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Prevalence of asymptomatic *Plasmodium falciparium* and *Plasmodium vivax* malaria carriage among school children of malaria endemic areas of Mirab Abaya district, Southern Ethiopia

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Asymptomatic malaria parasitemia has been reported in areas with high malaria transmission. Asymptomatic malaria carriers may play a significant role as an infection reservoir. Malaria elimination program have also faced challenges due to these parasite carriers and they should be considered in malaria-control programs in endemic areas for successful transmission interruption. The aim of the current study was to determine the prevalence of asymptomatic *Plasmodium falciparium and Plasmodium vivax* malaria among school children in malaria endemic areas of Mirab Abaya District, Southern Ethiopia. A cross sectional study design was employed from December 2014 to February 2015. A total of 422 school children aged 6 to 15 years were recruited using simple random sampling for this study, and blood samples were collected from asymptomatic school children residing in Mirab Abaya district kebeles. Malaria parasitemia was examined by using light microscopy and rapid diagnostic test (RDT). Asymptomatic malaria carriage was evaluated with the socio-demographic characteristics of the study participants. The data were analyzed using SPSS version 20 software. In this study, the prevalence of asymptomatic *Plasmodium* carriage was 1.2 and 3.6% with light microscopy and RDT, respectively. The overall prevalence of asymptomatic *Plasmodium* carriage (*P. falciparium* and *P.vivax*) were 15 (3.6%) (95%CI: 1.8-5.5). Of all *Plasmodium* carriage, 11 (73.4%) school children had *P. falciparium* and 4 (26.6%) had *P. vivax* infections. The prevalence of asymptomatic *Plasmodium* carriage (both in *P.falciparium* and *P.vivax*) did not correlate with gender and age group of school children in this study. The study revealed that the prevalence of asymptomatic *Plasmodium* malaria carriage is low. The result also indicates the ability of RDT to detect more asymptomatic *Plasmodium* malaria than microscopy. Therefore, treatment of asymptomatic carriers is very important and persistent malaria prevention and control strategies should be enhanced to achieve the elimination program, in endemic malaria areas.

Key words: Asymptomatic malaria, light microscopy, rapid diagnostic test, *Plasmodium falciparium, Plasmodium vivax*.

INTRODUCTION

Malaria is caused by a protozoan belonging to the genus, Plasmodium with five species: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi* infect humans

(WHO, 2012). Globally, an estimated 3.3 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk to acquire the disease. The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, which account for 78% of all deaths (WHO, 2014). The parasite burden is also highest in school age children due to low coverage of interventions, and infections may have consequences in school performance (Stevenson et al., 2013) and indicate school children as good proxy for transmission in a wide community (Stevenson et al., 2013).

As the new report indicates, the prevalence of malaria parasite infection, including both symptomatic and asymptomatic infections, has decreased significantly across sub-Saharan Africa since 2000. In sub-Saharan Africa, average infection prevalence in children aged 2–10 years fell from 26% in 2000 to 14% in 2013, a relative decline of 46% (WHO, 2014).

The clinical manifestation of *Plasmodium* infection varies from asymptomatic to severe and fatal malaria in endemic areas. On the other hand, asymptomatic infections can be associated with high levels of gametocytes, and likely serve as an important parasite reservoir, and it has a significant contribution by maintaining parasite for the transmission (Makanga, 2014).

Continuous *Plasmodium* parasites exposures makes to individual to produce partial immunity in high transmission areas (Kun et al., 2002) and it also creates asymptomatic carrier state to play a role for the persistence of malaria transmission in a given population (Stevenson et al., 2013; Staalsoe and Hviid, 1998). The asymptomatic malaria patients play critical role in the concept of malaria elimination program and it is a big challenge for the management of the elimination programme in any malaria endemic areas.

School-age children with malaria parasitemia do not have any symptoms because they have acquired some immunity. On the contrary, since young children with naive immune systems and pregnant women with potentially compromised immune systems are particularly vulnerable to this disease and so are considered to be the highest risk populations for malaria-related deaths. Malaria also mostly affect children in highly endemic areas with stable malaria transmission (Molineaux et al.,1998). Other study has indicated that asymptomatic infections can contribute to anemia and impairment of cognitive development in children (Nankabirawa et al.,2014).

