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Full length Research paper

Malaria-soil transmitted helminthes co-infection and associated factors among pregnant and non-pregnant women in Jimma Arjo district, western Ethiopia

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Malaria-soil transmitted helminthes co-infection is a major health problem among pregnant women in Ethiopia. Studies suggested that soil transmitted helminthes infections alone could increase the susceptibility of pregnant women to malaria. However, there is limited information on a community-based comparative study among pregnant and non-pregnant women with malaria-soil transmitted helminthes co-infection and associated factors in Jimma Arjo district, East Wallaga Zone, Oromia Region, Ethiopia. To assess the prevalence of malaria-soil transmitted helminthes co-infection and associated factors among pregnant and non-pregnant women in Jimma Arjo District. A community-based comparative cross-sectional study was conducted with a total of 291 pregnant, and 291 non-pregnant women from September to November 2023 in the study area. Rapid diagnostic tests and microscopy techniques were used for the malaria investigation. Infections of Soil transmitted helminthes and its intensities were determined using the Kato-Katz technique from stool samples. Anemia was determined using a portable spectrophotometer (Hemo Cue Hb 301). The prevalence of malaria-soil transmitted helminthes co-infections among pregnant and non-pregnant women was 6.1% and 3.6%, respectively. The odds of anemia among women infected with malaria-soil transmitted helminthes co-infection were higher compared with non-infected women. Pregnant women were more susceptible to malaria-soil transmitted helminthes co-infection than non-pregnant women.

Keywords: Malaria, Soil transmitted helminthes, Co-infection, Anemia, pregnant women, Plasmodium

INTRODUCTION

Malaria is a life-threatening disease caused by the protozoan parasites in Genus *Plasmodium.* It transmitted to humans via the inoculation of sporozoites by infected female *Anopheles* mosquitoes, during a blood meal. Although five species of *Plasmodium* infect humans, *Plasmodium falciparum* and *Plasmodium vivax* species contribute to adverse maternal and fetal outcomes in pregnancy (1). *P. falciparum* infection contributes significantly to maternal anemia, low birth weight of infants, intrauterine growth retardation, preterm deliveries, and infant mortality in sub-Saharan Africa (2-4).

Soil transmitted helminthes infection in humans is caused by an infection with *Necatoramericanus* and/or *Ancylostomaduodenale.* It transmitted often by larval skin penetration through contact with contaminated soil. It is one of the most predominant helminthic infections, with an estimated about 740 million cases in areas of rural poor tropics and subtropics (5). Soil-transmitted helminths (STHs) including soil transmitted helminthes infections are associated with cognitive impairment and lowered educational achievement, anaemia, stunted growth, malnutrition and responsible for about one million deaths per year (6).Soil transmitted helminthes is one of the most common intestinal nematode reported to have interactions with *Plasmodium* parasites (7). The hypothetical mechanisms for the apparent increase odds of malaria during soil transmitted helminthes infections could be due to the combination of immune modulation and soil transmitted helminthes-related blood destruction. The resultant level of anemia due to soil transmitted helminthes, increase cues that are attractive for mosquitoes (lactates, increased respiratory frequency, and CO2 exhalation, increased cardiac output) thus leading to a greater probability of infective bites (8).

Pregnancy increases the risk of malarial parasitic infection by uncertain mechanisms, but it may be partly because of shifts in cellular immunity from T helper type 1 to Th2 responses. During normal pregnancy, the woman's cellular immunity favors more Th2 cellular responses in the placenta, because the pro-inflammatory Th1 cytokines have adverse effects on the developing fetus (9, 10). This shift may protect individuals from clinical pathology due to inflammation, but on the other hand, affect malaria parasite clearance (11).

Helminths and *Plasmodium* species are co-endemic in many parts of sub-Saharan Africa. Hence, their co-infection is a common phenomenon (12). Also their co-endemicityis the most common public health problem in sub-Saharan Africa, where a significant proportion of the populations including pregnant women are exposed to infections. Out of several processes, the two core processes that define the distributions of helminths and *Plasmodium* species co-infection are exposure of hosts to parasites and susceptibility-related processes are common(13).

In Ethiopia, there is no adequate published literature on the prevalence of malaria-soil transmitted helminthes co-infection and resultant anemia among pregnant and non-pregnant women, where *Plasmodium* and STHs infections are expected co-endemic. Therefore it is necessary Malaria and hookworm transmission greatly depends on the same geographic conditions such as rainfall patterns, temperature, and humidity (34). In coupled with this environmental condition, hypothesized synergistic association in which immunological modulation induced by hookworm infection and pregnancy increases susceptibility to acquiring Plasmodium infection (9, 10, 35) is a very important problem that needs investigation among pregnant women.

Maternal anemia is associated with low birth weight and increased maternal morbidity and mortality. While the etiology of anemia is complex and multi factorial, parasitic diseases, including P. falciparum and hookworm infections, have been recognized as major contributors to anemia in endemic countries. Based on the distinct mechanisms by which they reduce hemoglobin levels, it can be speculated that their combined presence might synergistically to enhance the risk of anemia in pregnant women (36). Ethiopia is one of the African countries where approximately three-quarters of its territory is malaria endemic, and it is one of the most malaria-prone countries in Africa (37). The insect vector Anopheles arabiensis is the main malaria vector; An. pharoensis, An. funestus, and An. nili plays a role as secondary vectors (38).

Additionally, parasitic helminthiasis is the second most prevalent cause of outpatient morbidity in Ethiopia (39). The study conducted among pregnant women of Northwest Ethiopia reported that hookworm infection has the highest prevalence of 50% out of the intestinal parasites infected pregnant women (40).

