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GENETIC DIVERSITY OF *SCHISTOSOMA HAEMATOBIUM* IN SOHAG GOVERNORATE, UPPER EGYPT

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Abstract

Schistosomiasis is a chronic and debilitating disease especially in developing countries. *S. haematobium* of the urinogenital system is a major human health problem across Africa. Genetic variability recommended studying variation in disease burden and pathology, and epidemiological aspects. The present study investigated genetic diversity and polymorphism human *S. S. haematobium* isolated by using RAPD-PCR technique on 50/100 urine samples from different areas in Sohag Governorate.

Extracted DNA was used for molecular analysis using RAPD-PCR primers (A01, A02, A12, A13, & Y20). 27 *S. haematobium* DNA isolates (54%) were amplified by using specific primers. The isolates showed distinct variation in size and number of amplified fragments, indicating high genetic variation among them.

Keywords: Egypt, *Schistosoma haematobium*, Genetic diversity, RAPD-PCR

Introduction

Schistosomiasis is the third most widespread devastating parasitic disease worldwide and has a high mortality burden Zaqout *et al*, 2019). Generally, schistosomiasis is a snail-transmitted infectious disease caused by a long lasting infection with a blood fluke of genus *Schistosoma*, with *S. haematobium* and *S. mansoni* species endemic in Egypt (Abou-El-Naga, 2018). The estimates place the affected worldwide population for all schistosomiasis forms at 230 million, with an estimated 700 million at risk. 3 primary species of schistosomes affects human, *S. haematobium, S. mansoni* and *S. japonicum* (Lackey and Horrall, 2021).

S. haematobium infection caused carcinogenic led to poor bladder contractility (Ghobish *et al,* 1997), and *S. mansoni* and HCC (El-Tonsy *et al*, 2016), which opened new avenues to study mechanisms on how schistosomiasis lead cancer and other associated pathologies (Santos *et al*, 2021).

However, the diagnosis of active infection has been difficult because demonstration of infection depend on detecting parasite eggs in urine and/or stool. In the case of *S. haematobium* which parasitizes the urinogenital system; this method has low sensitivity in adults (Shiff, 2012). Detection of parasite-specific DNA in urine has been demonstrated and this has similar specificity but improved sensitivity (Webster *et al*, 2012).

The concept of detecting schistosome DNA in a host was initiated by Hamburger when he showed that schistosome genome contained a high proportion of tandem repeat fragments that were recognized by specific probes in *S. mansoni* worms (Hamburger *et al,* 1991). They later showed that a similar fragment DRA-1from *S. haematobium* could be used to detect developing schistosomes in the snail hosts, suggesting this may be of epidemiological importance (Hamburger *et al,* 2001). Parasite-specific DNA fragments can also be amplified from blood and stool taken from people infected with any of the major schistosomes. However, the use of urine residue after filtering through filter paper as a source of parasite-specific DNA has greatly simplified the process (Abbasi *et al*, 2012).

Molecular epidemiological studies of schistosomiasis have provided opportunities to investigate many important topics such as the contribution of parasite genetics to variation in disease burden and pathology, the genetic consequences of various control ac-

tivities for parasite populations, patterns of recruitment and transmission in endemic areas, and the likely evolution and spread of drug resistance (Gower *et al,* 2011).

The present study aimed to identify the different genotypes of *Schistosoma haematoium* in Sohag Governorate.

Patients and Methods

Urine samples of 100 out-patients were collected from the Clinical Pathology Laboratories, Sohag University's Hospital and Sohag Tropical Medicine Hospital, with symptoms suggestive for urinary schistosomiasis as burning micturition or terminal haematuria.

Urine samples were collected between 9am and 12pm in a sterile, clean and labelled urine container. Urine volume in each sample was about 30ml. Living *S. haematobium* eggs were received from Theodor Bilharz Research Institute, Giza and used as a positive control for the *S. haematobium* eggs for immunological study. The remaining urine was centrifuged, and deposit put in Eppendorf tubes at -20°C for DNA extraction process (Isenberg and Garcia, 2010). Extraction process was done from the preserved positive urine samples after defreezing using Thermo Scientific Gene JET

The whole blood genomic DNA purification Mini Kit (Thermo-Scientific, Cat. No. K0781). The functional quality of purified genomic DNA is evaluated by PCR amplification of a single-copy gene and by digestion with restriction enzymes. The extracted DNA from all positive samples showed variable DNA molecular weight ranging from low to high concentrations, so samples with very low DNA concentration were excluded before the reaction for all samples. Actual samples for RAPD-PCR were 27 samples. For amplification protocol using Taq PCR Master Mix Kit, Qiagen, Genomic DNA of *S. haematobium* (10ng) was used with a total reaction volume of 20µl in a DNA thermal cycler (Veriti ™ 96-well thermal cycler (9902, Singapore). Reaction was 40-cycles each for 1minute at 95°C, 2 minutes annealing at35°C, extension for 2 minutes at 72°C with transition time 1°C/s between different temperatures, and then for 7 minutes at 72°C and five the 10-bp oligonucleotide primers were designed (Shiff *et al,* 2000). Primers sequences were in (Tab. 1).