Malaria is a major public health problem and it is estimated that about 75% of the landmass of Ethiopia is malarious and 68% of the Ethiopian population, estimated at about 54 million live in malaria risk areas in 2010 (FMH,2010). Annually, approximately 4-5 million cases of malaria. *P.falciparium* and *P.vivax* are also the two most dominant malaria parasites in Ethiopia. They are prevalent in all malarious areas in the country with *P.falciparium* representing about 65 to 75% of the total reported malaria cases, relative frequency varying in time and space within a given geographical range. Prevalence of 20.5, 6.8 and 9.1% were reported among different study groups from east shewa (Haji, 2016), Sanja (Ligabaw et al.,2014) and Arba Minch town (Nega et al., 2015). Malaria outbreak was reported in some regions (Beffa et al.,2015).

Currently, the prevalence of malaria infection is declining even in high transmission areas with different prevention and control strategies. So, for a successful malaria elimination program study of parasite carriers, especially asymptomatic malaria is an issue to interrupt the transmission in a population. It is even more important to assess the situation of the malaria elimination and eradication measures. Therefore, this study aimed to determine the prevalence of asymptomatic malaria carriage among school children in the endemic areas of Mirab Abaya district, Southern Ethiopia.

MATERIALS AND METHODS

Study setting and periods

The study was conducted in Mirab Abaya district from December 2014 to February 2015. Mirab Abaya district is located in the Southern Nations, Nationalities and Peoples Region (SNNPR), in Gamo-Gofa zone, and is divided into 24 Kebeles (the smallest administrative units in Ethiopia), one urban and 23 rural. It has three major agro-ecologies: *dega* (high land), *woina dega* (mid-altitude) and *kolla* (low land, an altituide below 1220 asl). Out of the 24 Kebeles, 16 are in the *kolla* agro-ecology, six are in the *dega* agro-ecology and the other two are inthe *woina dega* agro-ecology. In the *kolla*, average annual rainfall ranges from 1,000 to 1,100 mm. Malaria is the most prevalent and cause of morbidity disease in *kolla* agro-ecology of the district (District Health Office Report). Birbir, Molle, Koyte, Yayeke and Algae kebeles are among the 16 *kolla* agro ecology kebeles and the primary schools also named based on the kebeles of the district.

Study design and population

A cross-sectional study was carried out in five primary schools of Mirab Abaya district school children aged between 5 and 15 years. The data were collected with questionnaire to assess the asymptomatic status of the children. Capillary blood sample were collected using finger prick to determine the prevalence of asymptomatic malaria using light microscopy and CareStart™

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Malaria Pf/Pv Combo Rapid Diagnostic Test (RDT) (Access Bio, Inc., New Jersey, USA).

Sample size and sampling technique

Sample size was calculated using single population proportion formula and considering the following assumption. The prevalence level of asymptomatic malaria was taken as 50% since there was no similar study in the school children of malaria endemic areas in the country and there were highly varied prevalence reports from other African countries, 95% confidence interval, 5% marginal error and a non response rate of 10%. Finally, a total of 422 children were included in the study from 5 primary schools of district kebeles. Five kebeles were randomly selected among the malaria endemic kebeles of the district and total number of school children was also proportionally recruited based on the number of students in selected kebeles primary schools. Study participants were allocated from each grade level considering their age using simple random sampling method. All *Kolla* kebeles primary schools are situated along the same route between 2 and 20 km far differences each other and the study participants homogeneity were considered in sample size determination.

Data collection procedures

School children were identified with previous history and clinical examination without having any malaria symptoms including chill, fever and sweating in order to evaluate asymptomatic *Plasmodium* infection according to questionnaire by professional nurses. After taking an informed consent, school children whose axillary body temperature was ≤37.5°C and at the age group between 6 and 15 were selected to be included as the study participant. Students treated with anti-malaria drugs, recently enrolled students from other district schools, dega kebeles dwellers and students with common malaria symptoms were excluded from the study. Finger-prick blood sample was collected from each students using heparinized capillary tube for RDT. A thick and thin blood smears were also prepared for the microscopic examination for all study participants.

Blood smear examination for malaria parasites

Thick and thin blood smears were prepared according to a standard method. The smears were air-dried, and then only the thin film was fixed using methanol. All slides were stained with 10% Giemsa and examined at 100x magnification with oil immersion for detection of malaria parasites by a trained microscopists. A total of 100 microscopic fields were read by the microscopists according to WHO protocol of malaria examination to reach a decision (WHO, 2010).

Quality control

All laboratory materials such as rapid test kits, slides, thermometers and sample transporting system were checked by experienced laboratory professionals. The specimens were also checked for serial number, quality and procedures of collection. The laboratory professionals involved in RDT and light microscopy examination were trained in malaria diagnosis and quality assurance training. In addition, to minimize missed parasite identification and discrepancy each microscopic slide was examined by the two trained professionals in Arba Minch College of Health Sciences, Medical laboratory centre. Rapid test kit was checked for expiration date, correct collection procedures and samples as well as in built control appearances. Inconsistent results of light microscopy were checked again to confirm the findings.

