Comparative Results Of Rapd-Pcr Genetic Polymorphism Of Schistosoma Haematobium

Amal A. Abd Elmawgood, Nada A. Nadi, Shaimaa M. Khalil And Eman Kh. Omran

*Department of Medical Parasitology, Sohag Faculty of Medicine, Sohag University. Egypt

Abstract

Schistosomiasis is one of the public health threats in Egypt. *S. hematobium* infection significantly increases the risk of urinary bladder metaplasia and cancer. The study of the genetic diversity of *S. hematobioum* might help predict the severity of the disease and the resistance to treatment. In the present study, the DNA of the *S. hematobium* was amplified using RAPD-PCR from 27 urine samples and in addition, a positive control sample was used. We used RAPD-PCR primers (A01, A02, A12, A13, and Y20). DNA electrophoresis was done on a 1.2% agarose gel and samples were run with high molecular weight and a low-molecular weight100pb ladder. The samples generated a total of 182 bands with 5 primers. The average band numbers were 3 with primer A01, 2 with primer A02, 1 with primer A12, 3 with primer 3, and 2 with primer Y20. The average molecular weight for the generated bands with each primer was 938pb, 738pb, 1425pb, 1006pb, and 866pb respectively. Our results show genetic polymorphism in the samples specifically in primers A02, A13, and Y20. This genetic polymorphism was evident by the differences in the band numbers and weights (allelic composition). Further research is required to assess the relationship between genetic polymorphism and pathology caused by *S. hematobium* infection and finally the response to treatment.

Keywords:- S. hematobioum, RAPD-PCR, genetic polymorphism

Introduction

Schistosomiasis could be a parasitic illness caused by the digenetic trematodes of the genus Schistosoma. Among the species of schistosomes infecing people, Schistosoma haematobium was mindful for the biggest number of infections in sub-Saharan Africa and one of the widespread causing urogenital schistosomiasis, a major human health problem in Africa [1].

Schistosomiasis is characterized by a variety of chronic symptoms, as well as acute manifestations, caused by the host's strong immune responses to parasite eggs lodged in host tissues of the mesenteric veins or nested in specific organs, such as the liver [2].

The occurrence of urinary tract schistosomiasis in the Nile Valley, from Delta to Upper Egypt is well known. Snails of the species B. truncates have also been found in water sources around Egypt. Previous surveys were carried out in villages spread along Qena Governorate, Upper Egypt to detect the prevalence of S. haematobium in human populations. It revealed that the estimated prevalence of S. haematobium was 13.9% [3].

Prior research has shown that the extent of pathology caused by *S. haematobium* in Sub-Saharan African and South African countries is less extreme than in Egypt. Despite high prevalence rates, morbidity of urinary schistosomiasis in these countries is moderate and does not pose a significant public health threat [4].

In Egypt, urinary schistosomiasis is commonly expressed as obstructive uropathy, which predisposes to pyelonephritis, lower urinary tract infections, and urinary calculi. It also significantly increases the risk for urothelial metaplasia. The latest research has opened new opportunities for understanding how parasite infection can lead to cancer and other pathologies [5].

A variety of diagnostic procedures to detect schistosome infection have been compared. These procedures include tests for circulating antigens, specific antibodies, egg detection, and ultrasound scans of the urinary tract. However, the diagnostic performance of these techniques is variable, and it is difficult to set anything like a "gold" standard in areas with variable S. haematobium prevalence [6].

Molecular epidemiological studies of schistosomiasis 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 activities for parasite populations, patterns of recruitment and transmission in endemic areas, and the likely evolution and spread of drug resistance [7,8].

Polymerase chain reaction (PCR) is a Nobel-prize-winning nucleic acid amplification technology that allows minute amounts of genetic material to be amplified into billions of copies in just a few hours. PCR tools were utilized in detailed insights into molecular epidemiology, analysis of whole genomes through sequencing DNA, and enhancement in sensitivity of parasitic detection [9].

