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Research Article

Epidemiological Assessment on Schistosomiasis in Southern Shan State, Myanmar

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Abstract

Background

Schistosomiasis is caused by blood flukes (trematode worms) of the genus *Schistosoma*. Its transmission has been reported from 78 countries over the world, affecting an estimated 250 million people and approximately 700 million are at risk. It was first serologically detected among the locals living around Inlay Lake, southern Shan State, Myanmar in 2013 but specific species and

vector snail were not identified yet. General objective of the present study was to assess schistosoma infection in the same area from an epidemiological point of view.

Method

An exploratory and cross-sectional analytic study was undertaken among the locals (n = 192) in selected rural health centre areas in February 2018. The participants were interviewed with pretested semi-structured questionnaires. Their blood samples (serum) were tested using Human Schistosoma IgG ELISA first to detect IgG antibodies and further confirmed by Schistosoma mansoni IgG ELISA (sensitivity 93.75%, specificity 98.55%). Their stool samples were tested with Kato-Katz techniques to search schistosma eggs. Freshwater snail surveys were also performed and collected snails were identified by zoologists with malacological experiences for species names. Questionnaire data collected were analysed using SPSS software 16.0 to determine the risk factors by Chi-squared test with a significant level of 0.05.

Results

Schistosoma seroprevalence (IgG) caused by *Schistosoma mansoni* was found to be 23.4% (95% CI: 17.4-29.4%). No schistosoma eggs were detected in stool samples. *Lissachatina fulica* (Ferussac, 1821) was identified as a vector snail in study areas. The risk factors were being male [OR = 5.05 (95% CI: 2.27-11.23), $p < 0.001$], residence [OR = 10.22 (95% CI: 3.27-31.92), $p < 0.005$ for *Khaung Daing* vs. *Min Chaung* and OR = 8.63 (95% CI: 2.7-27.63), $p < 0.005$ for *Ma Gyi Seik* vs. *Min Chaung*], water-related occupation [OR = 2.66 (95% CI: 1.34 - 5.26), p = 0.004], habits of not wearing boots when in contact with open water body [OR = 6.45 (95% CI: 1.49-27.78), p = 0.005], walking barefoot in workplaces [OR = 2.22 (95% CI: 1.11-4.44), p = 0.022], swimming [OR = 3.45 (95% CI: 1.16-10.28), p = 0.02], fishing [OR = 2.31 (95% CI: 1.17-4.59), p = 0.015], and playing in open water body [OR = 2.42 (95% CI: 1.21-4.83), p = 0.011] all factors classically associated with schistosoma infection.

Conclusions

There was local transmission of schistosoma infection at some time in study areas and disease prevention, control and surveillance are urgently needed.

Background

A chronic parasitic disease-schistosomiasis, also known as snail fever and bilharzia, is caused by blood flukes (trematode worms) of the genus *Schistosoma.* Its transmission has been reported from 78 countries over the world [1], affecting an estimated 250 million people and approximately 700 million are at risk [2]. About 85% of world's cases are in Africa where prevalence can exceed 50% in local populations [3] and annual deaths are almost 200,000 - 300,000 [2,4]. This disease is prevalent in tropical and subtropical areas especially in poor communities without access to safe water and adequate sanitation [5].

It is a socioeconomically devastating parasitic disease second only to malaria [6]. People in contact with infested water become infected when larval forms of parasites released by freshwater snails penetrate their skin. There are two major forms of human schistosomiasis - intestinal and urogenital. Species of intestinal schistosomias is are *S. mansoni, S. japonicum, S. mekongi* and *S. guineensis* and related *S. intercalatum.* Signs and symptoms are abdominal pain, diarrhea and blood in stool, hepatomegaly and splenomegaly. Species of urogenital form is *S. haematobium.* Haematuria, urogenital lesions and bladder cancer in late stage were noted in urogenital schistosomiasis [5]. In children the disease can cause anemia, stunting and reduced ability to learn [7]. Schistosomiasis caused by *S. japonicum* and *S. mekongi* is prevalent in China and Loas - the neighboring countries of Myanmar [5]. The infection due to *S. japonicum* was found in Shan State in Myanmar [8,9]. The Inlay Lake is situated in southern part of Shan State. Local people especially living on water-related work, such as, fisheries, floating vegetation etc. may contract this infection by intensive freshwater contact. An exploratory and cross-sectional analytic study was conducted among the local residents at risk living around the Inlay Lake in 2012 -2013. At that time schistosoma seroprevalence was found to be 23.8% (95% CI: 18.8-28.8%) among the local adult populations at risk [10]. But specific species name and vector snails were not identified yet. Therefore it was considered to undertake the present study in the same areas with the general objective of to assess schistosomiasis from an epidemiological point of view.