Data analysis

The data were analyzed using SPSS version 20.0 (IBM Corporation, USA). During data collection, completed results were checked regularly to rectify any discrepancy, logical errors or missing values. To describe data, mean and standard deviation for continuous variables and proportion for categorical variables were computed. The level of statistical significance was set as $p \leq 0.05$ and for each statistically significant factor, chi-square and 95% confidence interval (CI) was also computed.

Ethical consideration

The college research review committee revised the paper according to the rule and regulation. Accordingly, the study was approved by the ethics committees of South Nation Nationalities Peoples Regional Health Bureau Research and Technology Transfer Support Process Office (AMH-CHS-RPO-7/2014). Mirab Abaya Health office and the Educational office administrative authorities at district level were informed about the study and their consent was obtained with the letter. Participation was fully voluntary and informed written consent was obtained from school directors, each study participants and guardians of the selected children. Confidentiality of the collected information and laboratory test results was maintained. Children with positive result in microscopic examination and/or rapid test received standard anti-malarial drugs in accordance with standard treatment guideline of Ethiopia with the health post health professionals.

Socio-demographic characteristics of school children

A total of 422 school children aged between 6 and 15 years with approximately equal number of male (50.7%) and female (49.3%) were recruited for this study. Among the study participants, 160 (37.9%) age distribution was between 6 and 10 years old and 262 (62.1%) age was between 11 and 15 years. The mean age (SD) of study participant was 11.42 (1.962) years. The largest study participants were recruited from Birbir kebele primary school which is the district setting, 110 (26.1%) and the smallest study participants were 51 (12.1%) from Algae kebele (Table 1).

Parasitological data

A total of 5 school children (1.2%) had *Plasmodium* species parasite detected by light microscopy: 2 (1.0%) were females and 3 (1.4%) were male. Overall, 15 school children (3.6%) infected with *Plasmodium* species were detected by RDT: 8 (3.8%) were females and 7 (3.3%) were males. Age group wise; 6 (3.8%) school children had *Plasmodium* species in their blood in ages group between 6 and 10 years old and 9 (3.4%) school children had the parasite between 11 and 15 years old. The overall prevalence of *Plasmodium* species (*P. falciparium* and *P.vivax*) parasite were 15 (3.6%) (95%CI: 1.8 to 5.5) (Table 2).

Prevalence of Plasmodium spp. infection by light microscope

Overall, 5 school children (1.2%) had plasmodium species parasite

Table 1. Socio-demographic characteristics of school children.

Socio-demographic characteristics	Number (n)	%
Sex		
Male	214	50.7%
Female	208	49.3%
Total	422	100.0%
Age groups		
6-10	160	37.9%
11-15	262	62.1%]
Mean age in years+(SD)	11.42(1.962)	
Primary schools		
Koyte	101	23.9%
Molle	97	23.0%
Algae	51	12.1%
Birbir	110	26.1%
Yayeke	63	14.9%
Total	422	100.0%

Table 2. Prevalence of asymptomatic malaria in school children by light microscopy and RDT, by gender and age group.

Baseline characteristics	Microscopic result		RDT result		Total Positive (n/%)	Total
	Negative (n/%)	Positive (n/%)	Negative (n/%)	Positive (n/%)		
Sex						
Female	206(99.0%)	2(1.0%)	200(96.2%)	8(3.8%)	8(1.95%)	208
Male	211(98.6%)	3(1.4%)	207(96.7%)	7(3.3%)	7(1.65%)	214
Total [n[%]	417(98.8%)	5(1.2%)	407(96.4%)	15(3.6%)	15(3.6%)	422
Age group						
6-10	158(98.8%)	2(1.2%)	154(96.2%)	6(3.8%)	6(3.8%)	160
11-15	209(98.9%)	3(1.1%)	253(96.6%)	9(3.4%)	9(3.4%)	262
Total [n[%]	417(98.8%)	5(1.2%)	407(96.4%)	15(3.6%)	15(3.6%)	422

Table 3. Prevalence of asymptomatic malaria species in gender and age groups by light microscopy and RDT in school children.