METHODS AND MATERIALS

Study area

The study was conducted in Jimma Arjo District, which is the largest resettlement area located in East Wallaga Zone, Oromia Region, Ethiopia. The site is located approximately 319 km from Addis Ababa in the Southwest Oromia Region. The area is situated at altitude ranging from 900m to 1400 m above sea level and is categorized under the lowland (kola) agro-ecological zone. The annual temperature and rainfall vary from 37 to 40°c and 1000 to 1200 mm respectively. This resettlement area has seven major sub-sites and further subdivided into 28 (8 urban, 20 rural) kebeles. The 2013 Population and Housing Census conducted by the Central Statistical Agency of Ethiopia was reported a total population for this Woreda was 56,106, of whom 29,681 were men and 26,425 were women. This woreda has a total of 12,050 women in the reproductive age group (15-49 years) (77). According to the information obtained from the Jimma Arjo woreda Administration Office, the district has three health centers and one hospital (Jimma Arjo district annual health office report, unpublished). The main river in the area includes the Didesa which are tributaries of the Abay River.

Study period

The study was conducted from September to November 2023 in the study area.

Study design

A community-based comparative cross-sectional study was conducted.

Population

Source population

The source populations were all reproductive age women (15-49 years) of the JimmaArjo district.

Study population

All reproductive-age women (15-49 years) who fulfilled eligibility criteria.

Study subject

Randomly selected pregnant and non-pregnant women who were recruited from seven selected kebeles of the study area.

Eligibility criteria
Inclusion criteria

Being pregnant women of any trimesters and any non-pregnant women of reproductive age

Exclusion criteria

A woman who had taken anti-malarial drugs in the last 28 days and anthelminthic drug within two weeks before this study.

Being residents of the study area for less than 6 months before this study.

Sample size determination

The required sample size (*n*) was calculated by using a double population proportion formula with the assumptions of 95% confidence level, when sample are equal in both groups. We used prevalence assumptions; 13% and 6% prevalence of malaria-soil transmitted helminthes co-infection respectively for pregnant and non-pregnant women from the study previously conducted in a Semi-Urban Area in Anambra State, Nigeria (63).

$$n(\text{each group}) = \frac{(p_0q_0+p_1q_1)(z_1\text{-}\alpha/2+z_1\text{-}\beta)^2}{(P_1-p_0)^2}$$

Where:-

n = Minimum sample size

p_0=prevalence of malaria-soil transmitted helminthes co-infection in non-pregnant women=6%=0.06

$q_{0=}$ probability of malaria-soil transmitted helminthes co-infection not happened in non-pregnant women (q_0=1-$p_{0=}$84%, 0.84)

p_1=prevalence of malaria-soil transmitted helminthes co-infection in pregnant women.13%=0.13

$q_{1=}$ probability of co-infection not happened in pregnant women (q_1=1-p_1=87%, 0.87)

1-α =level of confidence at 95% (α = 0.05)

Z1-α/2= is the value of z from standard normal distribution=1.96

1-β=power 80%

Z1- β=is the value of z from standard normal table=0.84

❖ n= $(0.06\times0.84+0.13\times0.87)(1.96+0.84)^2/(0.13-0.06)^2$

n (each group)=261.6~262

Therefore the total sample size for two population =2×n (each group)

2×262=524

10% non-response rate, so the total sample size (n) was

n= 524 /0.9 = **582**

Sampling technique

Jimma Arjo District has 28 Kebeles (8 urban, 20 rural). Therefore, a population survey was conducted first stratified kebeles to rural and urban. After that two urban kebeles and five rural kebeles were selected by simple random sampling out of 28 Kebeles. Then the calculated sample size was proportionally allocated to the respective kebeles based on a number of pregnant women in a kebeles during the study period as follows; two urban 01 Kebeles 62/75 and 02 kebele42/51 and five rural kebeles; metta 34/41, Abote Didesa 41/49, lugama49/59, Harakeeku 28/34 and Gombo35/42) were selected by simple random sampling out of 28 Kebeles. To recruit individual participants before the actual data collection sampling frame had prepared for pregnant women in selected Kebeles and simple random sampling was employed to enroll the study unit. If a randomly-selected participant was not eligible for an interview or refused to be part of the study, the next eligible participant on the list was selected randomly. We sampled our study participants until we arrived at our desired sample size of 582. Non-pregnant women in the neighbor household of selected pregnant women present at home during data collection were included, if tested negative for HCG. Where there was more than one non-pregnant woman per household, one was selected randomly. Participants found with malaria, intestinal parasites and anemia were linked for treatment and health education on risks and management of infection to nearby health facilities.

Data collection procedures
Socio demographic characteristics

The principal investigator and trained field workers administered a pre-tested semi-structured questionnaire to participating women to obtain socio-demographic information and to assess associated factors for the co-infection.

Sample collection and laboratory diagnostic methods
Stool sample collection and processing

A freshly passed stool sample was collected from each of the subjects into a labeled screw caped stool cup by informing them on how to collect a stool sample. The stool specimens were processed following the standard procedure of the Kato-Katz concentration technique for microscopic examination (78) within the same day when the participants provide their stool sample. Two Kato-Katz slides were prepared for each sample; the slides were examined within 45 minutes of preparation for soil transmitted helminthes in jimma arjo health center laboratory.

Both Kato-Katz thick smears (A and B) were re-examined by a second reader who was blinded to the initial fecal egg counts (FECs) one after other. The internal recommendations of the Swiss TPH were used as a guide to define and classify discrepant results (79). The results were defined and classified into the following three types of discrepancies:(i) type 1: detection of a false positive or negative result, (ii) type 2: re-examined slide shows a difference >10 eggs when less than 100 eggs were counted during the first reading or (iii) finally type3: a difference >20% when 100 eggs or more were counted during the first reading. In the absence of discrepancies between both readings, the original FECs were kept. In case of discrepant results, a third examiner re-examined the slide.

