Original Article

Plasmodium falciparum and Schistosoma mansoni coinfection and the side benefit of artemether-lumefantrine in malaria patients

Solomon M. Abay[1], Mulugeta Tilahun[2], Nigus Fikrie[2], Abiy Habtewold[1]

[1]*Department of Pharmacology, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*
[2]*Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*

Abstract

Introduction: The distribution of both malaria and schistosomiasis exhibits a large geographical overlap in tropical environments, particularly in sub-Saharan Africa. This part of the world currently harbours more than 85% of the estimated global burden of these diseases. Studies showed that artemisinin derivatives used for the treatment of malaria also have an antischistosomal effect. This study aimed to investigate the extent of malaria-schistosomiasis co-infection and the antischistosomal effect of artemether-lumefantrine when administered to treat *falciparum* malaria in Kemise, Northeast Ethiopia.

Methodology: Stool samples were collected from 152 microscopically confirmed malaria patients and diagnosed for schistosomiasis using the Kato-Katz technique before treatment. The schistosomiasis cure rate and egg reduction were determined in co-infected patients, who were treated with artemether-lumefantrine,.

Results: Twenty-eight out of 152 malaria patients were co-infected (18.4%, n = 152) with schistosomiasis. All 28 co-infected patients were found stool-negative for *Schistosoma mansoni* eggs four weeks after treatment. The extent of co-infection was associated with age, sex and educational level. Cure rate and egg reduction rate following the treatment of artemether-lumefantrine were 100%.

Conclusion: The co-infection rate was associated with patient characteristics. Artemether-lumefantrine was effective against *S. mansoni* in co-infected patient. Multicenter and randomized trials, however, are needed for a better understanding of the efficacy of artemether-lumefantrine against schistosome infection with ranges of intensity.

Key words: schistosomiasis and malaria co-infection; artemether-lumefantrine; *S. mansoni*; *P. falciparum*; Ethiopia

J Infect Dev Ctries 2013; 7(6):468-474. doi:10.3855/jidc.2658

(Received 21 March 2012 – Accepted 28 August 2012)

Introduction

Malaria remains a challenge to both public health and socio-economic development, particularly in sub-Saharan African countries [1]. In Ethiopia, an estimated 65% of the population is reported to be at risk of acquiring malaria infection [2]. In malaria endemic areas, co-infection with multiple parasites, including *Schistosoma* species, is common [3,4].

In Kemise, surveys have been conducted on intestinal parasite and malaria coinfection [5, 6]. Even though malaria and schistosomiasis have been geographically co-endemic in Kemise as they are in many places in sub-Saharan Africa, studies on the prevalence of schistosome and *P. falciparum* co-infection is still limited. The present study therefore attempts to report the level of malaria and schistosomiasis co-infection.

Praziquantel is the drug of choice for the treatment of schistosomiasis. Although no resistance to praziquantel has been described, low cure rates of schistosomiasis have been reported [7]. Isolates from the resistant infections in Egypt are less susceptible to praziquantel-induced tegumental damage *in vitro*, suggesting that the worms are in some way less responsive to the drug [8,9]. Studies controversially indicated that resistance to praziquantel may be emerging. Higher treatment coverage in the framework of the roll-out of the neglected tropical diseases control program are expended to increase evolutionary pressure and hasten the emergence of resistance in *Schistosoma* species populations [10].

Efforts have been made by drug researchers to obtain alternatives to praziquantel, and artemisinin derivatives have found to be active against *S. mansoni* using *in vivo and in vitro* tests [11,12]. In an explorative study in Sudan investigating the antischistosomal effect of artemether-lumefantrine in patients taking the drug for the management of

malaria, all the patients were found to be stool-negative for schistosome eggs after treatment [13].

In Ethiopia, artemisinin based combination therapy is the drug of choice for the treatment of uncomplicated *falciparum* malaria. Particularly, artemether-lumefantrine has been used in Ethiopia since 2006. In addition to malaria-causing plasmodium species, artemether has been proved to have activity against several species of schistosomes [14]. In areas where malaria and schistosomiasis are co-endemic, the artemisinins taken by malaria patients may also exhibit antischistosomal properties though little is known about the impact of the co-infection state on the drug response [15].

The aim of the present work was to investigate the extent of co-infection of *P. falciparum* malaria and schistosomiasis, and investigate the effect of artemether-lumefantrine against schistosomiasis in the co-infected patients attending Kemise Health Center. This study aimed to determine the side benefits of artemether-lumefantrine based therapy in *falciparum* malaria patients co-infected with *S. mansoni*.

