Prevalence and Molecular Characterization of *Schistosoma haematobium* in the Pathogenesis of Human Urinary Schistosomiasis Among School Pupils in Cross River State, Nigeria

Abstract

Urinary schistosomiasis which is transmitted by schistosome species is the major cause of liver and bladder pathologies and still remains a serious threat in the underdeveloped and developing world. This study evaluates the prevalence of *Schistosoma haematobium*infection among school aged children in Biase, Obubra and Ogoja Local Government Areas of Cross River State. Five hundred (500) pupils were examined randomly from a public primary and secondary School in the study area. Freshly passed mid-day urine samples were collected and transferred to the laboratory where there were examined for the presence of *Schistosoma haematobium* eggs. Study participants were grouped into three age groups,8-10 years. 11-13 years, and 14-16 years old. Overall prevalence of *S. heamatobium*was (13.6%). Infection was more prevalent among the age group of 14-16years, the percentage of prevalence and intensity of infection were higher in males (14.1%) than in females (6.9%). Inter simple sequence repeats of PCR test performed for the collected urine samples using ISSR test reveals 73% study subjects had a polymorphism for UPA02 and UPA13 primers, while primer UPA13 showed 24% polymorphism. Total number of polymorphic bands were 2 each for primers UPA02 and UPA13 primers while UPA12 showed only one polymorphic band. Major allele frequencies (MAF) were 0.53 for each of UPA02 and UPA 13 primers but showed 0.71 frequency with UPA12 primer. Allele frequencies (AF) also varied slightly among the primers used. UPA02 and UPA 13 had allele frequencies of 8 each while UPA12 had 4 allele frequencies (Table 13). Nei's genetic diversity indices for the primers revealed variations among the different primers. UPA02 and UPA13 Nei's gene diversity of 0.64 each while primer UPA12 showed gene diversity of 0.28. Results of polymorphic information content showed that primers UPA02 and UPA13 discriminately revealed a PIC of 0.68 while UPA12 discriminated 0.28 PIC. This study therefore, revealed a critical need for targeting health campaign towards school age children and heads of households in order to empower them with the basic knowledge to recognize, treat and manage their health challenges.Applications of one to two doses of praziquantel considerably reduced the severity of urinary Schistosomiasis in the study area.

Keywords: Schistosomiasis, molecular characteristics, haematobium, pathogenesis

Introduction

Human *schistosomiasis* is a chronic disease caused by trematode worm of the genus *Schistosoma* that is prevalent in developing countries such as Nigeria. *Schistosomiasis* has a significant variation in morbidity and pathology among human hosts. However, the diversity among *S. haematobium* species may explain many ambiguous aspect of the parasite like epidemiology, susceptibility to infection and Response to treatment (Gower *et al*., 2013). The genetic variability within *S. haematobium* species is enormously understudied compared to that of its counterparts.Thus, little is known about the genetic diversity of natural *S. haematobium* population in the study area. All *S. haematobium* group species utilize *Bulinus spp*. For transmission, as the infective cercariae that emerge from snail cannot be identified easily by morphological examination. There is therefore the need to provide reliable molecular markers to enable for the identification of differentiate species. This is important in endemic areas targeted for transmission control wherever *S. haematobium* co-exist (Christensen *et al.,* 1983; Rollinson*et al.,* 2001). Although *S. haematobium* is considered morphologically uniform, it is known that the species adapt to different geographical locations. These characteristics could be due to differences in the population genetic.

Furthermore, using molecular tools for studying the population genetic structure will help to determine changes in gene frequencies, provide insight into the effectiveness of treatment, understanding the impacts of treatment on gene and population structure of *Schistosoma* parasites and establish whether movement of humans from endemic to known endemic areas would introduces new parasites into the local population as there is a link between *Schistosoma* infection intensity, transmission and the genetic structures of worm population (Agola*et al.,*2009).