In the case of a type 1 discrepancy, the presence or absence of eggs of the STH species in question was verified. In case of type 2 or 3 discrepancies, the FECs were compared to the FECs obtained by the first reader and the reader that performed QC. The FEC value of the reader that was closest to the FEC obtained during the third reading was considered correct. If the FEC of the QC reading was considered correct, the original was recorded FEC was changed to the FEC value obtained during QC. Average egg counts of the two slides multiplied by 24 to obtain egg counts per gram of stool for soil transmitted helminthes parasite(80).The intensity of soil transmitted helminthes infection categorized as follows: light, <1,000 eggs/gram of stool; moderate, 1,000–3,999 eggs/gram of stool; high, ≥4,000 eggs/gram of stool (80). Then soil transmitted helminthes parasitic load recorded in the pre-prepared laboratory format.

Blood sample collection and processing

Detection and identification of malaria parasite from Capillary blood samples were diagnosed both by Giemsa staining smear microscopy according to World Health Organization (WHO) criteria and RDTs (*Carestart*™ Malaria Pf/Pv (HRP2/PLDH) Ag Combo RDT). Asexual *Plasmodium* parasite density per µl of blood was determined by counting the number of asexual parasites per 200 white blood cells on a thick blood film assumed a total standard WBC count of 8000/µl. The parasite densities were calculated by averaging the two counts of qualified technicians. During malaria parasite blood film investigation, discordant of results, i.e., when differences between the two laboratory technicians species diagnosis, in parasite load of >50% discrepancy, or the presence of parasites (81)need the third investigator. The degree of parasite density graded as mild, moderate, and severe when the counts between 1–999 parasites/µl, 1000–9999/µl, and >10,000/µl, respectively, following the method described elsewhere (82).

$$\text{Parasite / µl} = \frac{\text{Number of asexual parasites} \times 8000 \text{ Leukocytes µl}}{200 \text{ Leukocytes}}$$

Determination of haemoglobin level

Hemoglobin concentrations were determined using a portable hemoglobin spectrophotometer, HemocueHb 301 analyzer (Hemo Cue Hb 301, Hemo Cue AB 16, Sweden), and a specially designed micro cuvette (the HemocueHb 301 Micro cuvette, Hemo Cue AB 16, Sweden). Then, the hemoglobin values were used to assess the status of anemia. For hemoglobin, the cut-off criterion levels below which indicating anemia by the WHO cut-off of 12 g/dl and 11 g/dl for non-pregnant and pregnant women above 15 years of age, respectively(83). Also, degree of anemia categorized using WHO standard; mild anemia Hb 10-10.9g/dl; moderate anemia Hb7-9.9g/dl; severe anemia Hb<7g/dl for pregnant and mild anemia Hb 11-11.9g/dl; moderate anemia Hb 8-10.9g/dl; severe anemia Hb<8g/dl for Non-pregnant women (15 years of age and above) (83).

Measurement and study variables

Dependent variables

- Malaria-soil transmitted helminthes co-infection
- Anemia
- Malaria

Independent variables

Data processing and analysis

All data from laboratory results and surveys was checked and cleared for completeness and inappropriate or illogical responses. Then data was coded, double entered into Epidata version 3.1, for quality control and export to SPSS for windows version 22 software package for analysis. Both descriptive and inferential statistics was employed for the analysis of data. One-way ANOVA was used to test mean differences of malaria parasites density and differences of mean hemoglobin with types of infection. Association of categorical variables with outcome variables was done using the chi-square (X^2) test. Binary logistic regression analysis was done for each independent variable then the candidate variable with a p-value less than 0.25 used in the multivariate logistic regression model. From the final model, Adjusted Odds ratios (OR) with a 95% confidence interval was reported. P-value < 0.05 was considered statistically significant.

Data quality assurance

Quality control

Two experienced laboratory technicians with principal investigators were examined the microscopic slides. If no asexual or sexual forms of the malaria parasites are seen after 2500 WBCs, we were declared the slide negative(84). When results between two microscopists discordant, a third senior Parasitologist and/or principal investigator read the slide blinded to determine the diagnosis and parasite count. The manufacturer's instruction strictly followed for the RDTs. Blood smear microscopy readers was blinded to the result of RDTs.

In case of discrepancies of parasite counts, the slides was re-examined by another senior technician and results discussed until agreement reached. For HemocueHb 301 accurately testing known levels of hemoglobin ensures that the system and technique used in testing give accurate results on patient tests.

The control solutions checked and if results for these solutions fall within a certain acceptable range to allow valid patient testing.

Data quality management

All laboratory materials such as rapid test kits, slides, HemocueHb 301, and sample transporting system was

Table 1: Measurement and study Variables

Variable name	Variable definition	Variable measurement
Age	Age in completed years	Discrete completed years
Educational level	Educational level reached	Nominal
Marital status	Current marital status	Nominal
Socio-economic status variables	Socio-economic status of study subject	Discrete
ITN	Ownership and sleeping under it	Dichotomous; yes No
Occupation	Professional activity	Nominal
Indoor residual spray (IRS)	Use of indoor residual spray	Nominal
Age of the pregnancy	Current Trimester of the pregnancy	Ordinal
Parity	The number of live born children a woman has delivered	Discrete
Plasmodium species	Types of plasmodium species	Nominal
Geophagy	Habit of eating soil	Dichotomous; yes No
Walking on bare foot	Walking on bare foot	Dichotomous; yes No
Night soil	Using human feces as fertilizer	Dichotomous; yes No
Stagnant water	Presence of stagnant water around house	Dichotomous; yes No
Pregnancy status	Pregnancy status of participant women	Dichotomous; yes No

checked by experienced laboratory professionals and principal investigators. The specimens also checked for a serial number, quality, and procedures of collection. The laboratory professionals who involved in the Kato-Katz concentration technique, RDT, light microscopy examination of malaria and HemocueHb 301 hemoglobin determination had trained before engaged. Also, to minimize missed parasite identification and discrepancy each microscopic slide was examined by the two trained professionals in the jimma Arjo health center medical laboratory room. The rapid test kit was checked for an expiration date, correct collection procedures and samples as well as inbuilt control appearances. Inconsistent results of light microscopy checked again to confirm the findings.

