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Original Research Article

Genetic Characterization of *Schistosoma haematobium* population in central Sudan

Abdelbasit Mohammed Ibrahim[1*], Imran Fadl Osman Fadl[2], and Mohammed Mubarak Elbasheir[1]

[1]Department of Parasitology and Medical Entomology, Alzaeim Alazhari University, Khartoum State, Sudan

[2]Department of Zoology, Faculty of Science University of Khartoum, Khartoum State, Sudan

***Corresponding Author's E-mail: abdelbasit00@gmail.com**

Abstract

***Schistosoma haematobium* is neglected and highly endemic parasitic infection in central Sudan affecting those who come in contact with the contaminated water of irrigating canals in the agricultural schemes and water pools of the seasonal rains. *S. haematobium* is the causative agent of the urinary schistosomiasis and the pathology and morbidity related to it. This study was conducted to characterize the population of *S. haematobium* in central Sudan by using pooled *Schistosoma* eggs and previously published microsatellite markers. 16 urine samples were selected from schoolchildren confirmed infected with *S. haematobium* by microscopic examination from Sinnar and Khartoum states. Samples were centrifuged and the sedimented eggs were pooled and preserved in Eppendorf tubes filled with 70 % alcohol and treated as one sample for the extraction of *Schistosoma* DNA. Six previously publish microsatellite markers were used for the population characterization. The study revealed a high genetic diversity level of *S. haematobium* population in the study area. However, the population was not exposed to any genetic force. All loci were checked for deviation from Hardy Weinberg equilibrium that was found to be ranging from 0.045 to 0.029. The study concluded that *S. haematobium* in the study area was in a high rate of genetic diversity however, it was not under any genetic force.**

Keywords: Genetic diversity, Microsatellite loci, Population structure, Schistosoma haematobium

INTRODUCTION

Schistosomiasis is a well-known and widely distributed disease in Africa, South America some parts of Asia, and in pockets at the coastal areas of Europe. It comes second to malaria in morbidity related to parasitic diseases (WHO, 2018, 2020). It affects more than 240 million persons in 78 countries 85% of them are in Africa particularly schoolchildren, farmers, animal breeders and women while performing washing or collecting water for their domestic works, this in addition to more than 600 million persons at risk of infection due to their living in disease highly transmission areas (WHO, 2018, 2020). *S. haematobium* or what is now being named as (urogenital schistosomiasis) is one of the two main species of *Schistosoma* infections in Sudan the other species is *S. mansoni*. It is estimated that 120 million persons are infected with *S. haematobium* in Africa alone with the associated pathology (WHO, 2020) led to more than 21,151 deaths in sub-Saharan Africa countries and 24,068 deaths globally in 2016 (WHO, 2018). *S. haematobium* is endemic in 53 countries in Africa and the Middle East countries, where more than 110 million peoples are infected (WHO, 2018 and 2020). Schistoso-

miasis is endemic in areas with poor or deficient sanitation facilities and humans are performing urination and defecation beside rivers, water pools and irrigating canals. Moreover, these areas either lack the facilities of clean water supplies or it may be insufficient and citizens are being forced to use these rivers or the irrigating canals for their domestic and recreational activities. In addition, children with their high rates of infection indiscriminate habits of swimming and playing in water ponds particularly schoolchildren after their schooldays and by this way they participate in propagating the situation (WHO, 2017). Schistosomiasis in Sudan constitutes one of the persistent socioeconomic and health problems, since it spreads over vast majority of the country, starting from the northern region at the borders of Egypt down to include the capital city (Khartoum) to the centre where most of the irrigating schemes and the reservoir dams were constructed such as the Gezira-Managil irrigating scheme, new Halfa agricultural scheme, the Sinnar dam and its extension the Rusaires dam this in addition to many other parts of Sudan such as Darfur and Kurdofan (Abdelrahman, *et al*, 2017, Ismail, *et al*, 2014, Tamomh *et al*., 2018; Alaa *et al*., 2013; Azzam *et al*., 2017; Ibrahim and Ibrahim, 2014).