Schistosomiasis otherwise known as Bilharzia is caused by *Schistosomes*parasitic trematodes worms of the genus *Schistosoma*. Five species infect humans, namely: *S. mansoni, S. japonicum, S. mekongi, S. intercalatum*and *S. haematobium*. *Schistosoma haematobium* is endemic in Africa. Varying in distribution and magnitude of disease burden, *S. haematobium* is widely distributed throughout Africa. Therefore, it is a major public health problem only second to malaria (Derbew *et al.,* 2013). *Schistosomiasis* apart from malaria is the most prevalent parasitic infection worldwide with up to 207 million people infected, and nearly 800 million currently at risk (Ross *et al.,* 2002). WHO (2000) reported more than 200,000 deaths per year due to Schistosomiasis worldwide.

In Nigeria, it is estimated to have 200 million people infected with Schistosomiasis giving an estimated 18.5% of infected population with school age children carrying the greatest burden of the disease (Useh, 2013). The endemicity of schistosomiasis in Nigeria has remained embarrassing and increasing since the last two and a half decades (Ejezie*et al.,* 1989). The most prevalent cause of Schistosomiasis infection in Nigeria is to *S. heamatobium* with as high as a prevalence of 60-75% among school children in some communities, Transmission is initiated by release of eggs into bodies of water. The eggs laid by the adult female worms pass in the host's faeces or urine and each hatches in water to release a miracidium. These free-swimming larvae must find and penetrate an appropriate freshwater snail (Bulinusglobosus). Once it has penetrated the snail, the miracidium transforms into a mother sporocyst which produces multiple daughter sporocysts through asexual reproduction. Urinary schistosomiasis is caused by *S. haematobium*, with some snails of the genus Bulinus acting as the intermediate hosts in turn produce cercariae which are released into water and are infective to human hosts, thus completing the life cycle. Although *schistosomes* are considered morphologically uniform, it is known that strains from the same or different geographical locations have shown differences in

egg production, infectivity, pathogenicity and susceptibility to chemotherapy(Allen *et al.,* 2002). These characteristics could be due to difference in the population genetic structure of *Schistosomes*.

Therefore, in elucidating the distribution of the parasite, genetic diversity is critical to the understanding and prediction of disease epidemiology. One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of parasite transmission and recruitment within the environment (Teesdate*et al*., 1985). Moreover, by using molecular tools, studying the population genetic structure will help to determine whether changes in gene frequencies provide insight into the effectiveness of treatment, understanding the impacts of treatment on the gene pool and population structure of *Schistosoma* parasites, and establishing whether movement of humans from endemic to non-endemic areas introduces new parasites into local populations (Gower *et al.,* 2001; Rollinson *et al.,* 2009). Genetic structuring has been reported in *Schistosom*a *mansoni* and *Schistosoma haematobium* populations from different countries in sub-Saharan Africa, indicative of isolation by distance (Coopan*et al*., 1986). In other studies in African countries, there is a link between *Schistosome*infection intensity, transmission and parasite geno-type and the genetic structure of worm populations, thegenetic structure of the parasite is believed to play a role in this phenomenon (Agola*et al*., 2009).

However, Genetic diversity is believed to have a major influence on many parasite-related characteristics, including the dynamics of transmission, host–parasite interaction, infectivity and virulence (Moorand*et al.,* 1996). The genetic variability within *S. haematobium* species is largely understudied compared to that of its more serious counterpart *Schistosoma mansoni* (Vandenboreck*et al.,*2011), primarily due to many constraints in the process of laboratory passage of the parasite and absence of sufficient specific markers (Golen*et al.,*2008). Studying the genetic diversity of *S. haematobium* is crucial for better understanding of the epidemiology of infection and for advancing novel treatment and vaccination strategies (Quan*et al.,*2015).Initial control measures for Schistosomiasis was based on the elimination of intermediate snail host, since the available drugs were too toxic for mass treatment. The use of molluscide for killing snail host, such as niclosamide, has become the molluscicide of choice (Jordan & Rosenfeld, 1983). Recent developments in the field of chemotherapy, has greatly improved the prospects of controlling the disease, with the aim of reducing prevalence and intensity of infection and reinfection. The current most effective drug for the treatment of schistosomiasis is praziquantel (Mutapi, Woelk&Mduluza, 2008). This drug has a wider spectrum of activity. It is effective against all the five species of human schistosomes and even cestodes. It has a minimal and transient side effects when administered. It is given as a single oral single dose treatment (Midzi, Sangweme, Zinyowera, Mapingure, Brouwer, Kumar, Mutapi, Woelk&Mduluza, 2008). The standard dose recommended by WHO (2002) is 40mg/kg body weight. It has been shown to achieve cure rates of 70-90%, with egg reduction rates of about 90% (Kumar &Gryseels, 1994).