Methodology

Study area and population

The study was conducted at Kemise Health Center in Kemise town, northeast Ethiopia. The town is in the Oromia Zone of the Amhara Region. Kemise is located at 325 km northeast of Addis Ababa, the capital. It has an altitude of 1424 m above sea level. The town has an estimated total population of 18,897, of whom 10,151 are males and 8,746 are females [15].

In Kemise, malaria transmission is seasonal and unstable [16]. The town is situated in an area where water collections are potential sites for the breading of anopheline mosquito larvae and infected snails, resulting in a high level of both malaria and schistosomiasis burden in the community.

Study subjects

Study participants, who fulfilled the inclusion criteria, were enrolled in the study for a follow-up period of one month. Patients above 5 years old, co-infected with *P. falciparum* and *S. mansoni*, were included in the study.

Study design

A prospective longitudinal study was conducted at Kemise Health Center, in Northeast Ethiopia during the high malaria transmission season, *i.e.* from September to November, 2009. The study was designed to determine the extent of *P. falciparum* and schistosomiasis confection and the effect of artemether-lumfantrine against *S. mansoni* in co-infected patients at a dosage regimen used for the treatment of *P. falciparum* malaria. As the study was non-comparative, randomization was not performed.

Sample size determination

The required sample size for the study was calculated using a formula for a single population proportion. We assumed 0.9% malaria prevalence [5]. Taking critical value at 95% confidence level ($Z\alpha/_2$ = 1.96) and degree of precision 0.05, the minimum sample size was 138. Ten percent was added to account for patients who were likely to be lost during follow-up, withdraw, or have incomplete records. The minimum desired sample size for the study was 152 patients with uncomplicated *P. falciparum* malaria.

Inclusion and exclusion criteria

The inclusion criteria were patients above five years old with uncomplicated *P. falciparum* malaria, diagnosed to have patent schistosome infection, mild *P. falciparum*, absence of danger signs or signs of severe and complicated *falciparum* malaria according to the definition given by World Health Organization, and absence of febrile conditions caused by diseases other than malaria. The exclusion criteria were pregnancy, infection with species other than *P. falciparum*, age younger than five years, complicated/severe *P. falciparum* malaria on screening/at study entry, praziquantel treatment before entry into the study, or severe clinical signs/symptom of the disease such as ascites and hepatosplenomegaly.

Detection of malaria parasite

Blood smears were prepared from each clinical malaria case to detect malaria parasites. These smears were then stained using the Giemsa staining method [17]. Each stained smear was then examined for detection of *P. falciparum* using light microscope.

Detection of schistosome infection

As soon as a malaria case was confirmed microscopically, a stool sample was collected from the patient for *S. mansoni* examination. Stool samples were examined for the presence of schistosome eggs using the standard Kato-Katz technique for three consecutive days [17]. After treatment with artemether-lumefantrine, stool samples of the co-infected cases were examined using the Kato-Katz method at days 28, 29 and 30 [18]. In an attempt to improve the sensitivity of Kato-Katz technique,

duplicate Kato-Katz thick smears were prepared from each sample collected for three consecutive days [19,20]. The World Health Organization cut of value [light 1 to 99 eggs per gram (epg), moderate 100 to 399 epg and heavy ≥ 400 epg] was used for classification of intensity of *S. mansoni* infection.

Treatment of malaria and schistosomiasis co-infected patients

Microscopically confirmed patients with P. falciparum and S. mansoni co-infection as well as uncomplicated P. falciparum malaria patients were treated with artemether-lumefantrine per national guidelines.

Study variables

The independent variables for this study were socio-demographic characteristics (age, weight, and educational status) and the artemether-lumefantrine intervention. The dependent variables were co-infection rate, intensity of schistosome infection, and treatment outcome.

Data processing and management

The laboratory results and clinical examinations were recorded for each patient. Intensity of infection of schistosomiasis was expressed as epg. Cure rate was expressed as the percentage of *S. mansoni* and *P. falciparum* co-infected patients who became S. mansoni ova negative after treatment.

Data quality control

Patients were recruited by trained clinical research assistants, including health officers and nurses. Both clinical and laboratory examinations were conducted on patients presenting with signs and symptoms of malaria.