Methods

Study Design, Areas, Populations, Period, and Sampling Method

An exploratory and cross-sectional analytic study was conducted in rural health centre (RHC) areas within approximately five kilometers of Inlay Lake, Nyaung Shwe Township, southern Shan State, Myanmar (c20.5863˚ N, 96.9102˚ E). The study areas were *Ye Oo* village (*Khaung Daing* RHC), *Pya Bin* village (*Min Chaung* RHC), *Ma Gyi Seik* village (*Ma Gyi Seik* RHC) and *Mine Thauk* village (*MineThauk* RHC). Study populations were 192 local residents apparently healthy and aged between 10 - 80 years as inclusion criteria. Exclusion criteria are ill persons and those who did not want to participate in the study. Study period was February 2018. A consecutive sampling method was used to obtain study populations.

Data Collection Methods

Firstly all eligible individuals were recruited consecutively in the morning at local RHCs or sub-centres in study areas. They were explained about the objectives of the study by the Principal Investigator. Then their written informed consents were obtained. For children under 15 years the consent was given from their parents or guardians. Next their whole blood (5ml) samples were taken to detect schistosoma antibodies. The collected samples were temporarily stored in cold boxes at 2-8˚C. Three tight screwed-cap plastic bottles (70ml) and three disposable plastic spoons were also given to each participant to collect their early morning stool samples for three consecutive days. After that, data on their sociodemographic and behavioural characteristics, self-reported health conditions within last six months were collected in face-to-face interviews by well-trained laboratory technicians of University of Community Health and Basic Health Staff members from local RHCs using pre-tested semi-structured questionnaires. In the evening after collection of data from about 50 participants in each day the research team went back to

Nyaung Shwe Hospital where collected blood sample were temporarily stored in a refrigerator at 2- 4°C. Then collected data were entered into a computer and saved. Next morning stool samples were collected by local health volunteers and sent to the hospital under cold chains at 2-8°C. In hospital they were kept temporarily in a refrigerator at 2-4°C. The research team members performed freshwater snail surveys in paddy fields, near open water bodies of ponds, ditches and creeks in study villages, and Inlay Lake. Collected snail samples were placed in water-filled buckets. Afterwards these snail samples were temporarily stored in a freezer in the hospital and then sent to Taunggyi University in Taunggyi, 30 km away from north-east of Nyaung Shwe, where they were identified by zoologists with malacological experiences for taxonomic classifications using snail keys [11]. After obtaining blood and stool samples and required data from 192 participants within one week period the research team left Nyaung Shwe for Yangon together with collected blood and stool samples under a strict cold chain in a large cold box at 2-4°C. In Yangon these samples were temporarily stored at National Health Laboratory at same temperature before laboratory tests.

Laboratory Tests

Serum from collected blood samples were tested with Human Schistosoma IgG ELISA* first and further confirmed by Schistosoma mansoni IgG ELISA‡ (with sensitivity 93.75%, specificity 98.55%) by consultant microbiologists and well trained laboratory technicians to detect specific schistosoma antibodies. Stool samples were tested with Kato-Katz techniques to detect schistosoma eggs.

Data Management

The data collected by questionnaires were entered into a computer and analyzed by Statistical Package for Social Science (SPSS) software version 16.0 to create frequency tables, use Chi-squared tests and calculate odds ratios (ORs) with 95% confidence interval (CI) to determine risk factors. A significant level was set at 0.05.

