haemoglobin, the cut-off criterion levels below which indicating aneamia was the WHO cut-off of 110 g/L for children 6-59 months; 115 g/L for children 5-11 years; 120 g/L for children 12-14 years; 120 g/L for non-pregnant women above 15 years of age and 130 g/L for men above 15 years of age[36].

2.5. Stool sample collection, and light microscope detection

2.5.1. Direct wet mount

Stool samples were collected by using labeled caps from all consented patients included in the study. A direct saline mount of each sample was checked for motile and easily observable intestinal parasites by microscope at 10× and 40× magnifications. A small portion of the stool samples (about 1 g) were also preserved in 10% formalin for repeating the tests by concentration technique when the direct wet mount would been negatives.

2.5.2. Formol ether concentration method

A portion of fresh stool sample about one gram was taken, crashed well and preserved in 4 mL of 10% formalin. Then the preserved stool sample sieved through double layer cotton gauze into conical centrifuge test tube. Next equal amount of diethyl ether added and hand-shaken or mixed by vortex for one minute and then centrifuged for another two minutes at 2 000 *g*. When the centrifugation ended the supernatant of the test tube discarded and the sediments examined for the presence of ova and/or parasites under the light microscope at a magnification of 10× and 40×.

2.6. Quality control

After data collection process, the data were checked for completeness and any incomplete or misfiled questionnaires were checked again by PIs. Then the result of laboratory examination was recorded on well-prepared format carefully and finally it was attached with the questionnaire. For quality purpose 10% of slides were reexamined by experienced laboratory technician who was blind for the first result.

2.7. Data analysis

Data were double entered and analysed using SPSS-15.0 statistical software (SPSS Inc. Chicago, 2007). Descriptive statistics were used to give a clear picture of background variables like age, sex and other variables. The frequency distribution of both dependent and independent variables were worked out and the association between the independent and dependent variables were measured and tested using *OR* and 95% *CI* and *P*-value less than 0.05 considered as statistically significant.

2.8. Ethical consideration

Ethical clearance was obtained from University of Gondar College of Medicine and Health Sciences department of Medical Laboratory Science ethical clearance committee. Additionally, after explaining the importance, purpose and procedure of the study briefly an informed written consent was obtained from adult participants and from their guardians in case of children. Anyone not willing to take part in the study had full right to do so and confidentiality of the study participants was also maintained. All cases with history of fever in the preceding three days and/or those who had fever on examination and positive for malaria parasite during blood film and those positive for STH were treated as per national guideline.

3. Results

3.1. Socio-demographic characteristics of study subjects

A total of 384 febrile patients (195 males and 189 females) were included in the study. The age range of study subjects was 1 to 80 years with median age of 23.8 years. The female male ratio of study subject was 1:1.03. The majority 279 (72.6%) of participants had age greater than 15 years, followed by 5-14 years 82 (21.3%) and <5 years 26 (6.1%). About 76% of study subjects were urban dwellers and private business was their main occupation. The majority 338 (88.0%) of the respondents was Amahara by ethnicity and Orthodox Christianity was the predominant religion in the area accounting for 92.0% (Table 1).

3.2. Malaria and soil-transmitted helminths infection

Out of 384 febrile patients examined for malaria parasites, 44 (11.5%) individuals were positive for malaria parasites, of which *Plasmodium vivax* (*P. vivax*) accounted for 75.0% (33), *P. falciparum* for 20.5% (9) infectious, whereas two person (4.5%) had mixed species infection. Prevalence of malaria

Table 1
Prevalence of malaria among fibril patients attending Azzezo health center in Gondar, 2011.

