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Genetic characterization of *Biomphalaria arabica*, the molluscan intermediate host for schistosomes in Saudi Arabia

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Abstract

The present study was a comparative analysis of genetic variability of *Biomphalaria arabica,* the intermediate hosts of *Schistosoma mansoni* from two different areas having different prevalence level of *Schistosomiasis* in Saudi Arabia. *Biomphalaria arabica* samples from Riyadh and Hofuf area were studied at the genotypic level by RAPD analysis in an attempt to associate possible polymorphic markers related to susceptibility to infection by the *Schistosoma mansoni*. A total of 9 primers were tested and polymorphic markers were obtained. The results showed polymorphism with 3 primers and suggested that RAPD represents an efficient means of genome comparison, since many molecules markers were detected as genetic variations between Riyadh and Hofuf snails. The present study confirms that genetic profile of *Biomphalaria arabica* is critically important for the success of *schistosome* life cycle and that snail host plays at least a part in prevalence of the disease.

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Introduction

Schistosomiasis or bilharzias is primary tropical parasitic disease that was first described in 1851 by Theodor Bilharz. It is caused by blood-dwelling fluke worms of the genus *schistosoma* that reside in the abdominal veins of their vertebrate definitive hosts. (Chitsulo et al, 2000). In Saudi Arabia *Biomphalaria arabica* is an intermediate host for *Schistosoma mansoni*. Of the 207 million people with schistosomiasis, 85% live in Africa. Other regions affected are the Americas (Brazil, Suriname and Venezuela, as well as several Caribbean islands); the Eastern Mediterranean (Islamic Republic of Iran, Iraq, Saudi Arabia, Syrian Arab Republic and Yemen); and eastern Asia (Cambodia, China, Indonesia, Japan, Lao People's Democratic Republic and the Philippines) (WHO fact sheet, 2010) Among human parasitic diseases, schistosomasis is the second most prevalent tropical diseases, after malaria, and is a leading cause of severe morbidity in many parts of the world. Human infection with both intestinal and urinary schistosomiasis has a wide distribution in the kingdom of Saudi Arabia (Ashi, 1989). The prevalence of schistosomiasis is clustered in the Eastern and Southwestern provinces, due to the preferable environmental conditions (Arfaa, 1976). Other factors may contribute to the increase in prevalence of the infection including the large number of expatriates, many from countries with higher prevalence of schistosomiasis, and hence the possibility of parasitic infection among them. (Abahussain 2005).

The analysis of the genetic variability to susceptibility of *Schistosoma mansoni* infection in the vector of the genus *Biomphalaria* is important in term of a better understanding of the epidemiology of schistosmiasis , the possible pathological implications of this interaction in the vertebrate hosts and the formulation of new strategies and approach to control the disease. (Spade et al 2002). For genetic control of schistosomiasis, one strategy is based on the premise that snail resistant to parasitic infection could be used as biological competitors to replace exisiting susceptible snail in endemic areas (Coelho et al.2004). This approach however, require a more thorough understanding of the genetic of the complex interrelationship between parasites and snails (Abdul Hamid *et al.,* 2006).

Several attempts have been made to determine the genetic variability among schistosomiasis intermediate hosts by using protein electrophoresis, allozyme phenotypes analysis and other molecular biology techniques (Mulvey and Woodruff, 1985., El-Khayat et al., 2008) Recently, several investigators showed that random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is useful for distinguishing the genetic differences between and within different *Biomphalaria* snail species (Abdel-Hamid et al., 1999., Knight et al., 1999., Oliveira et al., 2008) This technique (Welsh and Mc Clellan 1990, William et al 1990) has amplified the possibility of polymorphisms analysis and provide a screening method to identify region of genomic amplification ,deletion or rearrangement without the need of previous knowledge of genes and or genomic sequence being investigated. It has been successfully used for typing the geographical variants in *Schistosoma mansoni* (Pillay and Pillay 1994) and white fly (Perring et al 1993). In this study, we analyzed the genetic variability between *Biomphalaria arabica,* from Riyadh and hofuf areas by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) in an attempt to correlate possible polymorphic markers allied to susceptibility to infection by the *Schistosoma mansoni* as these two areas are having different prevalence level of Schistosomiasis in Saudi Arabia

Material and method

Snail collection and maintenance

Specimens of *B.arabica* snails were collected from Riyadh and Hofuf cities and were maintained in the laboratory under suitable conditions of aeration and temperature. They were fed with lettuce leaves. A sample of snails was randomly chosen and soft tissues were extracted according to the method previously described by (Nabih et al., 1989).