S. haematobium is restricted to their intermediate host or the snail that is responsible of it is transmission in the endemic areas. In the Sudan the intermediate host is *Bulinus truncatus*.

The genetic variation of *Schistosoma* as general and of *S. haematobium* in particular was documented in several studies (Afifi, *et al*, 2016, L. E. Agola, *et al*, 2009, Gasmelseed *et al*, 2014, Ekady, *et al*, 2020). The genetic diversity of human schistosome infections is influenced by a number of factors. Overlapping contact sites and snail and human movements may support the coexistence of a large number of genotypes in a given area. They can also engender the development of new parasitic strains or families through genetic interchange and recombination between local and introduced genotype, this can produce considerable genetic diversity in parasite populations among children, which may impact acquired immunity and the clinical outcome of the infection (Brouwer KC, *et al*., 2001). Further, human movements can promote co-existence of variable genotypes in certain regions. Moreover, the long lifespan of the definitive host, the human also contributes as a main factor for the diversity of the parasite (Angora *et al*., 2019; Kebede *et al*., 2018 and others). High genetic diversity may contribute to the emergence of new strains that are either non-susceptible or drug-resistant through genetic swapping and recombination between old and newly emerged genotypes (Brouwer KC 2001, Al-Mekhlafi HM, *et al.,* 2015). However, the sex of the infected host influences the density of infection e.g., boys in the endemic areas are always heavily infected than girls the fact that doesn't have any influence seen on the genetic diversity of the parasite (Betson *et al*., 2013, Gower *et al*., 2011).

MATERIALS AND METHODS

Ethical consideration

A written consent was obtained from the authorities of education and health directories from the two states. An educational lecture about the disease, its mode of transmission and how to avoid acquiring it was held in every school prior the commencement of collecting the samples. All positive children were given treatment for the disease as regular (Praziquantel 40 mg/kg).

This study was conducted to characterize the genetic population of *S. haematobium* in central Sudan

Study area

The study was held in central Sudan, Khartoum and Sinnar States. The Study area is located in the center of Sudan where the irrigating canals were constructed to provide water to the major agricultural projects in the region.

Sample size and study population

A study to evaluate the prevalence of schistosomiasis in this area was held in the year 2018, for that reason 229 urine samples were collected from Sinnar and Khartoum states and treated by centrifugation concentration technique to determine the prevalence of *S. haematobium*, then after the study was finished, a total of 16 urines were selected and used for this study. The participated population was schoolchildren from Elkreyyab Secondary School (from Khartoum North) and a Quran school (Khalwa) from Sinnar state. The ages of the study participants were ranged from 9 to 17 years with an average age of 13 years old. Both sexes were included in the study.

Samples collection procedure

Every study participant was provided with clean, capped labeled plastic container and requested him or her to bring at least 25 ml of urine. All urine samples were immediately centrifuged and examined for the disease. The positive samples were recorded and preserved for a while. Then 16 urines from the positive samples were selected and used for the study.

Table 1. Microsatellite markers that were used for the *S. haematobium* characterization

No	GenBank No	Sequence	BP
C131	EF057789	R: CATGGTGAGGTTCAAACGTG F: CTTGTCATTTGGGCATTGTG	288
SHA1	EF608039	F: TTGCATTCTCCTACCAACATG R: TCCATCAAACAACCAGTTGAC	702
SHA4	EF608040	F: CGAACTCCAACGAGCATC R: GGGTGTGGGAATGACTTG	896
SHC111	HS105664	F: CCCTTGTCTTCAATGCGTTA R: GAACGTCTAACTGGCGATCA	206
SHD3	EF608047	F: ATAGGATTCGATCTGCACTATG R: GACCACTTGTTGAGATTGATTT	561
SHC140	EF608046	F: TCCTTGAAGCAATGAATTTCAC R: CCAGGCAGTACCACAGTCC	676

