Full Length Research Paper

Malaria-intestinal helminthes co-infection among patients in Wolkite Health Center and Attat Hospital, Gurage Zone, Southern Ethiopia

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To initiate the prevention and control methods for overlapping distribution of intestinal helminthes and malaria, collecting adequate, updated and reliable information is required. Thus, the objective of this study was to assess the prevalence of Malaria-intestinal helminthes co-infection among patients attending Wolkite Health Center and Attat Hospital, Gurage Zone, Southern Ethiopia. Cross sectional parasitological study of 460 patients was conducted from April to June 2016. Giemsa-stained blood film was examined to detect malaria parasite, while the formal-ether concentration technique was used to diagnose intestinal helminthes. Data was entered and analyzed using SPSS version 16.0 soft ware. Overall prevalence of malaria infection was 18.3% (84). *Plasmodium vivax* 12% (55/460) and *Plasmodium falciparum* 5.9% (27/460) were the only malaria species identified. Mixed malaria species were 0.4% (2/460). The overall prevalence for at least one intestinal helminthes was 43% (198/460). *Ascaris lumbricoides* (16.7%), Hookworm (11.7%), *Hymenolepis nana* (12.4%), *Enterobius vermicularis* (8.0%) and *Taenia species* (13.0%) were the identified species. Malaria-intestinal helminthes co-infection was 10% (46/84). The most common among co-existed helminthes was *A. lumbricoides* (4.1%) followed by *Taenia species* (3.7%). The co-infection prevalence was higher in females 13% (29/224) compared to males 7.2% (17/236) (χ^2= 4.212, *P*- value= 0.04). Possible control methods such as public health education on bed net use and cleaning environment, provision of IRS and ITN/ILLN as well as providing community based control strategies should be the major focusing area of regional as well as federal health institutions in the country. This co-infection of malaria and intestinal helminthes may increase the risk to anaemia. Therefore, further studies on the association of co-infection with anaemia and assessment on the mechanism involved in such interaction is needed to support this current finding as well as provide useful information necessary to design control management for malaria in the context of co-infection.

Key words: Co-infection, malaria, intestinal helminths, Wolkite Health Center, Attat Hospital.

INTRODUCTION

A high rate of co-infection of intestinal helminths and malaria results because of their overlapping distribution

(Keiser et al., 2002; Adrienne et al., 2005), which may result both in synergism and antagonistic interaction between helminths and malaria parasites (Mathieu, 2002; Kirsten et al., 2005). Infection with helminths appears to polarize the immune response towards T-helper-2 type, characterized by high level of cytokines such as interleukin-4 (IL-4), IL-5, IL-13 and high serum level of immunoglobulin-E (IgE) (Hartgers and Yazdanbakhsh, 2006). This revealed that helminths could influence the host immunity to mediate immune responses that are beneficial to malaria parasites during co-infection (Nyamongo et al., 2015). Co-infections with helminthes and malaria parasites cause a significant problem against the host. For instance, they have negative impact upon host nutrition through a number of mechanisms which may have additive or multiplicative impacts, especially in childhood (Crompton and Nesheim, 2002). Another main impact of malaria and helminthes infections is anaemia. Malaria causes anaemia, among other mechanisms through haemolysis and increased spleenic clearance of infected and uninfected red blood cells and cytokine induced dyserythropoeisis (Crawley, 2004; McDevitt et al., 2004). Similarly, intestinal helminthes are significant causes of anaemia as a result of direct blood loss, nutritional theft and impairment of the appetite due to immunological factor (Stephenson et al., 2000; Hotez et al., 2004). Most gastrointestinal helminths and Plasmodium affect host nutrition in a similar manner. Hence, it seems plausible to consider different gastrointestinal helminth species together while assessing the impact of helminth coinfection on malaria (Abrahm and Berhanu, 2016). In general, individuals coinfected with more than one parasite species are at risk of increased morbidity (Kinung'hi et al., 2014).