Patients and methods:

This study was carried out in the period between December 2015 to December 2017. Urine samples were collected from 100 patients who were complaining of burning micturition or hematuria. Samples were collected from the Laboratories at Sohag University Hospital and from Sohag Tropical Medicine Hospital, which is considered a tertiary referral center covering almost all cities of Sohag Governorate. urine samples were concentrated by centrifugation at 1,500 rpm for 5 min, and the pellets were examined microscopically.

Fifty urine samples with positive microscopic examination for S. haematobium were used for this study. Of the 50 samples, 27 had adequate DNA concentration to proceed with RAPD-PCR analysis using 5 primers according to Shiff et al [10]:

Table 1: Selected Primers sequence

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

The primers were designed. A positive control sample was included (Theodor Bilharz Research Institute, Giza, Egypt.).

DNA extraction was performed using Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Cat. No. K0781). The amplification protocol

used the Taq PCR Master Mix Kit, Qiagen. Genomic DNA of S. haematobium (10 ng) with a total reaction volume of 20 μL in a DNA thermal cycler (Veriti ™ 96-well thermal cycler (9902, Singapore). The reaction was 40 cycles, each of 1-minute denaturation at 95◦ C, 2 minutes of annealing at35◦ C, and extension for 2 minutes at 72◦ C with a transition time of 1◦ C/s between the different temperature phases. Final extension for 7 minutes at 72◦ C.

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). The PCR products were visualized under UV light and photographed using Polaroid 667 film (Polaroid Corporation, Waltham, MA).

Lab™ 4.1 (Kapelan Bio-Imaging GmbH, Sun Microsystems Inc.) is an image acquisition and analysis software that was used to analyze digital image data from electrophoresis gels and blots. The molecular weights of the positive isolates were analyzed and plotted with each primer to assess the possible genetic groups available with each allele.

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 aimof the study.

Results

Twenty-seven urine samples from our laboratories and a control sample with microscopically positive S. haematobium had RAPD-PCR using the selected primers. There was an average of 2 positive bands with each isolate (mean 2.29 – range 1 to 7) (Table 2).

Table 2: Generated bands and average molecular weight with each primer

Primer	Total No of bands	Average no of bands	Average molecular weight (pb)
A01	61	3	938
A02	31	2	738
A12	7	1	1425
A13	56	3	1006
Y20	27	2	866

Positive control: The molecular weights of the generated bands were 1375 and 514pb for primer A01, 1375, 974, and 808pb with primer A02, 1550, and 875pb with primer A13, and 1027 and 611pb with primer Y20. It generated a single band with primer A12, and its molecular weight was 984pb.

Primer A01: CAGGCCCTTC : Twenty isolated samples had positive bands. The generated bands were between 1 to 7 (average 3) for each positive sample. The molecular weight of the generated bands ranged from 315 to 1662pb and on average 938pb. When Plotting the molecular weights of the positive bands with Primer A01, positive samples showed very similar bands including the positive control (Figure 1).
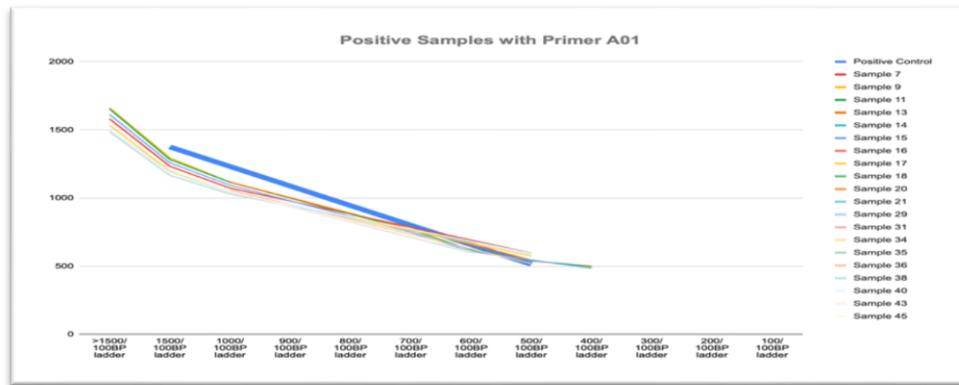
Figure 1: Plotting the molecular weights of the positive bands with Primer A01. Positive Samples showing very similar bands including the positive control (thick blue line)

