Journal of Bacteriology and Parasitology

Gebrehiwot et al., J Bacteriol Parasitol 2015, 6:2
DOI: 10.4172/2155-9597.1000220

Research Article Open Access

Association of Total Levels of Serum Antioxidants with Periportal Fibrosis and Intensity of *Schistosoma mansoni* Infections in Cheretee, North East Ethiopia

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Received date: January 02, 2015, **Accepted date:** Mar 23, 2015, **Published date:** Mar 27, 2015

Abstract

Schistosomiasis is only second to malaria in terms of public health importance among parasitic diseases. Hence morbidity and mortality associated with *Schistosoma mansoni* are mainly the result of periportal fibrosis of the liver. With the objective of evaluating the association of total serum antioxidants and intensity of *S. mansoni* infection a cross sectional study was conducted from February 2011 to June 2011 involving 414 individuals and 30 controls from *S. mansoni* non-endemic area. The study groups were selected using systematic random sampling technique and data were collected using a pre-tested clinical questionnaire, ulthersonographic examinations of the liver and serum ferric reducing antioxidant power (FRAP) assays. Prevalence of *Schistosoma mansoni* infection and periportal fibrosis in Cheretee was 36.72% and 9.42%, respectively. Prevalence of periportal fibrosis and intensity of infection had a sharp rise in the age group 11-20 years, reached its peak in the 21 to 30-year age group, and started to decline thereafter up to >40. Age, sex and intensity of infection were strongly associated with periportal fibrosis development ($p<0.05$). Mean total serum antioxidant concentrations were significantly lower in study subjects who were from *S. mansoni* endemic area (96.5 μM/L) compared with healthy participants from Addis Ababa (339.9 μM/L). However, mean total serum antioxidant concentration were not significantly different among PPF positive and negative individuals from *S. mansoni* endemic area. Finally, further studies are recommended on the cause of this low antioxidant concentration.

Keywords: Schistosomiasis; Ulthersonographic; Antioxidant; Periportal fibrosis; Cheretee; Cross sectional study

Introduction

Schistosomiasis is only second to malaria in terms of public health importance, with >200 million people currently infected worldwide, 85% of whom are in sub-Saharan Africa [1]. Based on recent estimates, and ~54 million people in Sub-Saharan Africa are considered to be currently infected with *S. Mansoni* [2]. This is associated with micronutrient deficiency which is estimated to affect about one-third of Sub-Saharan Africa's [3]. Schistosome worms live in mesenteric and portal veins of their human host, and schistosome eggs trapped in hepatic sinusoids induce an inflammatory granuloma that prevent toxic substances from diffusing from the eggs to the surrounding hepatic tissue which may lead to the development of periportal fibrosis [4,5].

Morbidity and mortality related to schistosomiasis mansoni are mainly the result of periportal fibrosis (PPF) of the liver associated with intensity of infection. However in some studies, disparities between community prevalence of infection and levels of PPF are often observed in various endemic areas [6-8]. Generally the possible determinants include variation in intensity of exposure, differences in parasite strains, nutritional status (including micronutrient deficiencies), the presence of other infections that may involve the liver (e.g., malaria, viral hepatitis, and brucellosis) and host genetics [9].

Oxidative stress contributes to the development of fibrosis in the liver either through direct stimulation or by promoting the production of pro-fibrotic cytokines [10-13]. Oxidative stress is a reflection of excess intracellular concentrations of reactive oxygen species (ROS) and one of the important indicators of cellular damage [14]. Furthermore, oxidative stress may also promote polarization of T-cell differentiation toward T helper-2 phenotype [12]. Using experimental models of the disease, it has been shown that oxidative stress occur at the site of inflammation (*S. mansoni* eggs entrapped). These are responsible for the damaging effects of schistosomiasis and indicate that free radicals (reactive oxygen species) are major component of the disease [15-19]. The ultimate result of ROS generation is killing of the parasite eggs; however, the process is potentially harmful for the host as production of ROS may initiate a fibrogenesis cascade in the liver or modulate tissue and cellular events responsible for progression of liver fibrosis. However, the path physiologic effect of ROS production associated with inflammatory response depends on a balance between pro-oxidant and antioxidant [14,20]. Thus antioxidants transform free radicals into less reactive species, thereby limiting their toxic effects [21-25].