Intestinal parasite infections still continue to be the major health problem worldwide. Such infection present a persistent and intolerable threat to the health of millions of people mainly in the tropic and subtropics and their cost in terms of human life and economic loss is incalculable. In Ethiopia, the prevalence and distribution of intestinal helminthes varies from place to place (Erosie et al., 2002; Mengistu and Berhanu, 2004; Jemaneh, 2000). This might be because of the diversity of the country environmental and living condition of individuals.

Malaria constitutes a major public health problem and impediment to socioeconomic development in Ethiopia. It is estimated that about 75% of the total area of the country and 65% of the population is estimated to be at risk of infection (Federal Ministry of Health (FMOH), 2007). According to WHO (2010) report, malaria is present everywhere in Ethiopia, except in the central highlands, and 56 million people are at risk. The disease is one of the country's leading health problems in terms of morbidity, mortality and impediment to socioeconomic development and top ranking in the list of common communicable diseases, consistently ranking in the top 10 causes of outpatient visits, admissions, and deaths at health centers and hospitals (Federal Ministry of Health

(FMOH), 2004). Though there has been a growing interest to investigate co-infections and their related clinical consequences worldwide, there is no previous study reported in the area on the concomitant occurrence of malaria and intestinal helminthes infections, their clinical manifestations and the association of the infections. Knowledge about the prevalence of malaria and intestinal parasites in particular areas is essential for the initiation and implementation of parasite control programmes in the region and give evidence-based propositions for timely interventions. Such information is required to guide policy makers in deciding on the type of preventive and control strategies in controlling intestinal helminthes induced anaemia. Therefore, this study investigates the prevalence of malaria and intestinal helminthes co-infection among patients attending in Wolkite health center and Attat hospital during the study period.

MATERIALS AND METHODS

Study area and subjects

This study was conducted at Wolkite Health Center and Attat Hospital in Gurage Zone located 158 km south west of Addis Ababa along the Jimma Road in the Southern Region of Ethiopia. Both the health center and hospital were found under Wolkite town, the capital of Gurage Zone. This town has a latitude and longitude of 8°17′N37°47′E and an elevation between 1910 and 1935 m above sea level. It is surrounded by Kebena Woreda and it was part of former Goro Woreda. Malaria and intestinal parasites are the most prevalent public health problems in the area. Malaria transmission in Gurage Zone is unstable, seasonal and depends on altitude and rainfall. 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 subjects were patients that attended Wolkite Health Center and Attat Hospital during the study period. Individuals who had no history of anti-malarial drug administration in the two weeks prior to screening, absence of any other serious chronic infection, had ability to give blood and stool samples were included in the study.

Study design and sampling procedure

This cross sectional study was carried out among patients that visited Wolkite Health Center and Atta Hospital during the study period. Systematic random sampling was used to select the individuals in the sample by selecting one of the elements at random from sampling frame at the starting point, and then onward from this point, the rest sample was selected systematically by applying pre-determined interval of every third elements.

Sample size calculation

Sample size was estimated using the statistical formula of sample size calculation $n = p(1-p)z^2/d^2$, where, n = required sample size, z = confidence level at 95% which is standard value of 1.96, p = estimated prevalence of intestinal parasite and d = marginal error at 5%, standard value of 0.05 (Danile, 1995). Since the overall prevalence of malaria-intestinal helminthes co-infection was not known for this study area, prevalence (p) was taken to be 50% and

Table 1. Age related prevalence of malaria and malaria species among patients in Wolkite Health Center and Attat Hospital in 2016.

Malaria species Characteristics		Pv {No. (%)}	Pf {No. (%)}	Total malaria {No. (%)}
Age group	≤ 5	1 (2.1)	0 (0)	1 (2.1)
	6-14	15 (12.3)	5 (4.1)	21 (17.2)
	≥15	39 (13.5)	22 (7.6)	61 (21)
	Total	55 (12)	27 (5.9)	84 (18.3)
	χ^2	5.071	5.233	10.395
	P- value	0.079	0.073	0.006

IHs= Intestinal helminthes, Pv= *Plasmodium vivax*, Pf= *Plasmodium falciparum*.

this gave the minimum sample size of 384. To lessen errors arising from the likelihood of non compliance or possible drop out, 20% of the sample size was added to the normal sample size. Thus, the minimum sample size for this study was 460 with 20% contingency for non- respondents.