Characteristics	Species of plasmodium by RDT			Species of plasmodium by Microscopy		
	Negative	*P. falciparium*	*P. vivax*	Negative (n/%)	*P. falciparium*	*P.vivax*
Sex						
Female	200 (96.2%)	7 (3.4%)	1(0.5%)	206(99.0%)	1(0.5%)	1(0.5%)
Male	207 (96.7%)	4(1.9%)	3(0.7%)	211(98.6%)	0(0.0%)	3(0.7%)
Total	407 (96.4%)	11(2.6%)	4(0.9%)	417(98.8%)	1(0.2%)	4(0.9%)
Age group						
6-10	154 (96.2%)	4(2.5%)	2(1.2%)	158(98.8%)	0(0.0%)	2(1.2%)
11-15	253(96.6%)	7(2.7%)	2(1.2%)	259(98.9%)	1(0.4%)	2(1.2%)

detected by light microscopy: 4 (0.9%) was *P. vivax* and 1 (0.2%) was *P. falciparium* infection. No *P. falciparium* and mixed infection were observed in age group between 6 and 10 years old, while 2 (1.2%) *P.vivax* infection was found in both age groups. One (0.4%) was *P. falciparium* infection in age group between 11 and 15 years old (Table 3).

Table 4. Association of the prevalence of asymptomatic malaria species with gender and age groups of school children.

Parasite species	Sex of school children		Total n=422	X^2	P-value
	Female(n=208)	Male(n=214)			
P. falciparium	7	4	11(2.6%)	0.9	0.3
P. vivax	1	3	4	0.25	0.6
Total	8	7	15(3.6%)	0.10	0.7
	Age group of school children				
	6-10[n=160]	**11-15[n=262]**			
P. falciparium	4	7	11(2.6%)	0.012	0.9
P. vivax	2	2	4	0.95	0.3
Total	6	9	15(3.6%)	0.29	0.8

Table 5. Blood stages of plasmodium species parasite density in microscopic study of school children.

Characteristics	Number of parasite/µl			Total (n=5)	X^2	P value
	<500	500-999	>1000			
Sex						
Female	0	1	2	3		
Male	0	0	2	2		
Total	0	1	4	5	1.032	0.5
Age Group						
6-10	0	0	2	2		
11-15	0	1	2	3		
Total	0	1	4	5	0.859	0.6

Prevalence of *Plasmodium spp.* infection by RDT

Using RDT, 11 (2.6%) school children had *P. falciparium* and 4 (0.9%) had *P. vivax* infections. Among *P. falciparium* infected study participants, 7 (63.3%) were females and 4 (36.6%) were males. One (0.2%) female had also *P. vivax* and 3 (0.7%) males were infected with *P. vivax*. Four (2.5%) and 2 (1.2%) of the school children whose age group is between 5 and 10 years old had *P. falciparium* and *P. vivax* infection, respectively. Seven (2.7%) and 2 (1.2%) school children were also infected with *P. falciparium* and *P. vivax* in the age group of 11 and 15 years old. In this study of all plasmodium carriage, the prevalence of *P. falciparium* infection was 11 (73.6%) and *P. vivax* infection was 4 (26.6%) (Table 3).

Prevalence of plasmodium parasite association with gender and age group

Among the infected school children, the overall prevalence of *Plasmodium* species in females were 8 (53.3%) and in males were 7 (46.6%). In this study, the prevalence of *Plasmodium* species (both in *P. falciparium* and *P.vivax*) did not correlate with gender and age group of school children, respectively (p=0.7 & p=0.8). However, *P. falciparium* infection was higher (7; 63.6%) in females than in males (4; 36.3%). Similarly, school children whose age group was between 11 and 15 years had higher *P. falciparium* infection than age group between 6 and 10 years (Table 4).

Density of *Plasmodium* parasite

In this study, the observed parasitemia level was >1000 parasite/µl and only 1 individual had parasitemia level between 500 and 999 using light microscopy. Among light microscopy positive school children, 4 (80%) had >1000 parasite/ µl. There was no significant association in the level of parasitemia and sex and age group of school children (P>0.05) (Table 5).

DISCUSSION

The study of asymptomatic malaria cases of school age children has been given little attention in the prevention and control program. Understanding of the burden of asymptomatic malaria in school age children has great implication in the interruption of malaria transmission. As evidence has indicated that asymptomatic parasitemia can impair cognitive and cause anemia in the host, it has also an important implication in preventing parasitemia (Nankabirwa et al., 2014).