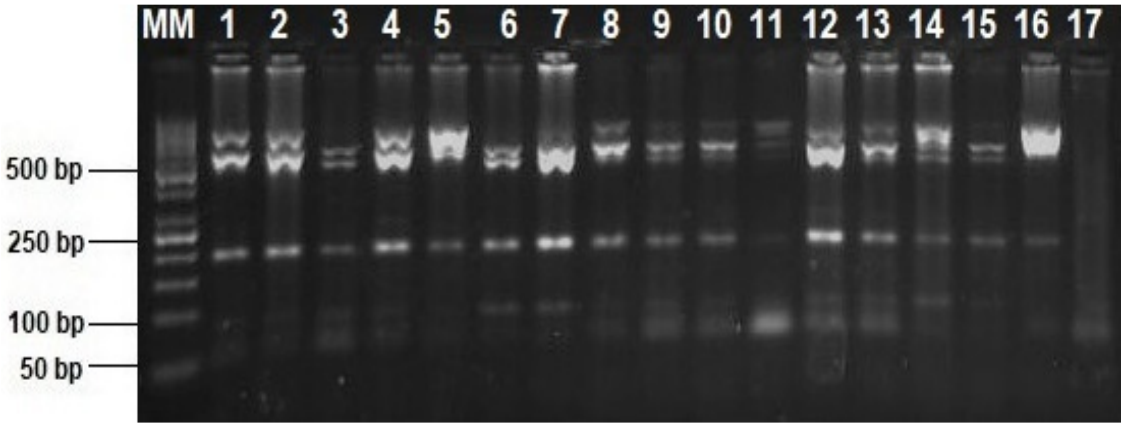

Figure 1. Result of microsatellite analysis of *S. haematobium* on agarose gel electrophoresis. From 1-16 were the samples, MM the ladder whereas 17 was the negative control (distilled water).

DNA extraction and PCR amplification

Sixteen urines were selected from the positive samples that were previously used for the study of prevalence of *S. haematobium* in this area and used for the population characterization of *S. haematobium*. From every selected sample 15 ml of urine was collected in a conical test tube; then the tubes were centrifuged at 15000 rpm for five minutes to precipitate the eggs at the bottom of the tube, then the eggs were collected, preserved in an Eppendorf tubes filled with 70% alcohol and stored for the study as it was the reliable protocol for the preservation of field collected *Schistosoma* eggs (Van der Broeck*et al*, 2011).

For the molecular test, all selected samples were analyzed using 6 previously published specific microsatellite markers for *S. haematobium* namely SH131, SHD3, SHA4, C111, SH140, and SHA1 (table1).

The genomic DNA of *S. haematobium* was extracted from pooled eggs of the parasite from all samples and done according to the kits manufacturer's instructions and procedure. All PCR preparations, DNA extraction and microsatellite amplification from *Schistosoma* eggs were done as per PCR conditions and quality control was assured avoiding any contaminations that could interfere with the PCR final products.

PCR was performed using the one-step (single tube) in a 25µl final volume using iNtRON's Maxime PCR PreMix Kit (iNtRONi-Taq, South Korea). Samples were applied for initial denaturation for 5 minutes at 95°C followed by 15 cycles of denaturation at 94°C for 30 seconds, annealing at 63.5°C for 30 seconds, decrease for 0.5 per each cycle and elongation at 72°C for 1 minute. Then after the completion of the first 15 cycles another 20 cycles of denaturation step at 95°C for 30 seconds, annealing step at 56°C for 30 seconds and elongation step at 72°C for 30 seconds was done. Then a final elongation step was done at 72°C for 15 minutes. PCR was performed on a thermocycler (MJ Research, USA). Distilled water was used as a negative control for each PCR run. Table 1

RESULTS

Sixteen urines from microscopically positive samples for *S. haematobium* eggs of children selected from schools in central Sudan were genotyped using 6 previously published microsatellite loci. The PCR results yielded a