Ethical considerations

This research was conducted after receiving ethical clearance letter from Wallaga University, institute of health science, institutional review board. Permission from the community was sought before initiating the study by communicating the responsible zonal and district administrative offices through official letters from Wallaga University. Community agreement and local oral consent obtained from village leaders through meetings with villagers. Individual informed oral and written consent sought from each pregnant and non-pregnant woman in the local language, Afaan Oromoo, for all who cannot write and read and literate women, respectively. To ensure confidentiality; interviews was conducted privately, each participant was assigned a unique identification number for anonymity of biological samples, questionnaires, and result record forms kept confidential and used only for the research purpose. Result of participants with parasitic infections and anaemia was sent to nearby health facilities for treatment and medical consultation.

RESULTS AND DISCUSSION

Socio-demographic and obstetric characteristics of respondents

A total of 582 reproductive-aged women comprising 291 pregnant women and 291 non-pregnant women were participated in the study. Out of 291 pregnant and 291 non-pregnant women who provided blood samples, 278 and 274 also provided stool samples, respectively.

The mean age of the study participants was 25.0 years (SD=5.8) for pregnant women and 25.3 years (SD= 6.4) for non-pregnant women, which has no statistical mean age significance difference with a p-value of 0.59. Among the pregnant women, 29.6% (86) were found in the age range of 15-20 years and also the majority of 33.3% (97) non-pregnant found in the same age group.

About 81% of the study participants' occupation was a farmer in both groups. The majority of the participants pregnant women attended primary education (52.2%), and (37.5%) had no formal education**.** Similarly, 48.8% and 37.8% of non-pregnant women had primary education and no formal education, respectively. All pregnant women were married whereas 93.1 % of non-pregnant women were married and 3.8 % were divorced. The gravidity of participants pregnant women were multigravidae 60.5 %, secundigravidae 16.2%, primigravidae22.7%, and the remaining were nulligravidae whereas 45%, 18.6%, 24.4%, and 12.0% for non-pregnant women respectively. During the study,

Table 2: Socio-demographic and obstetric characteristics of the study participants' in JimmaArjo district, East Wallaga zone, from September-November 2023.

Variables	Category	Pregnant women		Non-pregnant women	
		No.	%	No.	%
Residence	Urban	104	35.7	104	35.7
	Rural	187	64.3	187	64.3
Age	15-20 years	86	29.6	97	33.3
	21-25 years	86	29.6	81	27.8
	26-30 years	78	26.8	65	22.3
	31-35 years	30	10.3	29	10.0
	36-40	8	2.7	14	4.8
	41 and above	3	1.0	5	1.7
Ethnicity	Oromo	287	98.6	284	97.6
	Amhara	4	1.4	6	2.1
	Gurage			1	.3
Religion	Muslim	274	94.2	265	91.1
	Protestant	11	3.8	10	3.4
	Orthodox	6	2.1	16	5.5
Occupation	Farmer	236	81.1	237	81.4
	House wife	12	4.1	4	1.4
	Government work	14	4.8	21	7.2
	Student	1	.3	3	1.0
	Business man	28	9.6	26	8.9
Education	No formal education	109	37.5	110	37.8
	Primary school	152	52.2	142	48.8
	Secondary school	14	4.8	17	5.8
	Above secondary school	16	5.5	22	7.6
Marital status	Married	291	100.0	271	93.1
	Separated			5	1.7
	Divorced			11	3.8
	Widowed			4	1.4
Gravidity	Nulligravidae	2	.7	35	12.0
	Primigravidae	66	22.7	71	24.4
	Secundigravidae	47	16.2	54	18.6
	Multigravidae	178	60.4	131	45.0

45.7%, and 15.8% pregnant women were at their 3rd and 1st trimester, respectively.

About 87.6 % of the participant women live in corrugated iron sheet houses and 11.7 % of them dwelling in a thatched house. The main floor of most participant women's house was earth 545 (93.6%) and with 96% mud wall. All study participants reported they do have pit types of the latrine. One hundred twenty (20.6%) study participants were living in an area where there is stagnant water. Four hundred fifty-two (77.7%) of the women's house were sprayed with insecticides. ITN coverage in participant women in the study area was 519 (89.2%). However, Out of 519 only 88.8% of women was using ITN during the study for themselves. (Table 1)

Prevalence of malaria-soil transmitted helminthesco-infection

The prevalence of malaria-soil transmitted helminthes co-infection separately for pregnant and non-pregnant women was 17/278 (6.1%), and 10/276 (3.6%), respectively. The total prevalence of malaria infection and soil transmitted helminthes infection was 37 (12.7%) and 41 (14.7%), respectively among pregnant women. In contrast, the presence of malaria parasites, and soil transmitted helminthes infection among non-pregnant women was 49(16.8%) and 31(10.2%), respectively. From the total of 86 *Plasmodium*-infected study participants, 85(98.8%) were found positive for *P. falciparum* and only 1(1.2%) were *P. vivax*. Outof the microscopically confirmed malaria parasite cases, 96.5% (83/86) had trophozoites stage only and 3.4% (3/86) cases had both trophozoites and gametocytes. On RDT confirmed cases 98.8% (85/86 were positive for *HRP2* and also this was concordant with microscopically confirmed *P. falciparum*, while only one case was *PLDH* positive and concordant with *P. vivax* detected with microscopy. So during malaria parasite blood film investigation, there were no discordant results, i.e., there were no differences between the two laboratory technicians in species diagnosis, or the presence of parasites and in parasite load of >50% discrepancy.