Earlier community based studies in different parts of Africa have reported association of subjects with suboptimal antioxidant

micronutrients status and *S. mansoni* infection [21,22,26,27]. Furthermore; in Ethiopia to study the relationship between micronutrient malnutrition and *Schistosomiasis mansoni*, a cross sectional study was undertaken in selected parts of Ethiopia involving 421 school children. Compared with non-endemic controls, serum retinol concentrations were significantly lower and hydroperoxides were significantly higher in subjects from *Schistosomiasis mansoni* endemic areas. Furthermore, there was an inverse relationship between intensity of *S. mansoni* infection and concentrations of serum retinol in *S. mansoni*-endemic villages [28-32]. However the study did not directly assess the presence of PPF with serum total antioxidants and involved only children. The present study determined total serum antioxidants among PPF positive and PPF negative subjects from northeast Ethiopia study village of Wollo, cheretee with a recent evidence for increasing prevalence and intensity of *S. mansoni* infection.

Materials and Methods

Study subjects, morbidity questionnaire, and parasitological examination

The study was conducted during the months of February 2010 to June 2011 involving individuals from Cheretee village (Kemisse administrative zone, 320 km north of Addis Ababa at an altitude of 1500 meters above sea level (m.a.s.l.). Total population of Cheretee is 8486, which are predominantly Muslim whose livelihood largely dependent on subsistence farming. Controls were from Addis Ababa University Aklilu Lemma Institute of Pathobiology staff and students. Addis Ababa a capital city of Ethiopia is found at an altitude of 2200-2500 m.a.s.l. and 9° 2' 0" North and 38° 42' 0" East. The study subjects were individuals of rural peasant families or low-income farm laborers. From the result of previous communities surveyed, the prevalence of *S. mansoni* was 50% in the area [32]. Using the formula for comparison of proportions a minimum sample size of 385 subjects were involved to achieve 90% power with a level of significance of 0.05 [33]. To increase the power of the study 414 study participants were recruited. These 414 study participants were selected using systematic random sampling method generated from EPI-6 statistical module. Study participants were taken at random from the list of people surveyed by the project in titled with "The roll of antioxidants in the reversal of schistosomal PPF" from Cheretee community. In addition, healthy controls were recruited from the staff and students of ALIPB, Addis Ababa University, who had no travel history to schistosomiasis-endemic areas, and whose stool exams showed no ova or parasites. Stool specimens collected from each study subject were processed using 41.7 mg templates according to the modified Kato-Katz technique [34]. For each study subject, quintet Kato-Katz thick smears were prepared to optimize detection of *S. mansoni* infection [29]. Furthermore, for each participant, a pre-tested questionnaire on signs and symptoms of ill health was administered, in the local language, by trained local high-school graduates.

Clinical examination and ultrasonography

Periportal fibrosis positive subjects were determined using clinical examination and ultrasonography. The body weight was measured to the nearest 0.1 kg, with light clothing on, and body height was measured to the nearest centimetre with the subject wearing no shoes. After a brief clinical examination, ultrasonographic study of the liver was done using Hitachi (Tokyo, Japan) EUB 405 portable ultrasound equipment fitted with a 3.5 MHz convex abdominal probe. Standard ultrasonographic liver scans were performed for all subjects; if the liver scan appeared normal, no further examination was undertaken. In subjects with image patterns suggestive of PPF, the liver picture was compared with standard images and the corresponding image pattern score was recorded [31,35]. In addition, assessment of periportal thickening was made by taking inner and outer portal branch wall thickness (PBWT) measurements of 2-3 second branching portal veins. The summation of the image pattern and PBWT scores gave the final periportal thickening/fibrosis (PPT/F) grading of each individual. In subjects with PPT/F, inner to inner diameter of the main portal vein was measured at the entry point to the liver [31]. All PPF positive involved in this research are definite periportal fibrosis cases which are positive for the two methods used.

Sera collection, processing, and laboratory measurements

Venous blood samples were collected from study subjects using sterile needles and Vacutainer tubes (Becton-Dickinson Vacutainer SST tubes, cat. no. 367986, BD Diagnostics, Franklin Lake, NJ) and were kept in a dark room or in ice-box until clotting and sera separation were done after centrifugation at 1300 per minute. Sera were collected and temporarily stored in deep freezers (about -20°C) of the respective health institutions during survey periods. At the end of each survey, the sera were transported in cool icepacks to Addis Ababa and stored at -20°C for less than 6 weeks. The laboratory investigations were done at Aklilu Lemma Institute of Pathobiology, Addis Ababa University.