Table 2. Microsatellite results as shown on the electrophoresis gel

locus	Locus size	A	F	H	A_0
C131	686	11	0.687	0.54	6.3
SHA1	702	10	0.625	0.62	6.2
SHA4	896	9	0.562	0.69	5.0
SHC111	206	15	0.937	0.13	8.4
SHD3	561	9	0.562	0.69	5.0
SHC140	676	1	0.62	0.90	5.6
MEAN	621	9.16	0.571	0.59	6.0

Key: A: allele number, F: frequency, H: heterozygosity and A_0 allele richness

clear band for each locus used in the test. The samples were genotyped manually according to the number of sharing bands for each participant.

All participants were considered as one population and the characterization was based on this hypothesis. For the study of the population genetic diversity we followed the traditional procedure by counting the total number and percentage of each locus manually according to the PCR results. The mean number of allele per locus and the expected heterozygosity within each participant were used as indicators of the genetic polymorphism within the population under the study. The allele count per locus was performed by counting all alleles per participant and summed and the percentage was calculated for allele frequency and for the other parameters of allele richness and heterozygosity (table 3). The deviation from Hardy Weinberg equilibrium was checked for each locus to identify if the population was exposed to any genetic force. The deviation from HW equilibrium was found to be ranging from 0.029 for the locus SHC11 as it was scored the highest score of alleles and 0.045 for the locus SHC141.

According to the PCR results the total number of alleles per locus was ranging from 1 to 15, the locus SHC111 scored the highest number of alleles per locus (15) while the lowest number was scored by the locus SHC140 (1). The locus SHD3 and SHA4 were scored the same number of allele per locus (9). Figure 1, Table 2

DISCUSSION

The current study revealed a high proportion of *S. haematobium* genetic diversity among schoolchildren in the center of Sudan as it is a common characteristic that was confirmed in several studies held in this field. However, the same result was obtained in several researches conducted either locally in the country of the Sudan or in the neighboring countries or even worldwide. In the Sudan, a previously study held in the Gezira region showed a high diversity of *S.haematobium* parasite that was not associated with the severity of infection (Gasmelseed N. *et al* 2014), another study held in White Nile region in the central Sudan also showed a high diversity level of *S. haematobium* (Juan-Hua Quan *et al*., 2014). In the neighboring country of Egypt a study held in Qena province in Upper Egypt showed a high rate of genetic diversity of *S. haematobium* in human population, however, another study in Egypt revealed a high genetic diversity of *S. haematobium* that contributed to consistent virulence and diverse clinical outcomes (Afifi, *et al*., 2016). In addition, a study held in Yemen also confirmed the genetic diversity of *S. haematobium* (Al-Mekhlafi HM, *et al.,* 2015). Nevertheless, in Africa, more studies were performed that were revealed proportions of genetic diversity related to the hybridization between *S. haematobium* and other species exist in the same area. A study held in Cameron in central Africa declared that hybridization between *S. haematobium* and *S. mekongi* is a major source that increases the genetic diversity of the parasite (Tuekeng *et.al*, 2022). Another study held in Côte d'Ivoire revealed a high rate of genetic diversity of *S. haematobium* as a result of the high rates of hybridization between *S. haematobium* and *S. bovis* in the area of the study (EK Angora *et.al*, 2022). However, a study held in Zimbabwe proposed the high genetic diversity of *S. haematobium* in the area under the study for the rapid turnover of the genotypes (Brouwer *et.al*, 2001).

On the other hand, the genetic diversity of other species of *Schistosoma* was studied, particularly *S. mansoni* which was intensively checked and revealed a high proportion of genetic diversity in most areas where the studies were conducted (El-Kady, *et al*., 2020, Webster *et al*., 2012) and others.

CONCLUSION

As was confirmed in many other researches, this study revealed a high proportion of genetic diversity of *S. haematobium* population within schoolchildren in central Sudan.

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