Malaria parasitic infection was higher among non-pregnant women (16.8%) compared to pregnant women (12.7%). However, this difference was not statistically significant with X^2=1.651 at P=0.199. Reversely malaria-soil transmitted helminthes co-infection was higher in pregnant women 17(6.1%) compared to 10 (3.6%) in

Table 3: Prevalence of malaria-soil transmitted helminthes co-infection by pregnancy status in JimmaArjo district, September-November, 2023

Pregnancy status	Number of participants	Co-infection
Pregnant	278	17(6.1%)
Non-pregnant	276	10(3.6%)
Total	554	27(4.9%)

Table 4: *Plasmodium* parasite density among *malaria*-mono-infected and *malaria* -soil transmitted helminthes–co-infected pregnant and non-pregnant women inJimmaArjo, Southwest Ethiopia, from September to November 2023.

	Type of infection	Mean(±SD) parasitaemia	95%CI	Anova(one-way)
Pregnant women	*Plasmodium* mono-infected(15)	3008.00±2091.84	1849.57, 4166.42	F=16.14 P=0.0001
	Plasmodium-soil transmitted helminthes co-infected(17)	5872.94±1941.55	4874.68, 6871.19	
Non-pregnant	*Plasmodium* mono-infected(25)	5678.40±2534.33	4632.27, 6724.52	F=12.48 P=0.001
	Plasmodium-soil transmitted helminthes co- infected(10)	9086.40±2692.05	7160.61, 11012.18	

Note**: SD: standard deviation; CI: confidence interval.**

non-pregnant women. The mean age (±SD) of the study participants was 25 (±4.8) years and 25.5 (±7.5) years in pregnant and non-pregnant women respectively for malaria-soil transmitted helminthes co-infected patients.

The highest prevalence of malaria-soil transmitted helminthes co-infection 10 (37%) occurred among the age group of 21-25 years while the age group 36-40years had the lowest prevalence of 1(3.7%). The difference was not statistically significant with X^2=1.49 at P value 0.914. Pregnant women in their 3$^{rd.}$trimester had the highest prevalence of co-infection of 9(33.3%) while those in their 1st trimester recorded 2(7.4%). The difference in the prevalence of co-infection according to trimesters was not statistically significant(X^2=0.428 at p=0.807).

Among 554 participant women who provided stool samples and fulfilled inclusion criteria for soil transmitted helminthes detection with Kato-katz techniques, the overall intestinal helminthic infected study women was 174 (31.4%). The most frequent intestinal helminths identified were *S. mansoni* 73 (42%) followed bysoil transmitted helminthes 68 (39%), *E. vermicularis* 17 (9.8%), and *A. lumbricoides* 4(2.3%). The remaining participants had two or more intestinal helminthic infections which were mixed *S. mansoni*and *E. vermicularis* 8(4.6%), *S. mansoni*and soil transmitted helminthes 3(1.7%), and soil transmitted helminthes and *E. vermicularis* 1(0.6%). Out of 174 participant women infected with intestinal helminths; 97 (55.7%) and 77(44.3%) were pregnant and non-pregnant women respectively. Among the pregnant women, *S. mansoni 48(17.3%),* soil transmitted helminthes 41(14.7%), *E. vermicularis*9(3.2%),and *A. lumbricoides*4(1.4%), N=278were identified in the stool samples. Whereas, *S. mansoni 38*(13.8%), soil transmitted helminthes 31(11.2%)*, E. vermicularis 17*(2.5%), N=276, were also identified in the stool samples from the non-pregnant women. The prevalence of intestinal helminthic infection was higher in pregnant women than non-pregnant, However, it was not statistically significant different with X^2=2.64 at P=0.104.

From the total of 86 malaria-positive study participants, 40 were only malaria mono-infection and 27 were malaria-soil transmitted helminthes–co-infected patients. The remaining 19 malaria parasite infected patients were either not provided stool samples or concurrently infected with one or more intestinal helminths. The mean malaria parasites density in malaria-soil transmitted helminthes–co-infected patients was5872.94±1941.55 parasites/µl and 9086.40±2692.05 parasites/µl, respectively for pregnant and non-pregnant study subjects. But in malaria infection alone the mean density of malaria parasites was 3008.00±2091.85 parasites/µl and 5678.40±2534.33 parasites/µl respectively in pregnant and non-pregnant women. There were statistically significant differences higher mean parasite density of *Plasmodium* in malaria-soil transmitted helminthes co-infected than malaria-mono-infected in both pregnant and non-pregnant women (F=16.14, P<0.0001 vs. F=12.48, p=0.001) is shown in Table 4.

The highest mean malaria parasites density of 4938.46±2963.85 parasites/µl occurred among subjects in their 3rd trimester while the lowest mean parasite density of 4036.00±2088.72 parasites/µl of blood was observed among the participant in their 2nd trimester. The difference in the distribution of mean malaria parasite density according to the trimesters of pregnancy was not statistically significant (F=0.514 at p=0.603) data is not shown.