The ferric reducing antioxidant power (FRAP) assay was used to determine total antioxidants in serum. It was a recently developed, rapid, simplified and direct test of "total antioxidant power" adopted from Vitas and Rune Blomhoffs group. It can be run at 96 wells plate and corresponding ODs measured in a plate reader. At low pH, reduction of a ferric tripyridyltriazine (Fe3-TPTZ) complex to the ferrous form which has an intense blue color can be monitored by measuring the change in absorption at 593 nm. The change in absorbance, therefore, is directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture [36].

Statistical analysis

Statistical analyses were done using Excel and SPSS version 10, statistical software (SPSS, Inc., Chicago, IL). Excel was used for data entry. Normality and equality of variances were assessed before employing parametric tests for all out come variables. *S. mansoni* egg counts per gram of stool (epg) were also transformed to natural logarithms, using log (epg +1) to allow computing for subjects with zero counts. Mean intensities of *S. mansoni* epg expressed in text or tables was expressed in geometric means. One-way ANOVA was used for comparisons of mean serum total antioxidants by age categories and categories of *S. mansoni* egg excretion and also for comparisons of mean *S. mansoni* egg excretions by study group. Partial correlation analysis was used to assess the relationship between serum antioxidants and intensity of egg excretion after controlling for age among PPF positive and negative. Comparisons of proportions and means between groups were made using the X^2test and t test respectively. Results were considered significant for $P<0.05$.

Ethical clearance

The study was a component of a larger research project entitled "the role of antioxidants in the reversal of schistosomal periportal fibrosis," which had ethical clearance from Institutional and National Ethical Clearance committees of Ethiopia and from the Norwegian Board of Medical Research Ethics. All diagnostic and treatment procedures were carried out after obtaining informed consent from each subject or his/her guardians. Free treatment was offered to all subjects with schistosomiasis and/or other helminth infections. All subjects who were positive for *S. mansoni* were treated with a single dose of praziquantel at 40 mg/kg body weight. Subjects with *Taenia* spp. or *Hymenolepis nana* infections were treated with a 3-day course of albendazol 400 mg/day or with praziquantel if they had *S. mansoni* co-infection. Other helminth infections were treated with a single dose of albendazol 400 mg.

Results

Background characteristics

A total of 414 study participants were included in the study of which 210 were female and 204 male with mean (sd) age of 18.31 (17.3) years. 30 healthy subjects from non-endemic area (20 males and 10 female; mean (sd) age, 17.5 (4.97) years) also participated in the study as a controls. All non-endemic controls were free from *S. mansoni* infection and had normal ultrasonographic image patterns of the liver. Overall prevalence of *S. mansoni* infection in Cheretee was 36.7% and the geometric mean egg excretion of per gram of stool of all examined was 58.2 and geometric mean among egg positives was 82.7. On the other hand; 31 (7.42%) subjects harboured one or more other intestinal helminth infections. Among these, most subjects harboured *A. lumbricoids* (4.3%). Based on ultrasonographic image patterns and PBWT-for-height standard, among 414 study participants 375 (90.52%) had a normal image pattern and 39 (9.42%) had definite/advanced PPT/F. Among these subjects, more than half (61.5%) had demonstrable *S. mansoni* eggs in at least one of three Kato-Katz thick smear slides at the time of initial evaluation.

Prevalence and intensity of *S. mansoni* infection and associated variables

Prevalence of *S. mansoni* was higher in the age group 11-20 years and the infection status was strongly associated with categories of age group. On the other hand, the proportion of egg positives and intensity of egg excretions did not vary by sex. In addition, the prevalence of other helimentic infections was not related with PPF but prevalence and intensity of *S. mansoni* infection were strongly associated with PPF development (Table 1). After adjusting potential confounding variables, prevalence of *S. mansoni* infection was significantly associated with age of study participants. Compared to individuals younger than 10 years, those in the age group 11-20 and 21-30 years had an increased risk of being infected with *S. mansoni* (OR=2.7; 95% CI: 1.43 to 5.19 for 11-20 years old; OR=1.5; 95% CI: 1.89 to 6.21 for 21-30 years old). As indicated in Figure 1 intensity of infection among age groups, <10, 11-20 and 21-30 years were associated with higher infection of *S. mansoni*. The risks of getting infection were higher in the area in these age groups but lesser infection in the higher age groups. Moreover; among males and females there was no significant change in light and moderate infection but males had higher percent of heavily infected individuals.