Characteristics		Number(*n*)	Total malaria positive (*n* =44)	*OR*	95% *CI*	*P*-value
Sex	Male	195	28	0.552	0.288-1.057	0.070
	Female	189	16			
Age	<5 yr	26	6	2.885	1.064-7.823	0.037
	5-14 yr	82	12	1.648	0.792-3.433	0.182
	⩾15 yr	279	26	1.000	-	-
Residence	Urban	292	32	0.849	0.809-0.891	0.000
	Rural	92	12			
Religion	Orthodox	353	37	0.351	0.036-3.460	0.370
	Muslim	15	3	0.750	0.056-10.030	0.828
	Protestant	7	2	1.200	0.073-19.630	0.898
	Catholic	5	1	0.750	0.032-17.510	0.858
	Others	4	1	1.000	-	-
Ethnicity	Amahara	338	39	1.000	-	-
	Tigrea	40	4	0.852	0.288-2.522	0.772
	Others	6	1	1.533	0.175-13.467	0.700

Table 2
Prevalence of intestinal helminths among fibril patients attending Azzezo health center in Gondar, 2011.

Characteristics		Number (*n*)	Total intestinal helminths (*n*=207)	*OR*	95% *CI*	*P*-value
Sex	Male	195	98	1.066	0.714-1.591	0.755
	Female	189	109			
Age	<5 yr	26	10	0.744	0.326-1.698	0.482
	5-14 yr	82	70	7.684	3.901-15.134	0.000
	⩾15 yr	279	127	1.000	-	-
Residence	Urban	292	125	0.291	0.243-0.348	0.000
	Rural	92	82			
Religion	Orthodox	353	199	0.351	0.036-3.464	0.370
	Muslim	15	4	0.750	0.056-10.025	0.828
	Protestant	7	1	1.200	0.730-19.631	0.898
	Catholic	5	1	0.750	0.032-17.506	0.888
	Others	4	2	1.000	-	-
Ethnicity	Amahara	338	193	1.000	-	-
	Tigrea	40	12	0.332	0.158-0.655	0.002
	Others	6	2	0.376	0.068-2.079	0.262

was higher in males (28) when compared with prevalence in females (16). From the 44 malaria infected patients, 6 (23.1%) were in <5 years age group, 12 (14.6%) were in 5-14 years age group and the rest 26 (9.3%) were in >15 years age group (Table 1).

Prevalence of intestinal helminths was 207 (53.9%). The result shows that distribution of intestinal helminths was slightly higher in females (109, 52.7%) than in males (98, 47.3%). From helminths, *Ascaris lumbricoides* (*A. lumbricoides*) was the predominant isolate (62. 1%) followed by hookworms (18.4%) and *Hymenolepsis nana* (12.6%). *Strongyloides stercolaris (S. stercolaris)* and *Schistosoma mansoni (S. mansoni)* were common helminths isolated from study subjects. However, no significant difference was observed in helminths infection among males and females. Univariate analysis indicated with the exception of age, none of the studied sociodemographic variables had a significance association with helminths prevalence (Table 2).

From 44 malaria infected patients, 20 were positive for one or more STHs which give a co-infection prevalence of 5.1% (Table 3). The most common among co-existed helminths was *A. lumbricoides* (45%) followed by hookworm (30%), *Trichuris trichiura (T. trichiura)* (20%) and others (5%).

3.3. Hemoglobin measurement and aneamia

Mean haemoglobin concentration of the study participants was 129 g/L (ranging from 52 g/L to 207 g/L) with standard deviation of 2.34. *Plasmodium* alone or both *Plasmodium* and helminth infected females had significantly lower mean haemoglobin concentration than males. Children younger than 5 years lowest mean haemoglobin concentration as compared to the older age groups. From 384 individuals tested for aneamia 42(10.9%) individuals were found to anemic according to WHO classifications. Prevalence of aneamia was higher in females 22 (11.6%) than in males 20 (10.3%) and highest in children younger than 5 years 6 (23.1%) though it was not statistically significant. In general, data for the study patients indicate that co-infection with *Plasmodium* and helminth parasites is associated with significantly ($P < 0.000\ 1$) higher aneamia prevalence compared to individuals without any infection (Table 4).