Ethical Consideration

Ethical clearance was obtained from Ethics Review Committee of University of Community Heath, Magway, Myanmar.

Results

Schistosoma mansoni IgG was found in blood samples of 45 out of 192 participants. Therefore its seroprevalence is 23.4% (95% CI: 17.4 - 29.4%). There were no schistosoma eggs found in 131 stool samples examined, except hook worm eggs in four samples of two participants. Only 65 participants (33.9%) gave their stool

*Human Schistosoma IgG ELISA Kit, Cat. No: DEIA1792; Creative Diagnostics, 45-16 Ramsey Road Shirley, NY 11967, USA; www.creative-diagnostics.com; E-mail: info@creative-diagnostics.com

‡Demeditec Schistosoma mansoni IgG ELISA DESCHG0410;Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany); https://www.demeditec.com/en/products/schistosoma-mansoni-igg-elisa-deschg0410; www.demeditec.com; Email: info@demeditec.de

samples (ie. 28 participants for three consecutive days, ten for two days, and 27 for one day only) due to some reasons such as constipation in the day of collection, unfamiliar act of stool collection, and perceived being ashamed. The seven species of freshwater snails collected are shown in Table (1). *Lissachatina fulica* (Ferussac, 1821) was identified as a schistosoma vector (Photo). Participants' serological status, sociodemographic characteristics and self-reported health complaints in last six months are described in Table (2). The participants were aged between 10 and 80 years (mean ± SD age: 37.04 ± 16.34 years). Majority was in a 19 -59 year group (73.4%), lived in *Khaung Daing* and *Ma Gyi Seik* RHC areas (26% each), passed middle basic education (38%), was floating vegetation farmers (21.4%), obtained domestic water from tube well (60.4%), had unsanitary latrine (68.8%), and had none of health complaints within last six months (91.7%). The variables - gender, residence, water-related occupation, habits of not wearing boots when in contact with open water body, walking barefoot in workplaces, swimming in, fishing at, and playing in open water bodies are observed statistically associated with schistosoma positivity among the locals ($p < 0.05$) (Table 3).

Table 1: *Species of freshwater snails collected and their habits, RHC areas (villages) and schistosoma vector status in study areas*

Species of freshwater snail	Habitats	RHC areas (Villages)	Schistosoma vector status
Lissachtina fulica (Ferussac, 1821) (Synonym: *Achtina fulica*)	Inlay Lake	*Ma Gyi Seik (Ma GyiSeik)*	Yes
Pomacea canaliculata (Lamarck, 1819) (Synonym: *Ampullaria canaliculata,* Lamarck, 1882 and *Pila canaliculata,* Lamarck 1822)	Inlay Lake	*Ma Gyi Seik (Ma GyiSeik)*	No
Pila globosa (Swaison, 1822) (Synonym: *Ampullaria globosa*)	Inlay Lake	*Mine Thauk (Mine Thauk)*	No
Melanoides tuberculata (Muller, 1774) (Synonym: *M. tuberculata, Thiara tuberculata* and *Nerita tuberculata)*	Inlay Lake	*Ma GyiSeik (Ma GyiSeik)*	No
	Ditches	*KhaungDaing (Ye Oo)*	
Tarebia granifera (Lamarck, 1822) (Synonym: *Melania granifera* and *Tarebia lateritia*)	Inlay Lake	*Ma GyiSeik (Ma GyiSeik)*	No
	Inlay Lake	*Mine Thauk (Mine Thauk)*	
	Ditches and paddy fields	*Min Chaung (Pya Bin)*	

Pseudosuccinea columella (Say, 1817) (Synonym: *Lymnaea columella,* Say, 1817 and *Lymnaea ubaquensis,* Piaget, 1914)	Inlay Lake	*Ma GyiSeik (Ma GyiSeik)*	No
Pilsbryoconcha exilis (Lea, 1838) (Synonym: *Anodonta exilis*)	Ditches and paddy fields	*Min Chaung (Pya Bin)*	No