Periportal fibrosis and associated factors

Among all ultrasonography examined study participants 39 (9.42%) were positive for PPF. The prevalence of PPF was higher among *S. mansoni* positives than *S. mansoni* negatives. Among *S. mansoni* positive individuals egg excretion >100 were higher in number of PPF cases. Prevalence of PPT/F had a sharp rise in the age group 11-20 years, reached its peak in the 21- to 30-year age group, and started to decline thereafter up to >40. Age, sex and intensity of infection were strongly associated with periportal fibrosis development ($p<0.05$). After controlling for age and *S. mansoni* infection the odds of male subjects having PPF was 2.66 times higher than females (OR=2.66; 95% CI: 0.456 to 9.48). In a model that adjusts for sex and intensity of infection age was strongly associated with acquiring PPF infection with increasing age, except age groups <20 years. Compared to individuals younger than 10 years, those in the age group 21-30, 31-40 and >40 years had an increased risk of PPF development (OR=9.07; 95% CI: 0.456 to 9.48 for 21-30 years old; OR=9.91; 95% CI: 2.36 to 34.81 for 31-40 years old; OR=5.14; 95% CI: 2.51 to 39.12 for >40 years). Compared to light infection, heavy infection increase the odds of having PPF 4 times (OR=3.87; 95% CI: 1.12 to 12.98).

Study variables		Number (%) Sm* positive	Mean egg excretion per gram of stool	Number (%) positive for hl* other than Sm*
Age category	<10	118,3 (19.1)	27.9	9 (29.1)
	11-20	91, 5 (30.9)	109.2	9 (29.1)
	21-30	67,3 (21.1)	89.2	6 (19.4)
	31-40	63,2 (13.8)	30.1	2 (6.5)
	>40	75,2 (15.1)	40	5 (16.1)
p-value		0.000	0.002	0.359
Sex	Male	204 (52.6)	67.2	20 (64.5)
	female	210 (47.4)	52.8	11 (35.5)

p-value		0.298	0.299	0.110
PPF	Positive	39 (61.5)	150.76	3 (9.7)
	negative	375 (35.7)	48.57	28 (90.3)
p-value		0.000	0.03	0.959

Table 1: Prevalence, intensity of *S. mansoni* infections and other helminths infections by age, sex and PPF status among 414 subjects from Cheretee, 2011. (*S. mansoni*, hl= helminth).

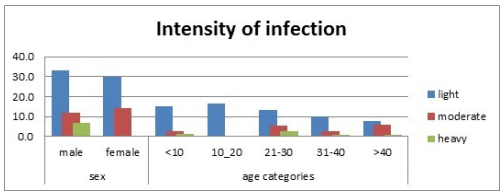

Figure 1: shows intensity of infection by age and sex among 150 *S. mansoni* positive study participants from Cheretee, 2011.

Determination of total antioxidant concentration

Of these 414 ultrasonography examined study subjects, sera were collected from 39 PPF positive, 39 PPF negative individuals and 30 controls from Addis Ababa. A total of 71 male and 37 female with a mean age of 26.28 ± 13.48 were included in the analysis. From the above mentioned participants 61.5% of PPF positive and 57.8% of PPF negative were also positive for *S. mansoni* infection with mean egg excretion of 278.6 and 141.7 respectively. PPF positive and PPF negative study participants were comparable in terms of their age, sex composition and *S. mansoni* infection. Mean intensity of *S. mansoni* infection was not significantly different among PPF positive and PPF negative study participants. None of the 30 healthy controls from Addis Ababa had *S. mansoni* infection or PPF. Mean total serum antioxidant concentrations were significantly lower in study subjects who were from *S. mansoni* endemic area (96.5 µM/L) compared with healthy participants from Addis Ababa (339.9 µM/L). However, mean total serum antioxidant concentration were not significantly different among PPF positive and negative individuals in the area (Table 2).