Table 1: Selected Primers sequence

DNA Primer sequence	
CAGGCCCTTC	A01
TGCCGAGCTG	A02
TCGGCGATAG	A12
CAGCACCCAC	A13
AGCCGTGGAA	Y20

DNA electrophoresis was done on a 1.2% agarose gel in TE buffer stained with ethidium bromide. Samples were run with two molecular weight standards: high molecular weight and a low-molecular weight 100-bp ladder (Gibco, Waltham, Massachusetts, USA). PCR products were visualized under UV light and photographed using Polaroid 667film by Polaroid Corporation, Waltham, MA. An analysis software program was used in Lab™ 4.1 (Kapelan Bio-Imaging GmbH, http://www.kapelanbio.com, Sun Microsystems Inc.), an image acquisition and analysis software to analyse digital image data from electrophoresis gels & blots. DNA bands from different isolates produced by RAPD-PCR were compared to positive control with each primer. Comparison between isolates was done by scoring the total bands of each isolate, determination of sharing bands between isolates. The similarity between isolates was estimated by index (F) = 2nxy (nx+ny) ×100, as nx & ny represented all bands generated by isolates x & y, respectively, and nxy represented the shared bands (Nei *et al,*1979)

Chi-square and Fisher's exact tests were used to compare categorical variables between groups. Continuous variables were compared using independent samples t-test and

one-way ANOVA test. $P < 0.05$ (two-tailed) was considered to represent statistical significance. SPSS version 26.0 (IBM, Armonk, New York) was used for the analysis.

Ethical considerations: The study was approved by the Ethics Committee, Faculty of Medicine, Sohag University. Consents were obtained from the patients or their guardians before data and sample collection with a brief explanation of the procedure and aim of the study.

Results

Fifty urine samples were positive after microscopic examination, patients were 29 males (58%) and 21 females (42%). The presenting symptoms were either haematuria in 21 patients (42%), dysuria in 18 (36%) or combined in 11 patients (22%). Samples with low DNA concentration were excluded. Out the 50 specimens, 27 samples were amplified by the selected 5 RAPD primers (A01, A02, A12, A13, & Y20) showed positive bands (54%). There were average 2 positive bands with each isolate (mean 2.29: range 1 to 7). Isolates shared in average 1 band with positive controls in each primer (mean 1.4: range 0 to 2). Total similarity index of positive samples was 56.2% (range 0-100%). Positive control was in average 2 bands with each primer (range 1 to 3) (Fig, 1).

Twenty isolates gave positive bands with primer A02, averaged 2 bands with each sample (range 1 to 4). Similarity index averaged 47.9% (range 0 to 80%). Six isolated samples had positive bands with primer A12, with an average 1 band with each sample (range 1 to 2). No shared bands with urine isolates with A12 primer and positive control without index similarity. 18 isolated samples had positive bands with primer A13. They had in average 3 bands with each sample (range 1 to 5). Similarity index averaged 62.5%. 12 isolated samples had positive bands with primer Y20. They had in average 2 bands with each sample (range 1 to 4). Similarity index was in average 60.8% (range 0 to 80%). For positive samples, similarity index was analyzed relative to sex & symptoms as well, but with neither significant between sex & positive bands with each primer (P=0.657), nor between positive bands with each primer & symptomatology (P= 0.546), or between similarity index and sex (P= 0.276), or between symptoms and similarity index (P= 0.535) as in (Fig. 2).

Discussion

Schistosoma genome is almost 270 Mbp (Simpson *et al,* 1982). Study of *Schistosoma* genetic variability and diversity is useful as a first step in understanding virulence of the parasites and the different clinical outcomes. Since major pathology of *Schistosoma* infection is linked to the immune reaction to the eggs, studying the genetic differences of different strains may reveal subtypes which are more immunogenic and hence causing more severe disease (Gasemelseed *et al,* 2014).