Table 2: *Schistosoma serological status, sociodemogaphic characteristics and self-reported health complaints in last six months of participants (n = 192)*

Variables	Category	Frequency (%)
Schistosoma serological status (*S. mansoni*)	Seropositive	45 (23.4)
	Seronegative	147 (76.6)
Age group (year)	≤ 18	32 (16.7)
	19-59	141 (73.4)
	60+	19 (9.9)
Gender	Male	101 (52.6)
	Female	91 (47.4)
Residence (RHC areas)	*Khaung Daing*	50 (26)
	Ma Gyi Seik	50 (26)
	Mine Thauk	43 (22.4)
	Min Chaung	49 (25.5)
Education	Illiterate	6 (3.1)
	3Rs*	12 (6.3)
	Primary	72 (37.5)
	Middle	73 (38.0)
	High	26 (13.5)
	Graduate	3 (1.6)
Occupation	Floating vegetation farmer‡	41(21.4)
	Paddy farmer‡	34 (17.7)
	Student	34 (17.7)
	Weaver	28 (14.6)
	Land vegetation farmer	21 (10.9)
	Casual worker	14 (7.3)
	Government servant/teacher	7 (3.6)
	Engine boat driver‡	1 (0.5)
	Others	12 (6.3)

Main domestic water source	Tube well	116 (60.4)
	Spring	46 (24)
	Pond	12 (6.2)
	Shallow well	11 (5.7)
	Creek	7 (3.6)
Latrine status	Sanitary	60 (31.2)
	Not sanitary	132 (68.8)
Reported health complaints in last six months	Abdominal pain	10 (5.2)
	Epigastric pain	4 (2.1)
	Urinary colic	2 (1)
	No complaint	176 (91.7

*Reading, Writing and Arithmetic, ‡Water-related work

Table 3: *Associations between some sociodemographic and behavioural characteristics and schistosoma serological status of participants (n=192)*

Variable	Category	Seropositive	Seronegative	OR (95%CI)	p value
Age group (year)	≤ 18	9	23	-	0.133
	19 - 59	35	106		
	60+	1	18		
Gender	Male	36	65	5.05(2.27-11.23)	< 0.001*
	Female	9	82		
Residence (RHC areas)	*Khaung Daing*	20	30	10.22 (3.27-31.92)	< 0.005*
	Ma Gyi Seik	18	32	8.63 (2.70-27.63)	< 0.005*
	Mine Thauk	4	39	1.57 (0.33- 7.46)	0.702†
	Min Chaung	3	46	1	<0.001‡
Occupation	Water related	26	50	2.66 (1.34-5.26)	0.004*
	Not water related	19	97		
Habit of not wearing boots when in contact with open water body	Yes	43	113	6.45(1.49-27.78)	0.005*
	No	2	34		
Walking barefoot in workplaces	Yes	29	66	2.22 (1.11-4.44)	0.022*
	No	26	81		
Swimming in open water body	Yes	41	110	3.45 (1.16-10.28)	0.02*
	No	4	37		

Fishing at open water body	Yes	22	43	2.31 (1.17-4.59)	0.015*
	No	23	104		
Playing in open water body	Yes	29	63	2.42 (1.21-4.83)	0.011*
	No	16	84		

*Statistically significant ($p < 0.05$)
†Fisher's exact test applied
‡Statistically significant overall ($p < 0.05$)

Discussion

In the present study current situation of schistosomiasis was assessed from an epidemiological point of view in terms of agent, host, environment, time, place and person. As *Schistosoma mansoni* seroprevalence was 23.4% (95% CI: 17.4 - 29.4), it can be considered that the whole study area is probably a moderate-risk community.

Agent: The causal agent was *S. mansoni,* first and foremost serologically detected in the study areas. This is one of three main species infecting human. Other two are *S. hematobium* and *S. japonicum*. More localized species are *S. mekongi* and *S. intercalatum* [12]. *S. mansoni* is the most prevalent being endemic in 55 countries, for example, Egypt, Sudan, Libya, sub-Saharan countries, Brazil, some Caribbean islands and Venezuela [2].