Primer A02: TGCCGAGCTG

Twenty isolated samples had positive bands with primer A02. The generated bands were between 1 to 4 bands and on average 2 bands. The molecular weight of the generated bands ranged from 462 to 1254pb and on average 738pb. Plotting the molecular weights of the positive bands with Primer A02 shows 3 groups of bands: (1) the positive control, (2) sample 17, (3) samples 7, 9, 11, 13, 15, 22, 29, 30, 31, 34, 36, 37, 38, 42, 44 and 45 (Figure 2).
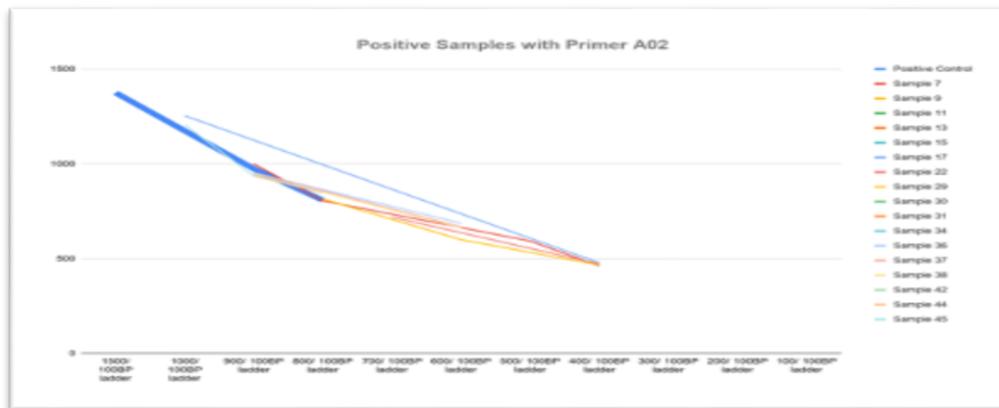

Figure 2: Plotting the molecular weights of the positive bands with Primer A02. Three groups of bands can be seen;
(1) the positive control (thick blue arrow), (2) sample 17 (light blue),
(3) samples 7, 9, 11, 13, 15, 22, 29, 30, 31, 34, 36, 37, 38, 42, 44 and 45

A12: TCGGCGATAG

Six isolated samples had positive bands with primer A12. The samples generated between 1 to 2 bands. The molecular weight of the generated bands ranged from 337 to 1914pb and on average 1425pb.

A13: CAGCACCCAC

Eighteen isolated samples had positive bands with primer A13. The generated bands were between 1 to 5 bands and on average 3 bands. The molecular weight of the generated bands ranged between 586 to 2594pb and on average 1006pb. Plotting the molecular weights of the positive bands with Primer A13 shows 3 groups of bands: (1) the positive control, (2) samples 7, 9, 11, 3, 34, 35, 36, 37, 42, and (3) samples 17, 21, 22, 29, 30, 38, 44, 45 and 47 (Figure 3).
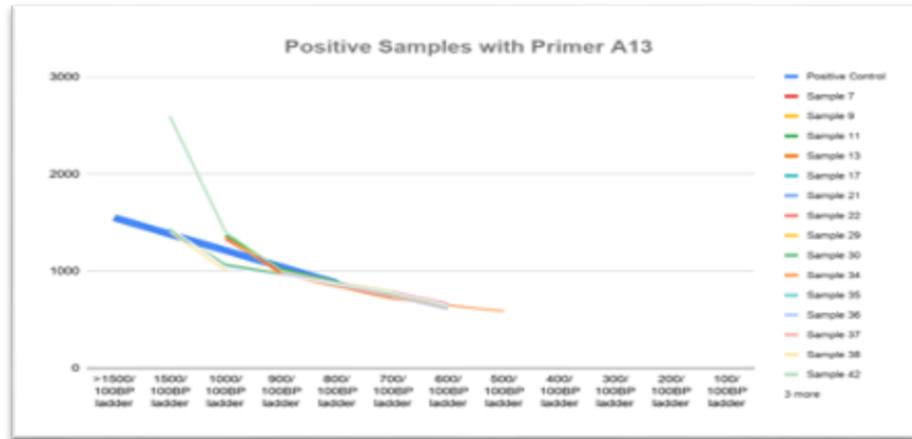
23