Study variables		**Study subjects**			**p-value**
		PPF positive	PPF negative	Control	
Number examined		39	39	30	
Mean Age		32.69	28.69	29.3	0.981
Sex	M	76.2%	66.7%	65.4%	0.961
	F	23.8%	33.3%	34.6%	
Mean egg excretion		278.6	141.7	-	0.424
Mean antioxidants		107.2	85.8	339.9	0.000
P-value		0.376		-	-
Positivity		61.5%	57.8%	-	0.145

Table 2: Demographic and parasitological characteristics of 108, whose total antioxidant determined study participants from study sites endemic and non-endemic to *S. mansoni*, 2011.

The number of individuals below detectable level of serum antioxidants were higher among PPF positive (17%) followed by PPF negative (8.5%) but there was no individual below detection value among the controls. Compared with PPF negative and positive, controls had scored highest value of mean total of antioxidant concentration. Mean total serum antioxidants had the highest value among individuals who were not excreting higher eggs in stool or among non-infected individuals. Intensity of infection was inversely correlated with mean total antioxidant concentration (r2, -0.756; P<0.05). Individuals with moderate and heavy *S. mansoni* infection had low mean total serum antioxidant concentration compared to *S. mansoni* negatives. Moreover, the mean antioxidant concentration of moderately and heavily *S. mansoni* infected individuals were one third and one fourth of non-infected, respectively (Figure 2). Intensity of infection was inversely correlated with antioxidant concentration in PPF negative (r2, -0.081, p=0.049) and PPF positive (r2, -0.075, p=0.014) study participants. Moreover, heavily *S. mansoni* infected PPF positive study participants mean total serum antioxidant concentration were one fourth of non-infected PPF positive study participants. Similarly, heavily *S. mansoni* infected PPF negative study participants had lower total serum antioxidant concentration than *S. mansoni* non-infected PPF negative individuals. The mean total serum antioxidants were not significantly different among PPF positive and PPF negative (P=0.376) individuals selected from the same community. Moreover; age and sex had no significance effect on antioxidant concentration among PPF positive and PPF negative study participants. However, prevalence and intensity of *S. mansoni* infection were significant predictors of the concentration of total serum antioxidants.

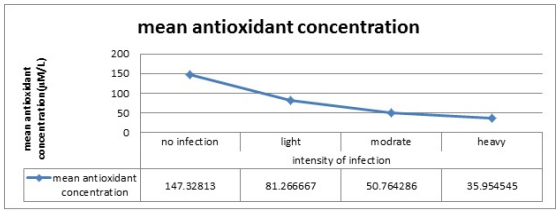

Figure 2: Distribution of mean total antioxidants with *Schistosoma mansoni* egg categories among 78 individuals from Cheretee, 2011.

Discussion

This study involved study participants from Cheretee community endemic to *S. mansoni* infection. The overall prevalence of *S. mansoni* and schistosomal periportal fibrosis estimated to be 36.5% and 9.42% respectively. The prevalence of *S. mansoni* infection is lower than previously reported from Cheretee 50% [31], this might be related to previous treatments and health education on intervention of infection. It was also lower compared to other parts of Ethiopia such as Methehara [28] and in different localities of northern Ethiopia [37]. But the prevalence of periportal fibrosis was comparable with similar study done elsewhere in Ethiopia (werkamado) [31,32] and Sudan [26] but higher than figures reported from some endemic parts of Ethiopia, (sille) [31,32] and neighbouring countries such as Kenya [38,39] and Tanzania [7], this might be associated with differences in nutrition, presence of other diseases like malaria, hepatitis and genetically associated factors in development of periportal fibrosis. The peak prevalence and intensity of *S. mansoni* infection is similar with other studies done in Ethiopia [32] and Kenya [39] and observed in the age category of 11-20 years and 21-30 years. Furthermore, infection prevalence and intensity is typically lower in older age groups, and it has been noted that risk for infection-associated disease is generally correlated with duration and intensity of infection [38]. Variation in infection and disease rates has been theorized to be due to age-related changes in exposure to acquired immunity and to age-related innate changes in susceptibility to infection over time, and it is likely that a combination of these factors all serve to regulate transmission and disease risk [27]. The peak for the proportion of subjects with PPT/F among those with high intensity of infection was observed in the age category of 21–30 years. These findings are in line with earlier studies [31,38,39]. This indicates the importance of intensity and duration of infection (as reflected by age of the subject) in the development of schistosomal periportal fibrosis.