Ethical considerations

Prior to data collection, consent was collected from participants as well as the stakeholders of the study area and only volunteer individuals were included in the study. Diagnosis was done using sterile and disposable materials. Only laboratory technicians were allowed to take the blood sample and all other activities on clinical examination as well as diagnosis was supervised by specialized healthcare personnel.

Data collection

Socio-demographic survey and clinical diagnosis were made by trained physicians of the health center and hospital. The laboratory techniques that were used in this study are: Blood film smear for malaria diagnosis and Formalin-Ether concentration techniques for stool parasite diagnosis.

Blood film determination for malaria parasites

Laboratory technicians collected the samples and malarial infections were determined from thick and thin films of finger-prick blood fixed and stained with Giemsa stain. Thick and thin blood smear wear prepared for each subject from capillary blood by finger prick using sterile lancet. The thick smear was stained with Giemsa solution and the thin smear was fixed with methanol before stained with Giemsa solution. Each blood smear was observed under the oil immersion objective of the microscope. The thick smear was used to determine whether the malaria parasite was present or not after observing 100 fields of vision. The thin smear was used to identify the *Plasmodium* species.

Formalin-Ether concentration technique for stool examination

Study subjects were provided with a dry, clean and leak proof stool cup labeled with identification number of each individual and applicator stick. Stool samples were preserved in 8 ml of 10% formalin solution, and transported to the Microbiology and Parasitology Laboratory of the Department of Biology, Wolkite University, for parasitic microscopic examination. Formalin-ether

concentration technique was used for laboratory examination of the collected samples (WHO, 1991). Stool examination was conducted by experienced medical laboratory personnel.

Data analysis

The data was computerized using Excel 2007, cleaned and checked against original document before analysis. All statistical analyses were performed using SPSS for windows version 16 statistical package. Descriptive statistical tests were applied to calculate the prevalence of Plasmodium species and intestinal helminthes as percentages and proportions. Pearson chi-square (x^2) test was used to verify the relationship between independent factors and the outcome variables. The 95% CI was used to show the accuracy of data analysis. P-value less than 5% was considered statistically significant.

RESULTS

Malaria and intestinal helminthes infection

Malaria infection

Out of 460 patients examined, 84(18.3%) were positive for malaria parasites. *Plasmodium vivax* 12% (55/460) and *Plasmodium falciparum* 5.9% (27/460) were the only malaria species identified in this study. Mixed malaria species were 0.4% (2/460).

Prevalence of malaria was higher among females 9.8% (45) when compared to males 8.5% (39) though there is no significant difference (χ^2= 0.978, P= 0.323). Age related malaria infections were observed (χ^2=10.395, P=0.006). The age group ≤5 years was the most affected (21%) followed by the age group 6-14 years old (17.2%) (Table 1).

The prevalence of malaria was higher among patients that visited Attat Hospital compared to Wolkite Health center though it was not significantly different (Figure 1).