In the present study, the genetic diversity of *S. haematobium* infections studied by the RAPD PCR amplification technique to detect DNA sequences with 5 primers. Calculation of the similarity index was used to assess the genetic diversity within our samples. The present results showed a total similarity index between samples of average 0.56 (range 0-100%). The highest similarity among samples was in isolated bands with primer A01 (similarity index = 0.693) and the similarity index was 0 with primer A12 (20 positive samples). Similarity index with other primers A02, A13 & Y20 were 0.479, 0.625 & 0.608 respectively. Thus, more genetic diversity of samples was within locus A12 followed by A02. Genetic diversity was lower and very similar in between primers; A01, A13 & Y20, without significant correlation between sex and urinary symptoms and positive DNA bands, as well as index.

El-Kady *et al*. (2020) in Egypt studied genetic diversity of *S. haematobium* in Qena Governorate, using *S. haematobium* eggs of 50 parasitological proved patients, 20 had positive bands with RAPD-PCR amplification using primers (A02: TGCCGAGCTG, A 07: GAAACGGGTG, A09: GGGTAACGCC & A10:GTGATCGCAG). They showed

distinct polymorphic patterns from DNA amplified with A02, A07, A09 & A10, with moderate to high variation in genomic schistosome populations. Calculated the similarity index (Nei and Li, 1979) showed bands ranged 0 to 95% indicating intra-species differences among the isolates.

Afifi *et al*. (2016) studied *S. haematobium* eggs genetic diversity from 3 urine samples of heavily infected patients from Egypt, Zimbabwe, & South Africa using RAPD-PCR, and reported severe pathogenicity of the Egyptian *S. haematobium* infections compared to the mild one in the 2 other countries. The primers were (P #2: TGCCGAGCTG, P #7: GAAACGGGTG, P #9: GGGTAACGCC, P #10: GTGATCGCAG). The similarity index was 0.566 between Egypt/Zimbabwe samples, 0.551 Egypt/South Africa & 0.721 Zimbabwe/South Africa. They concluded the possiblity 2 polygenetic ones; one from mainland Africa (Egyptian isolate) and the second one from Indian Ocean neighbouring coastal regions. But, they used only one urine sample from each region and didn't study the genetic diversity of *S. haematobium* strains in other Egyptian patients.

Ezeh *et al.* (2015) studied genetic variability of human *S. haematobium* eggs in Mali and Nigeria areas, 49 samples from 4 endemic locations in Mali and Nigeria (3 locations in Nigeria & 1 location in Mali). The genotyped individually pooled of *S. haematibium* obtained from infected primary school students (total 22 patients from Nigeria and 27 patients from Mali). They used the primers (A1, A6, B4, & C2). Mean allele number ranged from 2.3 to 5.9, and size ranged 110-232bp at locus A1, 103-364 at A6, 118-365 at B4, & 107-360 at C2. Mean alleles number was significantly higher in samples obtained from Mali compared to those from Nigeria in A6, B4 and C2 loci but not A1. No significant differences were detected between samples from the 3 location in Nigeria. They concluded that there was a high allelic diversity of *S. haematobium* in patients from Mali 7 Nigeria, and assumed that this genetic diversity was related to the prevalence and response to treatment.

Gasmelseed *et al.* (2014) in Sudan using same primers to study the genetic diversity of *S. haematobium* and pathogenic severity on 83 students with age range 6-20 years. Eggs were obtained by urine filtration technique, and trans-abdominal ultrasound evaluated pathology. *S. hematobioum* DNA was extracted from eggs in the collected samples, and genotyping was performed using RAPD PCR with the same 5 primers. In the study, 85% of the subjects had abnormal scans, and 72.3% (60 samples) had positive bands for A01 primer, while no product was detected for the other primers (A02, A12, A13, & Y20). A01 showed three different genotypes (A01-1, A01-2 & A01-3). They didn't give significant correlation between the severity of infection and the genotypes.

Sady *et al*. (2015) in Yemen reported that from pooled schistosome DNA from urine and stool samples gave information about the biology of human *Schistosoma* species, and *S. mansoni* showed a high genetic diversity, but *S. haematobium* showed a low one.

Conclusion

To the best of the authors' knowledge, this may be one of the few studies to characterize the genetic diversity of human *S. haematobium*. The amplification patterns of the *S. haematobium* isolates showed distinct variation in size and number of amplified fragments, indicating high genetic polymorphism among isolates in Sohag Governorate.