Table 5:Association of intestinal helminths with malaria parasite infection among pregnant and non-pregnant women of Jimma Arjo district, southwest, Ethiopia,from September to November2023

	Species of Intestinal helminths	Malaria Yes N (%)	No N (%)	Crude Odd ratio (95%CI)	Adjusted odd ratio (95%CI)	P-value
PREGNANT women	Soil transmitted helminthes					**0.001***
	Yes	17(41.5)	24(58.5)	7.68(3.55, 16.63)	7.83 (3.58, 17.12)	
	No	20(8.4)	217(91.6)	1	1	
	S. mansoni					0.686
	Yes	6(12.5)	42(87.5)	0.91(0.36, 2.33)	1.23(0.44, 3.38)	
	No	31(13.5)	199(86.5)	1	1	
	E.vermicularis					0.952
	Yes	1(11.1)	12(70.6)	0.80(0.09, 6.66)	1.06(0.12, 9.36)	
	No	36(13.4)	233(86.6)	1	1	
NOn-PREGNANt women	Soil transmitted helminthes					**0.014***
	Yes	10(32.3)	21(67.7)	2.85(1.24, 6.57)	2.44 (1.04, 5.71)	
	No	35(14.3)	210(85.7)	1	1	
	S. mansoni					0.166
	Yes	8(22.2)	28(77.8)	1.56(0.66, 3.70)	1.881(0.76, 4.59)	
	No	37(15.4)	203(84.6)	1	1	
	E.vermicularis					0.255
	Yes	5(29.4)	12(70.6)	2.28(0.76, 6.82)	1.95(0.61, 6.18)	
	No	40(15.4)	219(84.6)	1	1	

* Significant at P value < 0.05

The overall prevalence of soil transmitted helminthes in the study population was 13 % (72 of 554 subjects), with 70 subjects (97.2%) classified as having a light infection and only 2(2.8%) subjects were moderately infected and heavy infection was not found. The mean egg count of soil transmitted helminthes in malaria-soil transmitted helminthes co-infection was 290.82±168.83 EPG and 350.40±242.44 EPG among pregnant and non-pregnant respectively. However, in soil transmitted helminthes mono-infected it was 367.30±307.21 EPG and 214.80±124.21 EPG respectively among pregnant and non-pregnant. There were no statistically significant differences in mean egg intensity of soil transmitted helminthes among soil transmitted helminthes-mono-

NOTE: Data presented here included only 554 because women were excluded if not provided stool. After adjusted for education levels and dwelling kebeles

Association of malaria-soil transmitted helminthes co-infection with anaemia

From a total of 291 pregnant study participant screened for hemoglobin level, 29.6% (86/291) was anemic (hemoglobin level less than 11g/dl) whereas 25.8% of

infected and malaria-soil transmitted helminthes co-infected pregnant and non-pregnant women (p=0.36 vs. P=0.05) data is not shown.

As the intensity of soil transmitted helminthes ova increases, *Plasmodium* parasite density increases which is significant at r= 0.8 with a p-value of < 0.0001 among pregnant women, but not significantly correlated among non-pregnant women (P=0.157).

Soil transmitted helminthes infection significantly increased odds of malaria parasite infection[AOR: 7.83 (3.58, 17.12, p<.001)] and [AOR: 2.44 (1.04, 5.71) p=0.014]among pregnant and non-pregnant women respectively compared with no soil transmitted helminthes infection.(Table5)

non-pregnant women had anemia (hemoglobin level less than 12g/dl). Although mild anemia was commonly observed 49 (57%), moderate anemia 36(41.9%), and only 1 (1.2%) severe anemia (hemoglobin < 7 g/dl) was identified among pregnant women. While among non-pregnant women mild 58 (77.3%) and moderate 17(22.7%), respectively. The result shows anemia was more frequent in pregnant women related to non-pregnant. But the difference was not statistical significant (P=0.354).

Table 6: Association of anaemia with types of infection among participant women

Variables		Anemia		Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
		Yes	No			
Type of infection	Co-infection	10	17	8.3(3.5, 19.4)	7.9(3.4,18.2)	**0.0001***
	Malaria alone	18	22	3.8(1.9, 7.8)	3.8(1.9, 7.5)	**0.001***
	Soil transmitted helminthes alone	17	26	2.9 (1.5, 5.7)	3.0(1.5, 5.9)	**0.0001***
	Non-infected	60	280	1	1	
Parity	Not at all	30	74	1	1	
	Once	27	91	0.7(0.7,2.4)	1.2(0.6,2.6)	0.353
	Twice	28	78	1,2(0.6,2.2)	1.1(0.5,2.3)	0.786
	Three	26	44			
	Four and above	50	134	1.9(1.0,3.8)	1.9(8.7,4.1)	0.575
				1.2(0.7,2.1)	0.9(0.4,1.8)	0.067
Pregnancy status	Pregnant	86	205	1.2(0.8,1.9)	1.2(0.7,1.9)	0.347
	Non-pregnant	75	216	1	1	
Age	Age of participants			0.9(0.9,1.2)	1.0(0.4,2.7)	0.682

*** Significant at P value < 0.05**

The mean hemoglobin level in malaria-soil transmitted helminthes co-infected, malaria mono-infected, and soil transmitted helminthes mono-infected participant women were9.8g/dl, 10.8g/dl, and 10.3g/dl, respectively among pregnant women. Whereas, among non-pregnant it was 11.5g/dl, 12.0 g/dl, and 12.4g/dl respectively. The mean hemoglobin level difference was not statistically significant among malaria-soil transmitted helminthes co-infected, malaria mono-infected and soil transmitted helminthes mono infected participant pregnant, and non-pregnant women (P>0.05).

Anemia was found more in the third trimester but this relationship was not significant (P=0.958). The highest mean Hb levels of 11.9±1.7g/dl occurred among the pregnant women in their 2nd trimester while the lowest mean Hb levels of 11.7±1.6g/dl were, observed among the subjects in their 3rd trimester. However, the difference in the mean Hb was statistically not significant (F=0.225 at p=0.79). Amongst non-pregnant subjects, the highest mean Hb level of 13.7±1.5g/dl was observed among the subjects in 36-40 years while the lowest mean Hb of 12.7±1.8g/dl was observed among subjects in 31-35 years. The difference was not statistically significant (F=1.09, p=0.364).