Table 1. Primers used for the amplification gene from tissues.

Primer name	Sequence
RAPD1	5'-GGGTAACGCC-3
RAPD2	5'-CTGATGCTAC-3'
RAPD4	5'-AGTGCTACGT-3'
RAPD8	5'- CTCTCCGCCA-3'
RAPD9	5'-CAGGCCCTTC-3'
RAPD10	5'-GGTCCCTGAC-3'
RAPD12	5'-AGGGAACGAG-3'
RAPD14	5'- GTTGCCAGCC-3'
RAPD15	5'-TGCCGAGCTG-3'

DNA extraction

Genomic DNA was extracted from the soft parts of snails by using DNA tissue mini Kit from QIAGEN (Surrey, UK). The concentration of extracted DNA was determined by spectrophotometrically. Samples were labeled and stored at -70 °C .

DNA amplification by RAPD-PCR

DNA samples were amplified using RAPD-PCR. Each reaction was carried out in puReTaq Ready-To-Go PCR Beads which contain: stabilizer, BSA, dATP, dCTP, dGTP, dTTP, ~ 2.5 units of puReTaq DNA polymerase, and reaction buffer. Nine primers were used each with product size (10bp) as shown in Table-1. The amplification conditions were as follows: initial denaturation at 95°C for 5 min, followed by two cycles of: 95°C for 30 sec, at 30°C for 2 min and at 72°C for 1 min, followed by 33 cycles of 95°C for 30 sec, 40°C for 2 min, 72°C for 1 min. followed by 72°C for 5 min. The PCR products were stored at -20°C.

Gel analysis

After amplification, each PCR product and 100- base pair DNA ladder (Invitrogen) were run in a 1.5% agarose gel and the banding pattern of the randomly amplified DNA was visualized with silver staining The gel was visualized using the Alphaimiger imaging system (Alpha innotech Corporation) imaged using Mitsubishi P93D digital monochrome printer.

Analysis of polymorphisms

PCR products were also analyzed by eGene HDA-GT12™ obtained from genomic technology company e- Gene, inc. (Irvine, USA). The GC-5000 gel cartridge was used, as it can separate fragments sizes ranging from 15bp-5kb.the PCR products for each sample was diluted 5:5 with deionised water. The sample was run by 0M500 method with injection time 60 sec.

Similarity coefficient

The genetic variability between Riyadh *B.arabica* and Hofuf *B.arabica* was evaluated by analyzing the electrophoretic band patterns obtained on the gels and by determining the similarity coefficient as described by Dice (1945).

Statistical analysis

The statistical analysis of the data was carried out using one-sample t-test by Statistical Package of Social Science (SPSS) software (version 10.0), $p<0.05$ were considered significant.

Results

Analysis of RAPD in Riyadh and Hufuf sample

RAPD analysis was performed using a panel of 9 primers and DNA extracted from two different samples from Riyadh and Hofuf city. PCR products were analysed by silver staining polyacrylamide gel (Fig 5) and by eGene HDA-GT12™ (Fig. 3 and Fig. 4). eGene HDA-GT12™ was sensitive enough to detect polymorphisms between samples compared to standard silver stained polyacrylamide gels. Polymorphic pattern were observed between samples from the same regions as the genomic DNA amplified with primers 4(5'-AGTGCTACGT-3') 9(5'-CAGGCCCTTC -3')in Riyadh sample .and primer 9(5'-CAGGCCCTTC -3') ,10(5'-GGTCCCTGAC -3') in hofuf sample .three polymorphic bands represented in the genomic DNA amplified with primers(4,9,14) between samples from Riyadh and hofuf.

The results obtained by Dices coefficient points out that, by using different primers ,the similarity coefficient of Riyadh and Hofuf ranging from 0.71 to 0.89 (Table 2).

Table 2. Dices similarity coefficient (*) Between Riyadh and hofuf B.arabica snail tissue.