Figure 3: Plotting the molecular weights of the positive bands with Primer A13. Three groups of bands can be seen;
 (1) the positive control (thick blue arrow),   **(2)** samples 7, 9, 11, 13, 34, 35, 36, 37, 42 and **(3)** samples 17, 21, 22, 29, 30, 38, 44, 45 and 47.

Y20: AGCCGTGGAA

Twelve isolated samples had positive bands with primer Y20. The generated bands were 1 to 4 bands, on average 2 bands. The molecular weights of the generated bands were 192 to 1685pb and on average 866pb. Plotting the molecular weights of the positive bands with Primer Y20 shows two groups of bands: the positive control, samples 7, 9, 17, 21, 22, 29, 31 and 38 (Figure 4).
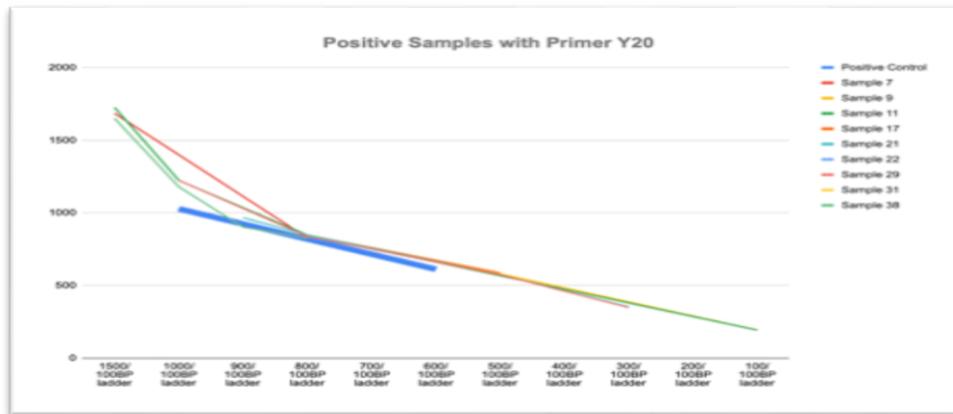

Figure 4: Plotting the molecular weights of the positive bands with Primer Y20. Two groups of bands can be seen; the positive control (thick blue arrow), samples 7, 9, 17, 21, 22, 29, 31 and 38.

Discussion:

Recent studies in Egypt were done to detect genetic polymorphism of S. haematobium in natural isolates using different PCR techniques as randomly amplified polymorphic DNA (RAPDs) markers which may be of great value to understanding pathogenesis and drug resistance of urogenital Schistosomiasis. These studies revealed that amplification patterns of the extracted S. haematobium isolates showed distinct variation in the size and number of amplified fragments which indicates that there is high genetic polymorphism among natural isolates in, Upper Egypt [11].

In the current study, 27 out of the 50 urine samples with microscopically

positive *S. hematobium* examination had adequate DNA concentration and produced positive RAPD-PCR bands. Our results show genetic polymerphism in the samples specifically in primers A02, A13, and Y20. This genetic polymorphism was evident by the differences in the band numbers and weights (allelic composition).