Table 3
Prevalence of malaria helminths co-infection among fibril patients attending Azzezo health center in Gondar, 2011.

Characteristics		Number (*n*)	Malaria helminths coinfection (*n*=20)	*OR*	95% *CI*	*P*-value
Sex	Male	195	12	0.674	0.269-1.688	0.390
	Female	189	8			
Age	<5 yr	26	3	2.440	0.654-9.117	0.184
	5-14 yr	82	3	0.711	0.199-2.536	0.599
	⩾15 yr	279	14	1.000	-	-
Residence	Urban	292	15	0.932	0.903-0.961	0.010
	Rural	92	5			
Religion	Orthodox	353	19	9.100	0.010-6.550	0.990
	Muslim	15	1	1.000	0.026-1.678	0.999
	Protestant	7	-	-	-	-
	Catholic	5	-	-	-	-
	Others	4	-	-	-	-
Ethnicity	Amahara	338	19	1.000	-	-
	Tigrea	40	1	0.430	0.056-3.305	0.418
	Others	6	-			

Table 4
Prevalence of anemia among fibril patients attending Azzezo health center in Gondar, 2011.

Characteristics		Number(*n*)	WHO defined anemic cases (*n*=42)	*OR*	95% *CI*	*P*-value
Sex	Male	195	20	1.153	0.607-2.190	0.664
	Female	189	22			
Age	<5 yrs	26	6	2.555	0.949-6.877	0.063
	5-15 yrs	82	7	0.795	0.335-1.888	0.063
	⩾15 yrs	279	29	1.000		
Infection status	No infection	153	15	1.000	-	-
	Malaria helminths coinfection	20	12	13.800	4.871-39.093	0.000
	Only malaria	24	10	6.600	2.489-17.349	0.000
	Only helminths	187	5	0.253	0.090-0.710	0.090

4. Discussion

Overlapping distribution of intestinal helminths and malaria parasites might results in a high rate of co-infection which may result both in synergism and antagonistic interaction between helminths and malaria parasites[8,10]. One of the main impacts of malaria and helminth infections is aneamia. Malaria causes aneamia, among other mechanisms through hemolysis and increased spleenic clearance of infected and uninfected red blood cells and cytokine induced dyserythropoeisis.

In the current study 44 (11.4%) individuals were positive for malaria parasites, of which 75.0% positive for *P. vivax*, 20.5% for *P. falciparum* and 4.5% for both species. This finding was smaller than study result done in Alaba Kulito (27.9%), Ethiopia[29]. The result variation might be due to seasonal variation where study conducted and other factors. In Ethiopia, epidemiological pattern of malaria transmission is generally unstable and seasonal, the level of transmission varying from place to place because of altitude and rainfall patterns. Some localities also experience perennial malaria, because the environmental and climatic situations permit the continual breeding of vectors in permanent breeding sites[30].

Among the etiology agents of aneamia, malaria parasites are the major one and the prevalence of aneamia among malaria-infected patients were 50% in our study. The simultaneous and consequential development of aneamia among malaria patients were higher in this study when compared with the study conducted in Jimma zone Asendabo (27.6%)[26] and in Alaba Kulito (31.4%)[29] in Ethiopia but lower in a study conducted in East Wollega Zone (65.5%)[28] in eastern part of Ethiopia. The difference between the distributions of aneamia among malaria infected patients Kenya and Ethiopia might be due to the difference in nutritional status between Kenya and Ethiopia and variation of other factors which contributes for development of aneamia. The occurrence of aneamia was statistically significantly associated with malaria parasites with *P* value of 0.000 1 (*OR*=16.95%, *CI*=7.605-33.664) (data not present here). Therefore, malaria infected patients are sixteen times high risk to develop aneamia when compared to non-infected individuals.