Similar to previous studies [31,39], the prevalence of schistosomal PPT/F were significantly higher among men than women. Because men do most of the farming activities in the community, the observed high prevalence of PPT/F is probably related to sex-related behavioral and occupational differences of exposures to potentially infected water bodies, which put men at higher risk of acquiring *S. mansoni* infection. Intensity of *S. mansoni*, age, and sex were significantly associated with the development of PPT/F. On the other hand, presences of other intestinal helminth infections were not associated with development of schistosomal PPT/F. Similarly, other researchers have also reported intensity of *S. mansoni* infection, sex, and ages as factors associated with the development of PPT/F [4,6,32]. Furthermore; these researchers have indicated the negative association of geohelminth with the development of schistosomal PPT/F. This could be associated with recent theory, concomitant infections can influence host immune response, and their interaction may result in augmentation or suppression of immunopathology of either or both infections [40].

The present study shows that total mean serum antioxidant concentrations were markedly low in both periportal fibrosis positive and negative study participants from *S. mansoni* endemic area, which was in sharp contrast to the normal concentrations found in study participants from non-endemic area as supported by different earlier reports in Ethiopia [31], India [25] and Turkey [14]. This low antioxidant level could be due to the fact that lack of multi-micronutrient supplementation, seasonal variation in food sources rich in micronutrients and infection in *S. mansoni* which results in decreased serum antioxidant [21-25]. In addition to this, presence of other endemic infections (such as malaria), genetic disorders and autoimmune disorders; in which earlier studies have shown that serum micronutrients concentrations are low in subjects with those diseases [41-46] and their concentrations improve after clearance of parasites in case of malaria [42,43]. In this study, however, although malaria is endemic in the study site, it would not expect to affect our estimates of serum micronutrients because sera were collected during the non-malaria transmission season.

In agreement with earlier reports [21,22], mean total serum antioxidants had the highest value among individuals who were not excreting higher egg per stool among PPF negatives and PPF positives. It is also similar with earlier report from Werkemado which have similar secio-cultular characteristics with Cheretee. It was stated that *S. mansoni* infection might be factor for PPF development in the area and intensity of infection was inversely correlated with antioxidant concentration [32]. Mean total serum antioxidants were not significantly different among PPF positives and PPF negatives. This could be because; in addition to mean total serum antioxidant concentration other factors may also be associated with development of PPF among study participants from the same community. Keeping the facts from earlier reports [31] and compared to total serum antioxidant of healthy participants in the current study, people from the current study area have low mean antioxidant concentration. However, when we compare the people from the same community in addition to luck of micronutrients other disease like malaria, hepatitis, inherited factors and Th2 polarization [38,17] might be associated with PPF development. Moreover, it also strengthens the fact that, dietary antioxidants other than the well-known sources may contribute significantly to antioxidant defenses [44]. On the other hand; prevalence and intensity of *S. mansoni* infection were significant predictors of the concentration of total serum antioxidants among PPF positives and negatives, as indicated by earlier researchers from Rural Zimbabwean and Kenyan school children [21,22].

Based on these findings, we recommend that, since children have the highest prevalence and intensity of infection, school age targeted chemotherapy combined with proper sanitation practices and provision of a safe water supply need to be implemented to substantially reduce the overall prevalence and morbidity caused by *Schistosomiasis mansoni*. On the other hand; since we only determine serum antioxidants from study participants further in-depth nutritional studies are needed to assess the association of the development of schistosomal periportal fibrosis and sources of micronutrients in the area. In conclusion, micronutrient malnutrition (low mean total of antioxidants) is associated with *Schistosoma mansoni* infection and *schistosomal* PPF as indicated from total antioxidant of the study participants from *S. mansoni* endemic and non-endemic areas.

Acknowledgements

Our sincere appreciation goes to School of Graduate Studies Addis Ababa University and Aklilu Lemma institute of Pathobiology for providing us different materials and resources during the research. We also thank the research project on morbidity studies of *Schistosomiasis mansoni* in Ethiopia led by Dr. Nega Berhe in collaboration with Oslo Universituy Hospital, Ullaval, Norway in which this work was financially supported. Our sincere thanks goes to Mr. Girmay Medhin, for his sincere collaboration in facilitating statistical advice and Mr. Abebe Nigusie and Hafty Haileslasie for their cooperation during laboratory work.

Financial Support

This study was financially supported by Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia and by the project entitled "Morbidity studies of *Schistosomiasis mansoni* in Ethiopia". Led by Dr. Nega Berhe in collaboration with Oslo Universituy Hospital, Ullaval, Norway.

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