According to Quan et al., regarding the genetic diversity of *S. hematobium* in Sudan, only 13 samples produced the expected PCR bands with A4 and B2 which was explained by the number of the *S. hematobium* eggs might not be adequate in urine or due to the genetic polymorphism [12].

Allelic composition in terms of numbers and molecular weight was used by Ezeh et al, to study the genetic diversity of *S. hematobium* infection in Mali and Nigeria. They couldn't find a significant difference in the allelic composition among samples collected from 3 different sites in Nigeria. On the other hand, when they compared the allelic composition of samples collected from Nigeria and Mali, they found significant differences at 3 loci out of 4. Furthermore, Ezeh et al., found that the high prevalence of the *S. hematobium* infection in Mali (60% in 2003) compared to 23.2% in Nigeria can be explained by the allelic richness of the samples collected from Mali. In other words, low infection prevalence in Nigeria would in turn reduce the genetic diversity [13].

Afifi et al., used infected urine samples with *S. hematobium* from Egypt, Zimbabwe, and South Africa to study the genetic diversity using RAPD-PCR. The used the primers (P #2: TGCCGAGCTG, P #7: GAAACGGGTG, P #9: GGGTAACGCC, P #10: GTGATCGCAG). The results showed 12 positive bands in the Egyptian sample with P#2, 0 with P#7, 13 with P#9, and 16 bands with P#10. On the

other hand, the samples from Zimbabwe and South Africa produced 15 and 14 bands respectively with P#2, 21, and 16 bands with P#7, 16, and 14 bands with P#9 and 13 bands for each country with P#10. Analyzing shared bands between isolates, great band sharing could be seen between Zimbabwean and South African isolates (44 bands) while there is moderate sharing between Egyptian and Zimbabwean specimens (30 bands) and Egyptian and South African isolates (27 bands). This might in turn explain the difference in virulence between the Egyptian disease and the Indian ocean neighboring coastal areas [14].

Gasmelseed et al., conducted another study of the genetic diversity of *S. hematobium* from 83 samples in Sudan. They used the same primers used in our study. Their samples showed positive products with primer A01 and no products were detected for the other primers. They could detect 3 genotypes with primer A01 due to polymorphic bands in markers 700, 800, 900, and 1100pb [15].

A cross-sectional study was carried on in five areas in Yemen. Schistosoma eggs were found in the samples after they were screened. To assess the genetic variation of the *S. mansoni* and S. haematobium infections, a partial fragment of the schistosome cox1 mitochondrial gene was analyzed from each human sample. The research discovered nineteen distinct haplotypes of *S. mansoni*, which were divided into four lineages. Furthermore, nine different haplotypes of *S. haematobium* were found, which could be classified into two classes [16].

In a recent study from Qena governorate conducted by El-Kady et al., they could amplify the DNA of 20 samples out of 50 positive *S. hematobium* urine samples using RAPD-DNA. A total of 40 generated bands could be seen with a range of 9 to 12 bands per primer and

the size of the bands was from 198pb with primer A09 to 1860pb with primer A10. The polymorphism of the generated bands was 100% with all primers and their results, therefore, showed a high degree of genetic variation [11].

References:

1 Barakat RMR. Epidemiology of Schistosomiasis in Egypt: Travel through Time: Review. J Adv Res 2013;4:425–32. https://doi.org/10.1016/j.jare.2012.07.003.

2. Bustinduy AL, King CH. Schistosomiasis. Manson's Tropical Infectious Diseases, Elsevier; 2014, p. 698-725.e6. https://doi.org/10.1016/B978-0-7020-5101-2.00053-4.

3. Sayed FG, Alsalahy M, Abd-el-Kader MS, Abdalla OH, Ahmed SS. Prevalence of schistosomiasis among patients attending Qena General Hospital over the years 2011 and 2012. J Egypt Soc Parasitol 2014;44:489–95. https://doi.org/10.12816/0006487.