Even if there has much common proposition on the association of aneamia with malnutrition, there has a considerable connection of aneamia with intestinal helminthes infections in addition to malaria. Different types of intestinal helminthes infection may affect nutritional status and RBC number and hemoglobin level in different ways (*e.g.*, nutrient absorption, and degree of mucosal

damage)[7]. Previous studies indicated various mechanisms through which hookworm, *S. mansoni*, *T. trichuira* and *A. lumbricoides*infections might alter nutritional status and reduce RBC number and Hgb levels[8-10].

In this study, the prevalence of aneamia was 10.94%. The prevalence of aneamia in this study is lowers in contrast of others that done in Cambodia (29.5%), Lao PDR (32.9%), and Vietnam (32.3%). Lower prevalence of aneamia in our study area might be due to the examination technique variation, study subjects difference, season's difference, and ecological factors[37].

Even though malaria and helminthes parasites do have different mechanisms to induce an aneamia, the aneamia cases among those only infected by *Plasmodium* are significantly higher (41.7%) than intestinal helminthes infected cases (8.2%) in this study. These might be due to the direct influence and damages of malaria parasites such as hemolysis, increased spleen clearance of infected and uninfected red blood cells.

The prevalence of intestinal helminthes parasites 54.0% in this study was lower when compared with that was done in Alaba Kulito HC (55.7%), southern Ethiopia[29]. Therefore, the different pattern of intestinal helminthic parasites distribution in our study area and other study might be due to the examination techniques difference, the study subjects' difference, and the number of specimens examined, parasitic distribution, ecological factors, personal and environmental sanitation, and health service coverage and program.

The aneamia prevalence among intestinal helminthes infected patients was 17(8.2%) in this study. Individuals co-infected with *Plasmodium* and helminth parasites reveled significantly ($P<0.000\ 1$) higher prevalence of aneamia compared to those without any infections. And this difference was also significant for haemoglobin concentration ($P<0.001$) (data not present here), in which patients co-infected with *Plasmodium* and helminth parasites showed lower mean haemoglobin concentration. This finding is similar with other study findings in Kenya and Ethiopia, Alaba Kulito[13,29]. The increased prevalence of aneamia in co-infected cases may be attributed to chronic blood and iron loss due to worm infections in addition to the loss due to malaria. And we would suggest that combined intervention would be particularly relevant for vulnerable populations who are at the highest risk for aneamia. Thus, antihelminthic treatment could potentially be co administered with malaria control to children younger than 5 years and adults $\geqslant$ 15 years (especial pregnant mothers) in areas of seasonal malaria transmission.

This study tried to determine malaria and intestinal helminthic infection from all febrile patients attending health center by standard laboratory techniques. But this is not without certain limitation as to failure to address other factors like diet and socio economic status which may have impact on haemoglobin levels and nutrition status are not considered in this study. And also, for each blood and stool specimens from participants only one slide was read to detect malaria and/or helminth infection and identify the type of species. In addition, there were small sample sizes in each infection types of different helminth intensity and *Plasmodium* parasitaemia level to make comparisons valid. Also this study did not assess association between helminth intensity and *Plasmodium* parasitaemia with aneamia.

Prevalence of malaria and soil transmitted helminths was high and the diseases were still major health problem in the study area which alerts public health intervention as soon as possible. Generally, malaria and soil-transmitted helminthiasis obviously contribute to aneamia and these conditions are more pronounced in individuals concurrently infected with malaria and soil-transmitted helminths. Hence, simultaneous combat against the two parasitic infections is very crucial to improve health of the affected communities in economically developing countries.

Conflict of interest statement

We declare that we have no conflict of interest.

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Abebe Alemu conceived the study, undertook statistical analysis and drafted the manuscript. Yitayal Shiferaw, Aklilu Ambachew and Halima Hamid initiated the study and made major contributions to the study design and statistical analysis. All authors contributed to the writing of the manuscript and approved the submitted version of the manuscript.

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