Host: Intermediate host of the disease was confirmed as *Lissachtina fulica* (Ferussac, 1821), (Synonym: *Achtina fulica*) in the present study. Its taxonomy is: Phylum: *Mollusca,* Class: *Gastropoda,* Subclass: *Pulmonata,* Order: *Stylommatophora,* Suborder: *Sigmurethra,* Superfamily: *Achatinoidea,* Family: *Achatinidae,* Subfamily: *Achatininae,* Genus: *Lissachatina,* Species: *Fulica* [11]. Its common names are giant African snail, Achatine, Escargot gent and Caramujo. In morphological features its shell is generally narrowly conic with 7-10 whorls and may attain a length of 200mm (averaging 50-100mm) and a width of 120mm when fully mature. The body is brown-grey in color and consists of alternating bands of brown and tan. From the native area of East Africa, this species was widely distributed to certain areas of North America, South and Central America, Indian Ocean, Pacific Islands, Caribbean, Australasia, Asia and Africa [13]. This type of snails can sexually reproduce in about one year and may live 3-5 years to maximum 9 years, and lay 400-1,000 eggs in one year. There is risk of snails moving long distances when they cling to cargo vehicles or machinery and even can survive for one year in unfavourable environments [14]. These snails were once moved across the countries, for example, being introduced into Brazil from Indonesia when they were sold in an agricultural fair in the former [15]. Its public health importance is that it harbors some parasites, namely, *Aelurostrongylus abstrusus, Angiostrongylus cantonensis,* (causing eosinophilic meningoencephalitis), *Angiostrongylus costaricensis* (causing abdominal angiostrongyliasis), *Schistosoma mansoni* (causing schistosomiasis), *Trichuris* spp., *Hymenolepis* spp. and *Strongyloides* spp. [16]. Other genera of intermediate snail hosts are amphibious freshwater *Oncomelania* spp. snails for the Oriental schistosome, *S. japonicum,* and aquatic freshwater *Bulinus* and *Biomphalaria* snails for *S. hematibium* and *S. mansoni* respectively [17,18]. Various animals, such as, dogs, cats, rodents, pigs, horses and goats serve as reservoirs for *S. japonicum* and dogs for *S. mekongi* [12]. In China, water buffaloes are major reservoir host for *S. japonicum* contributing up to 75% of human transmission. In the Philippines, this species infects up to 46 mammalian hosts including human and bovines [2].

Human is one of definitive mammalian hosts where schistosomes live an average of 3 - 10 years, but in some cases up to 40 years. Adult male and female worms live much of this time *in corpula* where female produce eggs and male fertilize them [18].

Environment: The environment of the study areas are full of open freshwater bodies such as Inlay Lake itself, ponds, springs, ditches and paddy fields all are receptive areas of vector snails. Water from catchment areas over the adjacent mountain ranges flows into these water sources. Water from the Lake flows down into *Bilu* Creek where a major hydroelectric power plant of Myanmar exists. Locals received their domestic water mainly from tube wells. Their latrines were mostly unsanitary and majority was built near the rim of and in the open water bodies. This human excreta disposal system should be modified to save the environment from being contaminated with.

Time: Regarding disease distribution, time may be all round the year whenever the locals are in close contact with infested open water bodies taking no personal protective measures (eg. wearing tall boots), living in the vector snail receptive areas lacking sanitary and proper human waste disposal and piped water supply systems, and effective snail control measures.

Place: The places of the present study are quite obvious as mentioned above in the environments favoring the high transmission of schistosoma infection.