Intestinal helminthes infection

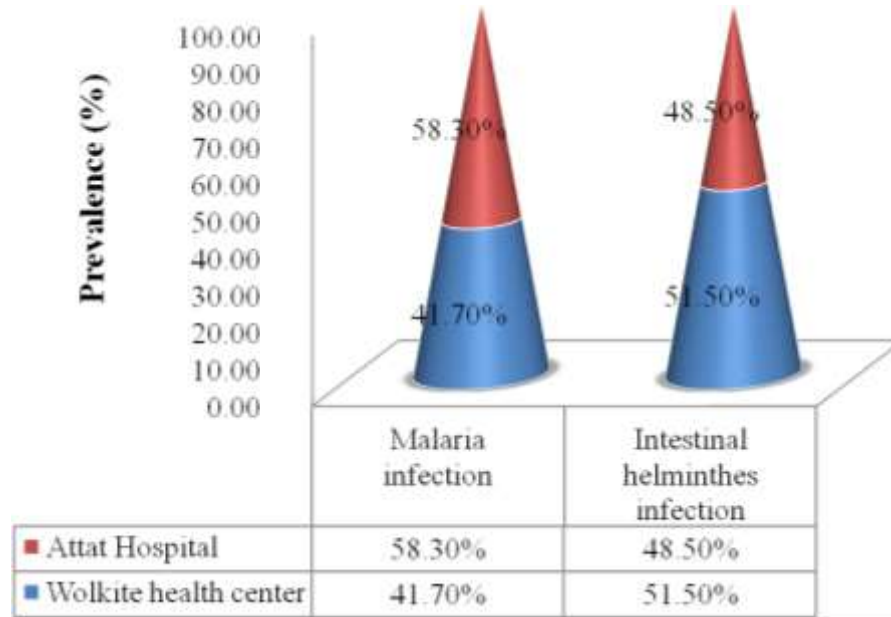From a total of 460 stool samples examined, 43% (198)

Figure 1. Comparing prevalence rate of malaria and intestinal helminthes among patients who visited Wolkite Health center and Attat Hospital in 2016.

Table 2. Gender related prevalence of Intestinal parasites (malaria species and Helminthes) among patients in Wolkite Health Center and Attat Hospital in 2016.

Intestinal helminthes infection	Gender		Total (n=460) {No. (%)}
	Males (n=236) {No. (%)}	Females (n=224) {No. (%)}	
Ascaris lumbricoides	44 (18.6)	33 (14.7)	77 (16.7)
Hookworm	33 (14.0)	21 (9.4)	54 (11.7)
Enterobius vermicularis	19 (8.1)	18 (8.0)	37 (8.0)
Hymenolepis nana	28 (12)	29 (13)	57 (12.4)
Taenia species	32 (13.6)	28 (12.5)	60 (13)
Plasmodium vivax	22 (9.3)	33 (14.7)	55 (12)
Plasmodium falciparum	15 (6.4)	12 (5.4)	27 (5.9)

were positive for one or more of intestinal helminthes. Five species of intestinal helminthes were identified with variable prevalence: *Ascaris lumbricoides* (16.7%), Hookworm (11.7%), *Hymenolepis nana* (12.4%), *Enterobius vermicularis* (8.0%) and *Taenia species* (13.0%).

The majority 134 (29.1%) of infected individuals had multiple infection and 64 (13.9%) were infected with single intestinal helminthes parasites. The result showed that distribution of intestinal helminthes was slightly higher in males (22.6%) than in females (20.4%) but it was not significant difference (χ^2=0.207, P=0.649) (Table 2). Age related difference was observed among intestinal helminthes species though only Hookworm showed statistically significant (χ^2 = 6.183, *P*- value=0.045 (Table 3).

Malaria-intestinal helminthes co-infection

From 84 malaria infected patients, 46 were positive for one or more intestinal helminthes which make a co-infection prevalence of 10%. The most common among co-existed helminthes was *A. lumbricoides* (4.1%) followed by *Taenia species* (3.7%) (Figure 2). Patients infected with intestinal helminthes were more likely to be infected with malaria 10.9% (50/460) compared to patients with no intestinal helminthes infection 7.4% (34/460) (χ^2= 11.385, *P*- value= 0.001).

DISCUSSION

The overall prevalence of malaria observed in the present

Table 3. Age related prevalence of Intestinal helminthes species among patients in Wolkite Health Center and Attat Hospital in 2016.