Molecular markers represent sensitive tools for exploring genetic variability and degree of genetic interplay among parasitic populations. The polymerase chain reaction based molecular markers has been developed in an attempt to discriminate between species, strains, and individuals (Williams *et al*.,1991). With such biotechnology, it became feasible to investigate population genetics of schistosomes with few available sequence data (Welsh *et al*., 1990). Molecular markers perform on a whole genome scan, is capable of identifying a large number of

loci and have emerged as a powerful tool in distinguishing both inter and intraspecific variations (Barral*et al.,* 1993; Dias *et al.,* 1993).The realization of the enormous influence that intraspecific variation may have on the epidemiology of parasitic disease has resulted in a great deal of research into reliable methods for identification and characterizing strains.

However because of the limitation of morphology as a means of separating parasitic organism at the specific or intraspecific level a great deal of interest has been given to the usefulness of other different criteria such as molecular DNA sequencing which provides a direct measure of genotypic variation and has also made it possible to assess probably many inter and intraspecific variant s which have remained undetectable because of the inadequacy of existing identfication technique of *Schistosomes* species (Ayala, 1982). Several studies have utilized molecular markers to explore the genetic variability within *Schistosome* populations in different hosts (Minchella*et al.*,1994). The small amount of DNA required for such a technique make it feasible to use cercarial or miracidial DNA, rather than DNA derived from adult *schistosomes*, to investigate genetic background, thus reducing the expected selection exerted by atypical hosts (Lorverde, 1985; Shiff, 2000).

This study is aimed at adopting microsatellite markers to examine the possible diversity among *S. haematobium* natural isolates from some selected region of endemic areas in cross river state, Nigeria.

MATERIALS AND METHODS

2.1 Study area

The study was conducted in five communities through random selection five settlements situated near water bodies in Cross River State,South-Southern Nigeria, where *Schistosomia, haematobium* is endemic. The schools includes Apostolic primary school and Solomon memorial secondary school Agwagwune both in Adim LGA, The Adim community located at Latitudes 5.76283 and Longitudes 7.99272, Christ the king primary school in Ogoja, with latitude 6.6528 longitude 8.7918 are in Cross-River state while community secondary school Abuochiche and community primary school Ibii , Bekwarra LGA are in State with an Area of 240km^2 and a population of 1566011(NPC, 2006) and lies on latitude $5^0 53^0$E and longitude $7^0 57^0$ N Geomorphologically, the areas are composed of an alternation of high and low land with some of the low occupied by surface water bodies . Most of the inhabitants of thesecommunities are farmers and the villagers depend on various streams and ponds for their water-related activities. There are also no proper toilet facilities, hence individuals defecate and urinate indiscriminately in their surrounding environments as well as in water bodies.