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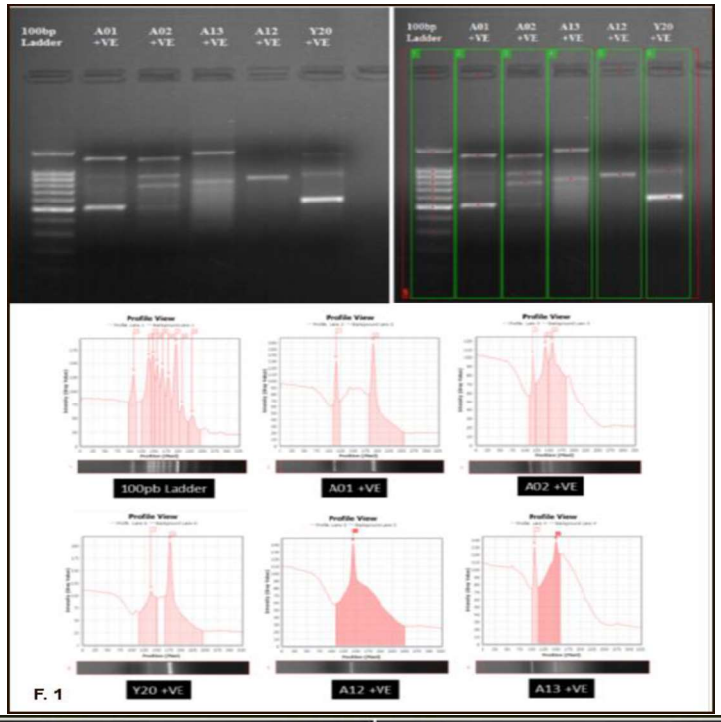
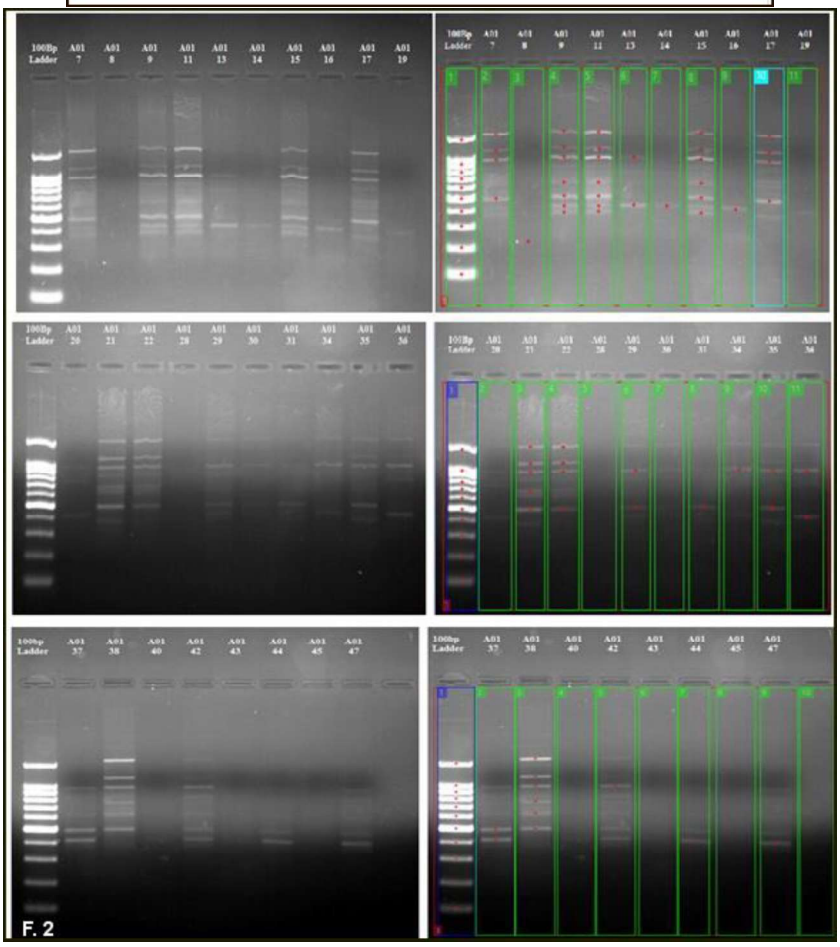
Explanation of figures

Fig.1: 1.2% agarose gel showed random amplified polymorphic DNA, PCR pattern generated by Primers A01, A02, A13, A12 & Y20 for positive controls with image analysis of individual bands using Lab™ 4.1 (Kapelan Bio-Imaging GmbH, http://www.kapelanbio.com/Sun Microsystems Inc.).

Fig. 2: 1.2% agarose gel showing random amplified polymorphic DNA, PCR pattern generated by Primers A01


100bp Ladder
A01 +VE
A02 +VE
A13 +VE
A12 +VE
Y20 +VE
100pb Ladder
A01 +VE
A02 +VE
Y20 +VE
A12 +VE
A13 +VE

F. 1


100Bp Ladder
A01

F. 2