The odds of anemia among study participants infected with malaria-soil transmitted helminthes co-infection, were 8 times higher than women who had no parasitic infection [AOR:7.9, 95.5% CI: (3.4-18.2)]. The odds of anemia among women infected with malaria alone was four times [AOR: 3.8, 95% CI: 1.9-7.5)] than non-infected participant women. Soil transmitted helminthes mono-infection increases odds of anemia by 3 times [(AOR: 3.0, 95% CI: 1.5, 5.9)], compared to non-infected women after adjusting for pregnancy status, age and, parity (Table 6)

Associated factors for malaria-soil transmitted helminthes co-infection

Variables with P-value <0.25 were entered into the multivariable logistic regression model.These variables were a combination of variables (walking barefoot and non-usage of IRS, walking barefoot and non-usage of ITN, walking barefoot and presence of stagnant water nearby house), for malaria-soil transmitted helminthes infections have not co-exposure. That is their mode of transmission is very different. For this reason, after analyzed for each parasite separately we had found an association of walking barefoot for soil transmitted helminthes infection while non-usage of ITN, non-usage of IRS, and presence of stagnant water nearby house for malaria. Then by computing their combination we analyzed for their co-infection.

On a multivariate logistic regression analysis (Table 7), the variables associated with malaria-soil transmitted helminthes co-infection in order of their strength of association includes a combination of (habit of walking barefoot and non-usage of ITNs [AOR=5.0(95% CI 1.73, 14.81), P=0.003], habit of walking barefoot and non-usage of IRS AOR= 4.0(95% CI 1.60, 10.23), p=0.003], habit of walking barefoot and presence of stagnant water nearby participant's house [AOR= 3.2(95% CI 1.24, 8.70), p=0.017], and being pregnant [AOR= 2.8 (95% CI 1.02, 7.61), p=0.044] were significantly increases odds of co-infection after adjusting for participant dwelling

Table 7: Factors associated with malaria-soil transmitted helminthes co-infection among women of reproductive age (pregnant and no-pregnant women) in the Jimma Arjo woreda, from September-November 2023

Variables		**Malaria-soil transmitted helminthes Co-infection**		**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**P-value**
		Yes	**No**			
		N (%)	**N (%)**			
Walking barefoot + presence of stagnant water	Yes	12 (15.2)	67 (84.8)	5.4 (2.46, 12.23)	3.2 (1.24, 8.70)	**0.017***
	No	15 (3.2)	460(96.8)	1	1	
Walking barefoot + non-user of IRS	Yes	13 (14.0)	80 (86.0)	5.1 (2.35, 11.44)	4.0 (1.60, 10.23)	**0.003***
	No	14 (3.0)	447(97.0)	1	1	
Walking barefoot + non-user of ITNs	Yes	8 (18.6)	35 (81.4)	7.4 (2.93, 19.03)	5.0 (1.73, 14.81)	**0. 003***
	No	14 (3.0)	458(97.0)	1	1	
Being pregnant	Yes	17(6.1)	261(96.4)	1.7 (0.77, 3.85)	2.8 (1.02, 7.61)	**0. 044***
	No	10(3.6)	266(93.9)	1	1	

Key: CI; confidence interval; AOR Adjusted odds ratio; N=number; * Significant at P value < 0.05

kebeles and parity.

DISCUSSION

The current study revealed pregnant women were more susceptible to malaria-soil transmitted helminthes co-infections than non-pregnant women with a prevalence of 6.1% and 3.6%, respectively. The prevalence of malaria-soil transmitted helminthes co-infection and anemia was higher among pregnant women; this might be due to normal physiological and immunological changes during pregnancy. This observation was similar to that reported 7.7% prevalence of malaria-STHs co-infection where soil transmitted helminthes was the most prevalent in pregnant women, Gilgel Gibe dam Area, Southwest, Ethiopia (51), 6.8% malaria-STHs co-infection among pregnant women in Kenya (85) and, 5% among pregnant women in Osun State, Nigeria (66). It was lower than 13% and 6% prevalence of malaria-soil transmitted helminthes co-infection respectively for pregnant and non-pregnant women in, Nigeria(63), and 10% in Southern Ethiopia (86). But our finding was higher than 3% reported in Calabar, Nigeria (62), and, no cases of co-infection and 1.3%, respectively for pregnant and non-pregnant in Kumasi, Ghana (87). The highest prevalence of co-infection obtained in this study also consistent with another study that showed the same finding in Nigeria (65). The differences among these studies may be in respect to endemic city levels of parasites, and also most of them conducted on malaria-STHs co-infection rather than specifically for malaria-soil transmitted helminthes co-infection.

Contrary to malaria-soil transmitted helminthes co-infection, malaria prevalence was higher among non-pregnant than pregnant women in this study i.e. 16.8% and 12.7%, respectively. But this was not statistically significant. This result contradicts with a study that shows the prevalence of malaria infection was higher in pregnant women 12.6% compared to non-pregnant women (6.6%)in Ghana (87).But the prevalence of malaria was similar to the results observed among pregnant12.72% among pregnant women in Ethiopia (53), 11.6% in the Gilgel Gibe dam Area (51), and 12.6% in Ghana (87). This may be even though pregnant women more susceptible to malaria infection, susceptibility only could not sufficient to cause infection. The reason might be a difference in exposure status; awareness of how to prevent and proper use of control tools between pregnant and non-pregnant women. For most pregnant women follow antenatal service periodically they are expected to have updated awareness of how to prevent mosquito bites.

Our study found statistically significant differences in the higher mean parasite density of malaria parasites between malaria-soil transmitted helminthes co-infected and malaria-mono-infected pregnant women and non-pregnant women (F=16.14, P<0.001 vs. F=12.48, p=0.001). These findings in line with the study reported the mean malaria parasite load was significantly higher in malaria-STHs–co-infected patients than in patients infected with only malaria parasites in Ethiopia (64).