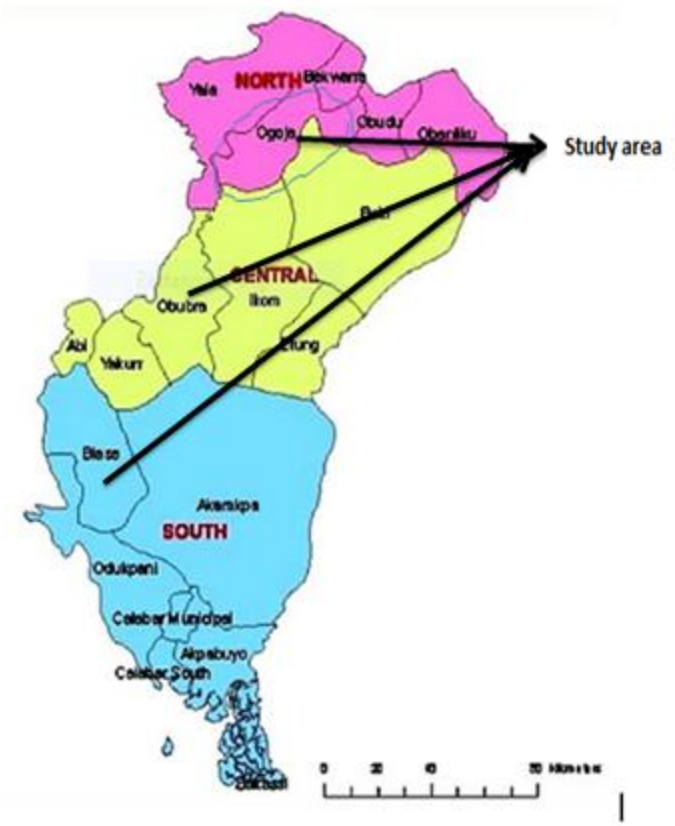


Image 1: Study area

2.2 Participant selection (inclusion/exclusion criteria)

One hundred pupils were randomly selected from each school thereby bringing the total number of participating pupils to five hundred. However, pupils under the age of four and those above the age of twenty were not selected. At the end of the selection the study participant from the study area where made up 310 (62% male) and 190 (38% female).

2.3. Ethical approval

Ethical approval was obtained from the Cross-River and Ebonyi state ministries of health ,the school school heads were informed on the significant of the study and consent was obtained from the parents/ guardiance of the children .

2.4 Parasitological analysis

2.4.1 Collection of samples

Urine samples were collected from each participant in a labeled transparent 20 ml specimen bottle between 10h00 and 14h00 on the collection days to WHO recommendation as the period coincides with when excretion of haematobium egg is highest (the optimal period of egg load in urine). Participants will be instructed to drop the last part of the urine stream into the labelled bottle. The urine samples weretransferred immediately to the laboratory for analysis .

2.4.2 **Detection of Haematuria and Proteinuria**

Each urine samples were checked for Haematuria and Protinuria using reagent dipstick (Medi-test Combi 9 Macherey- Nagel, Germany) The reagent end of the diptick was dipped into fresh urine for 45 seconds after which the areas were compared with the standard coloured chart

provided by the manufacturers. Haematuria was graded as positive when 3 to 20 erythorcyteswere dectected per microlitre (µl) of urine, moderately positive by presence of 50RBC/µl and highly positive by presence of more than 250 RBC/µl .Proteinuria was graded according to the concentration of protein per µl of urine . Therefore the presense of 30mg/µl was regarded as trace and 100mg /µl as positive and 300mg/µl as strong positive after the detection of haematuria and Proteinuria.

2.4.3. Detection of ova of *Schistosomia haematobium*

The urine samples were examined for Schistosomia haematobium eggs were subjected using the urine sedimentation method. 10ml of urine was taken from each specimen bottle and centrifuged at 3000 rounds per minute, the supernatant was discarded and one drop of the sediment spread on a grease- free glass slide and covered with a cover slip and viewed under the x10 magnification to detect the presence ova *S.haematobium* ova describe as golden yellowish and elliptical in shape ,with characteristics terminal spines (Montressor*et al.,* 2002). The eggs were counted and expressed as number of egg per 10millilitres of urine ().the intensity of infection was graded as low if less or equal to 50 eggs/10ml and high when more 100 eggs/10ml of urine (WHO, 1983)

2.4.4. Urine cell pellets preparation

Each urine sample were centrifuged at 5000 xg for 10min, the supernatant decanted and cell pellets washed three times with 25 mlPBS(0.8% Nacl, 2.7 Mn KCL,1.8 Mm KH_2PO_4,8 Mm Na_2HPO_2, pH 7.4). The cell pellets were stored immediately at -80^0c until used

2.4.5 DNA preparation

Genomic DNA extraction from the urine pallet collected samples were performed utilizing QIAamp DNA Mini kit (QIAGEN, Hilden, Germany). Genomic DNA pellet were dissolved in Tris-EDTA (TE) buffer to a working concentration of 5 ng/µL.