Gel analysis	Primers		
	P4	P9	P14
Number of marked band in Hofuf snails	6	5	5
Number of marked band in Riyadh snails	10	6	6
Number of shared bands between hofuf and Riyadh samples snail (a)	5	4	4
Number of bands in hofuf but not in Riyadh snail (b)	0	0	0
Number of bands in Riyadh but not in hofuf snail (c)	4	1	1
Similarity coefficient (S)	0.71	0.89	0.89

(*) S=2a/2a+b+c

Discussion

The analysis of the genetic variability related to susceptibility to *Schistosoma mansonia* infection in the vector of the genus *Biomphalarid* is important in term of a better understanding of the epidemiology of schisomiasis itself ,the possible pathological implication of this interaction in vertebrates hosts, and the formation of new strategies and approaches for disease control. (Abdel-Hamid etal, 2006)

In the present study RAPD analysis was performed using DNA extracted from *Biomhalaria Arabica* snail tissue in Riyadh and Hofuf city .Nine primers were used , and generated a considerable number of amplification products for comparison of each profile for each primer were used based on the presence / absence of the bands

Since any shift in band is considered as a sign of difference. Among the nine primers ,three of them allow to detected polymorphic marker between Riyadh and Hofuf ,also polymorphic bands were detected using primer 4 and primer 9 samples from Riyadh city (fig 3) and in two samples from hafuf city using primer 9 and 10 (Fig. 4).

Our results indicate that RAPD primers were useful for distinguishing snail population with respect to compatible and less – compatible properties since many molecular markers were detected as genetic variation between Riyadh and Hofuf snail. This could be support through considering the work of Abdel – Hamid et al (1999) which suggested that RAPDs should be highly useful for phylogenetic analysis among closely related individuals .This suggestion is in agreement with both Barral et al. (1991)and vidigal et al.(1994) who indicated that RAPD markers are a highly resolving and helpful tools for investigation of variability. They provide a simple technology that can be used to rapidly distinguish species, strains and sexes in laboratory conditions. Also, Simpson et al (1993) proved that RADP is undoubtedly a powerful approach for analysis of genetic variation and identification of genetic markers.

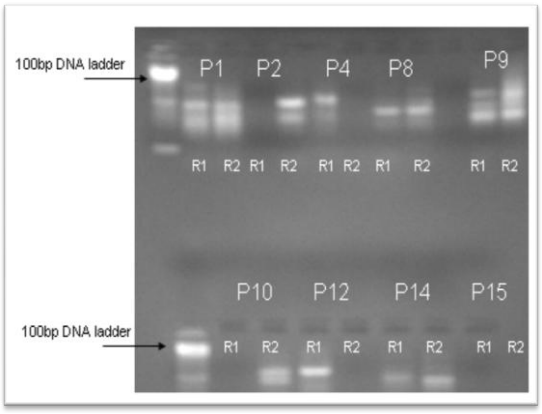

Fig. 1. RAPD using primers (P1,2,4,8,9,10,12,14,15) for PCR product from two different samples from Riyadh city after being analyzed on agarose gel electrophoresis.

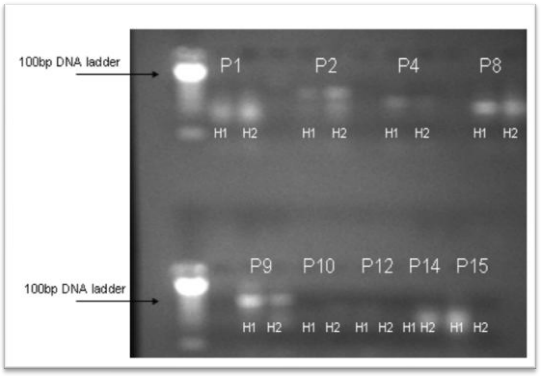

Fig. 2. RAPD using primers (P1,2,4,8,9,10,12,14,15) for PCR product from two different samples from Hofuf city after being analyzed on agarose gel electrophoresis.