Age group	No. of examined {No. (%)}	Intestinal helminthes species (His)				
		Al {No. (%)}	Hw {No. (%)}	Hn {No. (%)}	Ev {No. (%)}	Ts {No. (%)}
≤5	48 (10.4)	9 (18.8)	8 (16.7)	8 (16.7)	6 (12.5)	7 (14.6)
6-14	122 (26.5)	26 (21.3)	7 (5.7)	21 (17.2)	12 (9.8)	14 (11.5)
≥15	290 (63.1)	42 (14.5)	39 (13.5)	28 (9.7)	19 (6.4)	39 (13.5)
Total	460 (100)	77 (16.7)	54 (11.7)	57 (12.4)	37 (8)	60 (13)
χ^2		3.029	6.183	5.421	2.691	0.407
P- value		0.220	0.045	0.067	0.260	0.816

Al= *Ascaris lumbricoides*, Ev= *Enterobius vermicularis, Hn= Hymenolepis nana,* Hw= Hookworm, Ts= *Taenia species*, IHs= Intestinal helminthes.
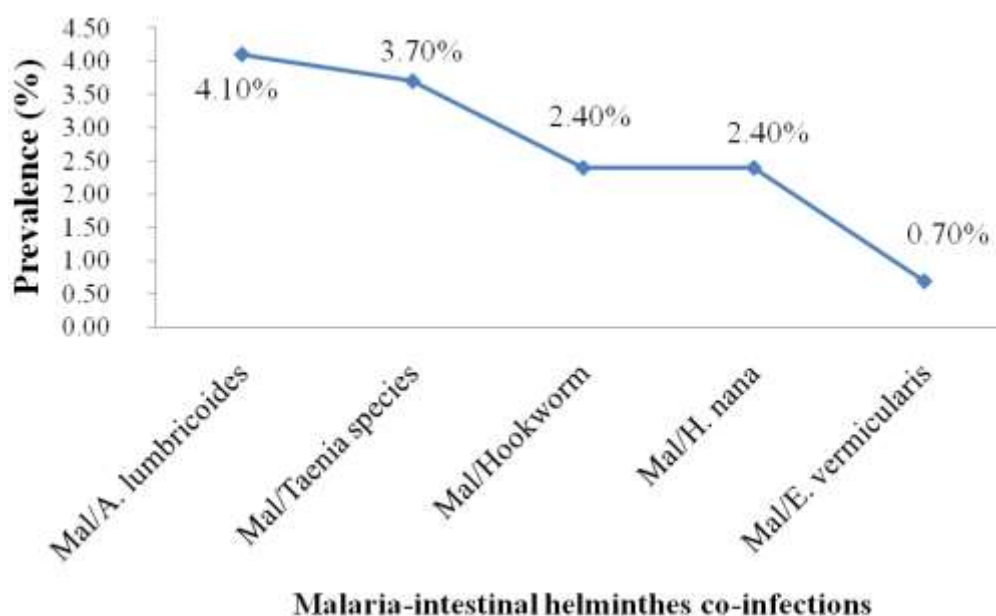

Figure 2. Prevalence of malaria-intestinal helminthes co-infections among patients who visited Wolkite Health Center and Attat Hospital in 2016. Mal/*A. lumbricoides*= Malaria-*Ascaris lumbricoides* co-infection, Mal/*Taenia species*= Malaria-*Taenia species* co-infection, Mal/Hookworm=Malaria-Hookworm co-infection, Mal/*H. nana*= Malaria-*Hymenolepis nana* co-infection and Mal/*E. vermicularis*= Malaria-*Enterobius vermicularis* co-infection.

study was relatively lower than study conducted in Alaba Kulito Health Center (27.9%), Southern Ethiopia (Abraham et al., 2012), Bumula District (46.4%) in western Kenya (Kepha et al., 2015) and in two rural communities in the mount Cameroon are (33.3%) (NKemnji et al., 2017) but higher than reported from Azzezo Heath Center (11.4%), Northwest Ethiopia (Abebe et al., 2012). The observed difference might be due to seasonal variation where the study was conducted, climatic condition that might influence malaria vector breeding and distribution in different areas. In Ethiopia, epidemiological pattern of malaria transmission is generally unstable and seasonal, with 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 (Abraham et al., 2009). Furthermore, the low prevalence of malaria in the present study might be due to the time when the study was conducted since the present study samples were collected during the dry season. Brooker and Michael (2000) and Hay et al. (2000), have shown that peak transmission of malaria occurs following the main rainy season and a minor transmission peak occurs following light rainy season in the tropics.