Data analysis

SPSS version 15 software (SPSS IBM, Chicago, Ill, USA) was used to analyze data. Pearson's χ^2 test and paired t-test were used for statistical analysis of categorical and continuous data, respectively. The a priori statistical significance level was 0.05. Egg reduction rate was assessed by the change in geometric mean egg count per gram (GMEC) and calculated as [1-(GMEC after treatment/GMEC before treatment)] x 100. Treatment outcome was expressed as cure "for patients whose status changed from schistosome egg-positive to negative after treatment," and non-cure "for those whose status remained schistosome egg-positive after treatment."

Ethical considerations

The study protocol was submitted to the Institutional Review Board of the Faculty of Medicine, Addis Ababa University, and obtained ethical clearance to conduct this study. Before enrollment, written and informed consent was obtained from each adult patient and from the guardians/parents of each child. Illiterate participants had the written and informed consent after they chose a literate witness to read the consent document; agreement of illiterate participants was indicated by their thumb prints on the written consent form.

Results

Among the 152 malaria cases with uncomplicated *P. falciparum* infection, 28 (18.4%) were found to be co-infected with *S. mansoni*. Males were more co-infected with *P. falciparum* and schistosomes compared to females (15.1% versus 3.3%) (P = 0.01). Participants aged 6 to 14 years were the most co-infected (9.9%; 15/152), followed by 15- to 24-year-olds (5.3%; 8/152), while the least co-infected group was in the age range of 25 to 34- years 1.3%; (3/152) (P = 0.008). Considering level of education, children in the first to the eighth grades (11.8%; 18/152) were the most co-infected group, followed by illiterate participants (3.9%; 6/152), while participants in grades 11 and above were the least co-infected group (0.7 %; 1/152) (P = 0.024). The co-infection rate of rural residents (11.84%) was not significantly different from that of urban residents (6.57 %) (P = 0.177), as shown in Table 1.

Of the 28 co-infected patients, 19 had light *S. mansoni* infection (67.7%) and 9 (32.1%) had moderate *S. mansoni* infection (32.1%). The overall GMEC was 83.6 epg. Intensity of *S. mansoni* infection was relatively higher in study participants who were female (92.6 epg), aged 15 to 24 years (94.1 epg), or enrolled in the first to the eighth grades (146.2 epg). However, gender (P = 0.678), age (P = 0.315) and educational level (P = 0.680) were not significantly associated with intensity of *S. mansoni* infection (Table 2).

The cure rate after treatment was calculated as the percentages of individuals who had tested as schistosome egg free after treatment. The results showed that both the cure rate and the percentage of egg reduction were 100% at the end of the follow-up period (Table 3). Though the findings for the malarial

Table 1. Extent of *Schistosoma* and *falciparum* malaria co-infection among patients Kemise Health Center, Oromia Zone of Amhara Region, December 2009

Variables	Sub-category	Malaria positive patient	Co-infected patients Number, (%)	P-value
Sex	Female	60	5 (3.3%)	< 0.05[a]
	Male	92	23 (15.1%)	
Age group	6-14	42	15 (9.9%)	< 0.01[b]
	15-24	50	8 (5.3%)	
	25-34	30	2 (1.3%)	
	35 and above	30	3 (2%)	
Educational level	Illiterate	59	6 (3.9%)	< 0.05[c]
	1-8 grade*	59	18 (11.84%)	
	9-10 grade**	25	3 (2.0%)	
	11 & above***	9	1 (.7%)	
Residence	Rural	83	18 (11.84%)	> 0.05[d]
	Urban	69	10 (6.57%)	
Total		152	28 (18.4%)	

*Elementary & junior school; **Secondary school; ***Preparatory/vocational school, College
[a]P = 0.010 for the comparison of co-infection in males and females
[b]P = 0.008 for the comparison of co-infection in age groups
[c]P = 0.024 for the comparison of co-infection in educational level
[d]P = 0.177 for the comparison of co-infection in rural residents and urban

infection response are being prepared for publication, all the 28 co-infected patients were cured from their uncomplicated *P. falciparum* malaria.

Discussion

The current study revealed that Kemise town is co-endemic for S. *mansoni* and *falciparum* malaria with a co-infection rate of 18.4%, which is similar to the rate reported in an investigation from Tanzania (22.6%) [21]. Males have been shown to have higher co-infection rates compared with females. The observed differences among males and females might be exposure related.