4.] Brouwer KC, Ndhlovu PD, Wagatsuma Y, Munatsi A, Shiff CJ. Epidemiological assessment of Schistosoma haematobium-induced kidney and bladder pathology in rural Zimbabwe. Acta Trop 2003;85:339–47. https://doi.org/10.1016/s0001-706x(02)00262-0.

5. Santos LL, Santos J, Gouveia MJ, Bernardo C, Lopes C, Rinaldi G, et al. Urogenital Schistosomiasis-History, Pathogenesis, and Bladder Cancer. J Clin Med 2021;10. https://doi.org/10.3390/jcm10020205.

6. Koukounari A, Webster JP, Donnelly CA, Bray BC, Naples J, Bosompem K, et al. Sensitivities and specificities of diagnostic tests and infection prevalence of Schistosoma haematobium estimated from data on adults in villages northwest of Accra, Ghana. Am J Trop Med Hyg 2009;80:435–41.

7. Ibironke OA, Phillips AE, Garba A, Lamine SM, Shiff C. Diagnosis of Schistosoma haematobium by detection of specific DNA fragments from filtered urine samples. Am J Trop Med Hyg 2011;84:998–1001. https://doi.org/10.4269/ajtmh.2011.10-0691.

8. Gower CM, Gabrielli AF, Sacko M, Dembelé R, Golan R, Emery AM, et al. Population genetics of Schistosoma haematobium: development of novel microsatellite markers and their application to schistosomiasis control in Mali. Parasitology 2011;138:978–94. https://doi.org/10.1017/S0031182011000722.

9. Ahmed M, Singh MN, Bera AK, Bandyopadhyay S, Bhattacharya D. Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. Asian Pac J Trop Med 2011;4:589–93. https://doi.org/10.1016/S1995-7645(11)60152-1.

10. Shiff C. Accurate diagnostics for schistosomiasis: a new role for PCR? Reports in Parasitology 2015. https://doi.org/10.2147/RIP.S74319.

11. El-Kady AM, El-Amir MI, Hassan MH, Allemailem KS, Almatroudi A, Ahmad AA. Genetic Diversity of Schistosoma haematobium in Qena Governorate, Upper Egypt. Infect Drug Resist 2020;13:3601–11. https://doi.org/10.2147/IDR.S266928.

12. Quan J-H, Choi I-W, Ismail HAHA, Mohamed AS, Jeong H-G, Lee J-S, et al. Genetic Diversity of Schistosoma haematobium Eggs Isolated from Human Urine in Sudan. Korean J Parasitol 2015;53:271–7. https://doi.org/10.3347/kjp.2015.53.3.271.

13. Ezeh C, Yin M, Li H, Zhang T, Xu B, Sacko M, et al. High genetic variability of Schistosoma haematobium in Mali and Nigeria. Korean J Parasitol 2015;53:129–34.

https://doi.org/10.3347/kjp.2015.53.1.1
29.

14. Afifi MA, Jiman-Fatani AA, Al-Hussainy NH, Al-Rabia MW, Bogari AA. Genetic diversity among natural populations of Schistosoma haematobium might contribute to inconsistent virulence and diverse clinical outcomes. J Microsc Ultrastruct 2016;4:222–7. https://doi.org/10.1016/j.jmau.2016.04.002.

15. Gasmelseed N, Karamino NE, Abdelwahed MO, Hamdoun AO, Elmadani AE. Genetic diversity of Schistosoma haematobium parasite IS NOT associated with severity of disease in an endemic area in Sudan. BMC Infect Dis 2014;14:469. https://doi.org/10.1186/1471-2334-14-469.

14. Sady H, Al-Mekhlafi HM, Webster BL, Ngui R, Atroosh WM, Al-Delaimy AK, et al. New insights into the genetic diversity of Schistosoma mansoni and S. haematobiumin Yemen. Parasit Vectors 2015;8:544. https://doi.org/10.1186/s13071-015-1168-8.