The prevalence rate of intestinal helminthes was 43% whereas the observed overall prevalence rate of the present study was found to be relatively lower than studies done in Alaba Kulito Health Center (55.7%),

Southern Ethiopia (Abraham et al., 2012) and Azzezo Heath Center (53.9%), Northwest Ethiopia (Abebe et al., 2012). The differences in findings among the studies might be due to variations in socio-economic conditions, individual behavioral habits of selected population, the methods employed for stool examination, the sample size taken as well as the time of study conducted. Further, Mengistu and Berhanu (2004) stated that the distribution and prevalence of various species of intestinal parasites also vary from region to region because of several environmental, social and geographical factors.

In the present study malaria-intestinal helminthes co-infection was 10%. This figure is higher compared to study conducted in Azzezo Heath Center (5.1%), Northwest Ethiopia (Abebe et al., 2012) and Gilgel Gibe dama area (7.7%), Southwest Ethiopia (Million, 2013). However, it was lower than the prevalence of co-infection reported from Thailand (Boel et al., 2010), Ghana (Yatich et al., 2009) and Nigeria (Egwunyenga et al., 2001). The observed difference might be due to seasonal variation where study conducted, climatic condition that might influence malaria vector breeding and distribution in different areas.

Patients infected with intestinal helminthes were more infected with malaria compared to patients with no intestinal helminthes infection. This finding was in line with study conducted in Gilgel Gibe dam areas (Million et al., 20013), Southern Ethiopia (Andargachew et al., 2013) and Ghana (Yatich et al., 2009). In addition, the findings of a meta-analysis by Naing et al. (2013) reported positive association between uncomplicated malaria and STH co-infection among school age children based on studies conducted globally. Furthermore, a systematic review and meta-analysis by Degarege et al. (2016) showed that *Plasmodium falciparum* density tended to be higher among children infected with STH than those uninfected with intestinal helminths. This might be due to the fact that some helminthes modulate the host response both to themselves and to concurrent infections. It has been suggested that the immune response evoked by helminthes infections may modify immune responses to *Plasmodium* and consequently alter infection and disease risk (Diallo et al., 2010; Hartgers et al., 2009; Sangweme et al., 2010). The biology of the parasite and the host, climate, socioeconomic status of the population and the like in the area are the major factors that influence the epidemiological and geographical patterns of infections and co-infections. Climate determines the survival of the mosquito vector of the malaria and the free living and infective stage of the helminthes (Brooker and Michael, 2000).

The most common among co-existed helminths was *A. lumbricoides*. This outcome was similar with study conducted in Southwest Nigeria (Dada-Adegbola et al., 2013) and in two rural communities of Cameroon (Zeukeng et al., 2014). Furthermore, different research outcomes revealed that positive association between *A. lumbricoides* infection and prevalence of malaria among patients in Ethiopia (Abrahm et al., 2012) and pregnant women in Ghana (Yatich et al., 2009). This might be due to the fact that it is the most prevalent helminthes that infects patients in the area. Furthermore, Natcher et al. (2002) and Hartgers and Yazdanbakhsh (2006) suggested that it could be that Th-2 profile-associated immunoglobulin E production seen in *Ascaris* infection may down-modulate Th-1 anti-malaria immune response, resulting in increased risk of malaria infection.

Conclusion

The present study showed that malaria co-exists with intestinal helminthes infections among the studied patients found in Wolkite Health Center and Attat Hospital and may give a warning signal for the regional health office authorities to start focusing attention in this area by providing community based control strategies. This co-infection of malaria and intestinal helminthes may exaggerate the risk to anaemia. Therefore further studies on the association of co-infection with anaemia and assessment on the mechanism involved in such interaction needed to support this current finding as well as provide useful information necessary to design control management for malaria in the context of co-infection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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