Person: The persons at risk were all locals in the study areas and some are more prone to get infection. With regard to age group, it was not associated with seropositvity. But among 32 school age children (ie. eighteen year and below group), nine were seropositive (28.1%). If stool samples of these seropositive children contained schistosoma eggs detected by parasitological methods (ie. Kato-Katz techniques), the study areas could be recognized as a moderate-risk community [19]. In the present study, males were five times more likely to contract schistosoma infection than females due to their work nature more exposed to infested water like fishing and swimming. This finding is compatible with those of other studies [2,20,21]. Another risk factor for the infection is residence in which those living in *Khaung Daing* RHC and *Ma Gyi Seik* RHC areas were ten and eight times more likely to get infection when compared to those in *Min Chaung* RHC respectively. In this case, *Khaung Daing* RHC area was also a highest risk area found in previous study [10]. Naturally, distribution of schistosoma infection is very focal and determined by presence of competent snail vectors, inadequate sanitation and infected human [3]. So this area can be considered as a disease focus area or a pocket of high morbidity for which further study should be undertaken. Those with water-related occupations such as paddy field farmers, floating vegetation farmers and engine boat drivers were at a twofold higher risk of infection because of more exposure to water bodies. Lack of wearing tall boots and walking barefoot in the water-exposed places gave rise to six- and twofold chances of being infected respectively. Those with behaviors of swimming in, fishing near and playing in open water sources had three-, two- and twofold odds of contracting infection respectively. Swimming was a risk factor found in one of the studies [22]. Regarding reported health complaints among the participants within last six months, only 16 individuals (8.3%) reported they had signs and symptoms of gastrointestinal and renal diseases. Out of these 16 individuals, only four made complaints about gastritis. These complaints were not related with seropositivity.

Although schistosoma infection was serologically first identified five years ago and now, Myanmar is shown still as a non-endemic country area in the world map of its geographical distribution [23] that might need to be revised. The prevention and control measures, followed by surveillance, should be urgently taken using chemotherapy (ie. single oral dose of paraziquantel - 40mg/kg body weight), provision of potable water and adequate sanitation, hygiene education and snail control [7]. Intervention could be carried out at three different points: (i) reducing human contact with infested water, (ii) reducing availability of snail hosts, and (iii) preventing snail infestation by discontinuing contamination of freshwater by parasite eggs in human feces [24]. Health education should be delivered individually or in mass to avoid swimming, wading, playing in and washing near open water sources. They should also be advised to drink water boiled for at least 1 minute and use water stored for at least 1 - 2 days for bathing if water is fetched from infested water sources [25]. It is also noted that World Health Assembly resolution 65.21 called on all countries to intensify interventions to control the disease and to strengthen surveillance of its transmission [18]. Limitations of the present study are that vector snail was only identified by keys, not by detecting parasite DNA from collected snails, sampling method to recruit participants was a consecutive sampling method and sample size was also undersized thus results were not representative to the whole population in the study areas. Only 33.9% of participants provided their stool samples decreasing the probability of finding the eggs.

Conclusions

There was local transmission of schistosoma infection at some time among local people of study areas. Therefore disease prevention, control and surveillance measures are urgently needed to cut off the local transmission and to prevent further spread of vectors to other receptive areas in the country Myanmar.

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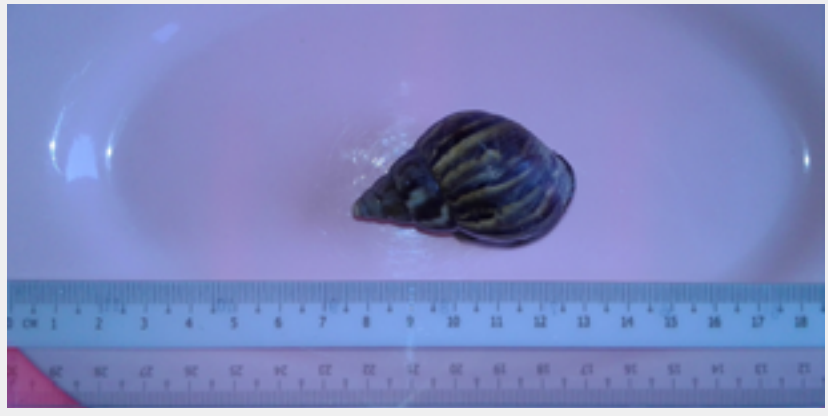

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