In this study, we found the presence of soil transmitted helminthes infection was significantly associated with increased odds of malaria parasitic infection in both pregnant and non-pregnant women [AOR: 7.83 (3.58, 17.12, p<0.001)] and [AOR: 2.44 (1.04, 5.71) p=0.014] respectively. The association of soil transmitted helminthes infection with malaria shown by this study was consistent with previous studies that suggest soil transmitted helminthes infection increases susceptibility for malaria (28, 30). Another study showed pregnant women who had any intestinal helminth infection were almost five times as likely to be infected with *P. falciparum* compared with women with no worm infection(55).However, it is not in agreement with another study that reported no association was found between malaria and soil transmitted helminthes infection, which may be due to the spatial overlap of both parasites in Africa as suggested by Shapiro et al (88).

Additionally, whether these differences are only due to immunological factors, or, as hypothesized elsewhere, soil transmitted helminthes-related anaemia leads to increased attractiveness for the vector, need to be further study (8).

Therefore the association of soil transmitted helminthes infection with increased prevalence of malaria observed by this study may be due to alteration of the immune response by down regulating the Th1 pathway toward an anti-inflammatory and type 2 responses both by soil transmitted helminthes infection and during pregnancy. Furthermore, their biological mechanisms of association require further study to reach on the same conclusion.

Also this study found as the intensity of soil transmitted helminthes ova increases, malaria parasite density increases which were significant at r=0.8 with a p-value of < 0.0001 for pregnant women which in line with other studies in Ethiopia (64, 89). However, no similar relation was found among non-pregnant women (P=0.157) in our study. This finding indicates soil transmitted helminthes infection may result in severe malaria if not early diagnosed and treated, especially in pregnant women. Therefore this finding implies treating soil transmitted helminthes infection early might have double impact protection from soil transmitted helminthes's direct morbidity and may be it impacts on severe malaria infection.

The prevalence of anemia in this study was 29.6% among pregnant and 25.8% in non-pregnant women is similar with WHO report for pregnant and slightly greater in non-pregnant i.e., the worldwide prevalence of anemia was 30% in pregnant women and 20% in non-pregnant women, with higher levels in women living in developing countries (90). However, these results contradicted with the higher prevalence of 83%, and 66% of the pregnant and non-pregnant women respectively reported from Nigeria (91) and 34.6% of anemia associated with asymptomatic malaria among pregnant women in the rural surroundings of Arba Minch Town (76). This variation may be due to differences in demographic, immune status, and lifestyle of study populations.

This study indicated that malaria-soil transmitted helminthes co-infection was associated significantly [AOR: 7.9, 95.5% CI: (3.4-18.2)] higher anaemia prevalence than malaria parasites [AOR: 3.8, 95.5% CI (1.9, 7.5) and/or soil transmitted helminthes alone [AOR: 3.0 95.5% CI (1.5, 5.9). This indicates the frequency of anaemia is further aggravated by the additive effect of malaria-soil transmitted helminthes co-infections in this study was consistent with another study that revealed malaria-soil transmitted helminthes co-infection greatly increase the odds for anaemia (92). Another study reported co-infection with geohelminthes and malaria parasites were four times likely to have anaemia compared with those infected with geohelminthes or malaria parasites alone where co-infection of *Necatoramericanus*and *P. falciparum* was associated with maternal anaemia(55). A study in Kenya also in line with our findings showed women who had malaria parasites alone were 3-fold likely to have anaemia and who had co-infected with geohelminthes and malaria parasites were 4-fold likely to have low haemoglobin levels compared to those infected with either geohelminthes or malaria parasites (85). In contrast to our finding study conducted on malaria-helminth co-infection in Southern, Ethiopia show a higher prevalence of anemia in co-infected participant patients (93).

The present study found odds of having anemia higher in malaria-mono-infected than soil transmitted helminthes-mono-infected women (AOR 3.8, 95% CI: 1.9-7.5) and (AOR: 3.0, 95% CI: 1.5, 5.9), respectively. This implies odds of causing anemia were slightly higher among malaria alone than soil transmitted helminthes-infected women which in line with the study suggested, the odds for having anaemia in pregnancy for malaria than soil transmitted helminthes infections (92). The finding of this study showed mean hemoglobin level in participant women infected with *Plasmodium*-soil transmitted helminthes co-infected was lower than malaria and soil transmitted helminthes mono-infected women although not statistically significant which in agreement with studies in Ethiopia (93), Nigeria (65). However, it contradicts with higher mean hemoglobin reported among Children aged 8 years who had *P. falciparum*-soil transmitted helminthes co-infection (94).

At the most basic level, exposure to parasites and infection is a spatial problem. This is because hosts and parasites must come into contact for infection to occur. Indeed, co-exposure is a necessary pre-condition for co-infection. However, malaria and soil transmitted helminthes infections have very different modes of transmission (no co-exposure at all). Thus we combined associated factors found for each infection together to analyze their association with co-infection. We found walking barefoot plus (non-usage of ITNs,non-usage of

IRS, presence of stagnant water nearby participant's house), and being pregnant increases odds of malaria-soil transmitted helminthesco-infection.

This implies women who had a habit of both walking barefoot and not using ITNs and/IRS and/or presence of stagnant water nearby house were at a risk of this co-infection. As early recognized stagnant water (89) is a favorable environment for the breeding of mosquitos. Including this also not using ITNs and IRS (44) were associated with increasing malaria infection. Walking barefoot suggested risk factor for soil transmitted helminthes infection (43). Also we found being pregnant increased the odds of co-infection. This may be due to immunological factors during pregnancy and/or soil transmitted helminthes interaction with malaria parasites (49).

CONCLUSIONS

This study illustrates higher prevalence of malaria-soil transmitted helminthes co-infection among pregnant women than non-pregnant women in study area that associated with increased odds of anaemia. The study also revealed positive correlation of soil transmitted helminthes eggs load with *plasmodium* parasites density which might result in severe infection particular among pregnant women that cause adverse maternal outcome. Additionally, we found soil transmitted helminthes infection associated with increased odds of malaria parasite infection in women irrespective of their pregnancy status.

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