The aim of this study was to determine the prevalence of asymptomatic malaria carriage in school children of Mirab Abaya district, Southern Ethiopia. The study

revealed that the prevalence of *Plasmodium* carriage (*P. falciparuim* and *P. vivax*) was 1.2 and 3.6% with light microscopy and RDT, respectively. This prevalence was higher than other study of African school children using RDT (0%) (Strom et al., 2013). The result was consistent with the study on children in Dakar (Diallo et al., 2012), Tanzania (Nzobo et al., 2015) and Pakistan (Awan et al., 2012).

The prevalence was also lower as compared to studies done in Northern Ethiopia (Ligabaw et al.,2014), Cameroon (Tientche et al., 2016) and Ghana (Sarpong et al., 2015) in which prevalence of 6.8, 74.2 and 41.5% were reported, respectively. In this study, the lower *Plasmodium* carriage prevalence might be due to the sociodemographic factors, population movements and metrological conditions as well as malaria prevention and control strategies of the district using household sprays and distribution treated bed nets focusing on this endemic areas. Even though, the prevalence of asymptomatic malaria is low, there could be a potential for the transmission of malaria parasite due to the presence of plasmodium carriers in this study area.

A ten years of retrospective morbidity data of Mirab Abaya district revealed that malaria was highly endemic with a higher incidence in age group of between 2 and 80 years (Eskindir and Bernt, 2010). The prevalence of asymptomatic malaria parasite carriage was lower and far as compared to other endemic regions, however, there could be the chance to be infected with parasite and developing immunity against malaria parasite is common due to the frequent exposure in the endemic areas (Gudo et al., 2013). This malaria parasite prevalence variation might be due to the intensive prevention and control strategies to halt malaria transmission and climatic variations and the laboratory test used as well as other factors in this study area.

Prevalence of asymptomatic malaria carriage with gender and age groups was not observed in the present study. But other studies (Ligabaw et al., 2014; Golassa et al., 2015; Singh et al., 2014) have indicated that there are associations with the two variables. Females were more *Plasmodium* carriage in this study as compared to other study (Diallo et al., 2012) without statistically significant difference. In addition, high prevalence of asymptomatic malaria carriage was observed in age groups between 11 and 15 years than the lower school age groups without significant association. In contrast to this study, other studies (Nankabirwa et al., 2014; Ligabaw et al., 2014) showed that asymptomatic carriage decreases as the age increases with the development of immunity against parasite. Moreover, there is also research which showed adults develop immunity to malaria parasite with age and repeated exposure to mosquitoes vector (Ganguly et al., 2013). The present study difference as compared to the other studies might be the small number of asymptomatic cases detected in the study, the sampling techniques in the selection, the habit of exposure to risk factors in gender and the effect of the climate the school children are exposed with the parasite at the study sites.

In this study, prevalence of *P. falciparium* infection was predominant with statistically significant difference as compared to *P. vivax* infection using RDT. However, carriage of *P. falciparium* was higher than *P.vivax* using light microscopy without statistically significant difference. In agreement with the other study (Golassa et al., 2015), the detection of parasite antigen using RDT method was higher than in light microscopy. Light microscopy method showed lower detection ability as compared to RDT (Matangila et al., 2014). This could be due to lower parasitemia level in children detected by microscopy as compared to RDT.

Based on the findings of this study, light microscopy detected only school children whose parasitic densities are greater than 500 parasite/µl. No positive results were detected in school children whose parasitic densities were less than 500 parasite/µl using light microscopy. In agreement with other study, all light microscopy positive cases were detected by RDT (Golassa et al., 2015). The inconsistency of the result in the two methods might be due to the fact that the density of parasites in the blood of school children detected by light microscopy was lower as compared to that of RDT. Therefore, RDT is an alternative to screen asymptomatic malaria cases in the large community such as for kebeles dwellers and school children to treat the infected community and to enhance malaria prevention and control strategies to achieve malaria elimination program.

The present study also has its own limitations; the study did not include polymerase chain reaction (PCR) method to increase the chance of detecting the malaria parasite carrier. In addition, entomological assessments were not conducted on mosquitoes vector to detect the presence of parasite to support the impact of elimination strategies.

Conclusions

The study revealed that the prevalence of asymptomatic *Plasmodium* malaria carriage is low. The result also indicates the ability of RDT to detect more asymptomatic *Plasmodium* malaria than microscopy. Therefore, treatment of asymptomatic carriers is very important and persistent malaria prevention and control strategies should be enhanced to achieve the elimination program, in endemic malaria area.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations: RDT, Rapid diagnostic test; **PCR,**

polymerase chain reaction; **ITNs,** insecticide treated nets; **SNNPR,** South Nation Nationalities People Region.

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