School-aged children are one of the high-risk groups for the overlap of two infections [22]. The current findings also revealed that the 6- to 14-year-old group (school-aged children) had the highest co-infection rate. In the current study, the co-infection rate decreased as age and educational level increased. This inverse relationship might be due to awareness in avoiding any exposure to the two disease-causing agents, *Plasmodium* and *Schistosoma* species, through minimizing contact with infected water bodies and proper use of bed nets. The other possible age-specific reason could be that children have incompetent immunity against malaria and schistosomiasis. The association between age and co-infection rate has also been reported somewhere else [21,23].

The demographic variables (age, gender and educational level) considered in the study were not significantly associated with the intensity of *S. mansoni* infection. These findings, except the association between age and intensity of infection in one report, are not unusual and have been previously described in other settings [24,25].

Artemether-lumefantrine was introduced in Ethiopia for the treatment of *P. falciparum* infection in 2006. From experimental studies, artemisinin

Table 2. *Schistosoma mansoni* egg load of malaria patients attending in Kemise Health Center, Oromia Zone of Amhara Region, December 2009

Variables	Sub-category	Number co-infected cases	Cases with light infection (< 100 epg)	Cases with moderate infection (100-400 epg)	Overall epg
Sex	Female	5	10.7% (3/28)	7.1% (2/28)	92.6
	Male	23	57.1% (16/28)	25% (7/28)	81.7
Age group	6-14	15	35.7% (10/28)	17.9% (5/28)	71.6
	15-24	8	14.3% (4/28)	14.3% (4/28)	94.1
	25-34	2	7.1% (2/28)	0% (0/28)	79.4
	35 & above	3	10.7% (3/28)	0% (0/28)	77.2
Education level	Illiterate	6	17.9% (5/28)	3.6% (1/28)	82.3
	1-8 grade	18	39.28% (11/28)	4.6% (7/28)	146.2
	9-10 grade	3	7.1% (2/28)	3.6% (1/28)	94.2
	11 & above	1	3.6% (1/28)	0% (0/28)	72
Over all infection intensity		28	67.86% (19/28)	32.1% (9/28)	83.6

Note: Gender (P = 0.678), age (P = 0.315) and educational level (P = 0.680) were not associated with intensity of infection.

Table 3. Effect of artemether-lumfantrine against *S. mansoni* in *falciparum* malaria co-infected patients

Variable	Pre-treatment	Post-treatment	P-value[a]
Schistosomiasis cases	28	0	0.000
Cure rate (%)		100	
Overall epg	83.6	0	0.000
Egg load reduction (%)		100	

[a]P = comparing pre-treatment and post-treatment period; Categorical variable was analyzed using Pearson's χ^2 test. Continuous variable was analyzed using paired t-test.

derivatives act against other parasites, as well as against tumor cells [26]. Hence it is cogent to investigate the clinical side benefits of artemisinins when used in the treatment of malaria. The present study revealed that all co-infected patients became *S. mansoni* egg negative following artemether-lumefantrine therapy at doses used for the treatment of malaria. This is comparable to the results of a study conducted in Sudan, in which malaria patients treated with artemether-lumefantrine were negative for *S. mansoni* eggs after a month check-up [13]. In addition, artemether alone showed significant prophylactic action against *S. mansoni* in the Côte d'Ivoire study [27].

The cure rate of schistosomiasis in the present study was 100%, unlike the treatment outcome of the Kenya study that was based on patients with a high intensity of *S. mansoni* infection [28]. The results of the current study may be due to the light schistosome infection of the participants, as our findings and those reported elsewhere have shown that artemisinins act better against light infections [13,29,30].

Conclusion

Demographic characteristics (gender, educational level, and age) influence the P. falciparum and S. mansoni co-infection rate in Kemise. Artemether-lumefantrine is highly effective against schistosomiasis in uncomplicated falciparum malaria co-infected patients at a dose used for the treatment of falciparum malaria. Artemether-lumefantrine may make possible the use of a single regimen to treat patients with S. mansoni and falciparum malaria co-infection. However, before the use of artemether-lumefantrine alone in the treatment of schistosomiasis in malaria co-infected patients, there is a need for multicenter and randomized trial evidence on the range of S. mansoni infection level.

Acknowledgements

The authors are grateful to Addis Ababa University Graduate School for funding the project, and to all the health professionals and patients who participated in the study for their cooperation.

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Corresponding author
Solomon Mequanente Abay
Department of Pharmacology
School of Medicine
Addis Ababa University
PO Box 55151
Addis Ababa
Ethiopia
Telephone/Fax:
Email: solomonabay@gmail.com

Conflict of interests: No conflict of interests is declared.