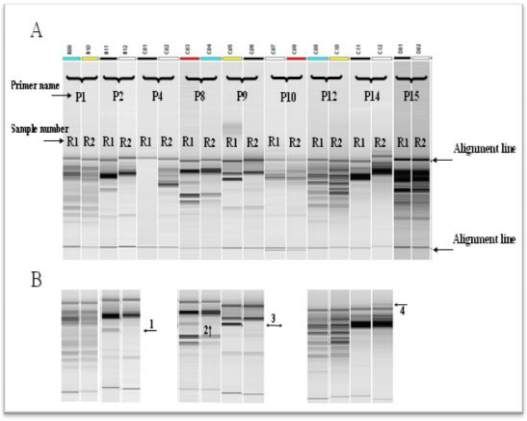

Fig. 3.

A-RAPD using primers (P1,2,4,8,9,10,12,14,15) for PCR product from two different samples from Riyadh city after being analyzed on eGene's HDA-GT12™ all samples amplified with all primer sets apart from P4 with sample 1 didn't amplify

B-represent the realigned samples of same primers, arrows 1and 2 points at a faint band at positions indicating that both samples from Al- Riyadh show the same pattern. Arrow 3 points to the position where there is difference between samples. Arrow 4 points to the band above alignment line which represents noise that is excluded. Results above prove that both samples from Al- Riyadh show have similar pattern with all markers except maker 9.

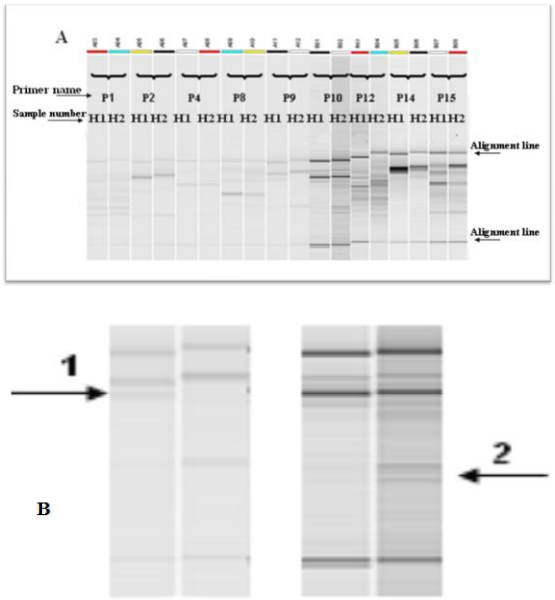

Fig. 4.

A-RAPD using primers (P1,2,4,8,9,10,12,14,15) for PCR product from two different samples from Hofuf after being analyzed on eGene's HDA-GT12™

B-represent the realigned samples of same primers, arrows 1and 2 points to the position where there is difference between samples. Results above prove that both samples from Hofuf show similar pattern with all markers except maker 9 and10.

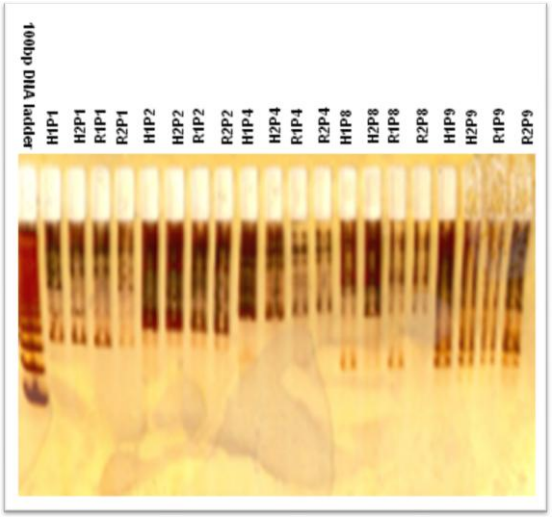

Fig. 5. RAPD using primers (P1, 2, 4, 8, and 9) for PCR product from two different samples from Riyadh city and two different sample from Hofuf city after being analyzed on polyacryamide gel followed by silver staining.

Conclusion

The comprehension of genetic basis susceptibility of snail to *S.mansoni* may, as it was proposed by Rollinson et al (1998), basis for the evolution of new strategies for the control of schistosomiasis. The polymorphic bands was detected in samples of Riyadh and Hofuf shows that there are some genetic differences between samples, which should be investigated more and optimized to be useful for further and wider analysis .the present study confirmed the previous report of El-Ansary and Qurashy (1994) and Mubila and rollison (2002) that the snail genetic profile is critically important for the success of schistosome life cycle .

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