2.5.1 Genomic DNA Extraction from samples

Genomic DNA was extracted from each sample using a modified method of Stothardet*et al* (1996). Samples weremascerated and DNA was extracted from each snail using CTAB extraction buffer containing 2-merccaptoethanol, hexadecyltrimethyl-ammonium bromide (CTAB) (solid), tris (hydroxyl-mehyl) amino-methane, ethlenediamine-tetraacetic acid, disodium salt solution (EDTA), and sodium chloride. Each snail were soaked in TE (10mM tris HCL and 1mM EDTA) to get rid of the remaining ethanol. Tissue from each of the snail were placed in sterile 1.5ml Eppendodorf tube, 500 *µl*of CTAB solution added and 10 *µl* of proteinase was added too. The genomic DNA were extracted from the CTAB buffer by adding an equal volume of chloroform and isoamylalchohol to each tube , the organic and the aqueous layers were gentle mixed for 5mins and spun at 13,000 rpm for 20 mins.the upper aqueous layer were removed into another sterile eppendorf tube and equal volume of 100% ethanol were added , mixed and incubated at -20℃ overnight to enhance DNA precipitation, it were be spun again for 13,000 rpm for 20mins.The pallet were washed with 70 % ethanol and spun for another 20 mins ,the supernatant were removed and the pellets dried at room temperature.

2.5.2 Methods for Molecular characterization ofschistosomiasis

Genomic DNA extracted from snail was subjected to PCR amplification of schistosome*Dra1 gene*sequence repeats using forward primers

5’GATTCACCTATCAGACGAAAC3’ and reverse primers 5’TCACAACGATACGACCAAC3’. All the PCR amplifications were performed with the thermal cycler(bio- Rad cycler) and the amplified products were visualized on 1.5% agarose gel. Photo documentation was performed with Gel documentation.

2.5.3 Drug Administration

Individual whose samples were confirmed in the laboratory as positive for parasite ova,were included in the drug administration routine. Their body weight were obtained using balance scale to determine the appropriate dose to be dispensed as recommended by WHO (2002).

2.5.4 Inclusion criteria

i. Pupils within the age range of <5-15 years.
ii. Pupils with detectable ova of *Schistosoma haematobium* in their urine
iii. Subject resident of the community and attending the designated schools.
iv. Subjects that healthy to take part in the study.

2.5.5 Exclusion criteria

i. Pupils who are receiving treatment for any ailment
ii. Person outside the specified age range.
iii. Person treated for schistosomiasis with praziquantel in the previous 30 days in the community.

Pre-treatment and Post-treatment were assessed by repeating previous parasitological analysis, 90 days post treatment. The effect of treatment on the indicators of infection and morbidity, namely egg count, haematuria and other pathological condition usually show rapid and significant reduction and regression from about 90 days post treatment according toMungai&Ouma, 1999.

2.6 Genotyping of *S. haematobium* DNA using ISSR PCR Reaction

DNA extraction was done for all collected samples using QIAamp DNA Mini kit (Germany). Genotyping of *S. haematobium* DNA using PCR test the amplification conditions were based on the original method using Thermal cycler. .In this study five universal primers were used for genotyping of *S. haematobium* strain (UPA02, 5'-CAGGCCCTTC-3'; UPA12, 5'-TGCCGAGCTG-3';UPA13, 5'-TCGGCGATAG-3';USHY20, 5'-CAGCACCCAC-3';UPA13 ,5'-AGCCGTGGAA-3';), primers were selected according to Shiff et al (2000). Amplification reaction was carried out in a final volume of 25 µl and the PCR product determined on 2% polyacrylamide by electrophoresis.

2.7 Data analysis

DNA bands from different isolates produced by the PCR reaction were compared to a DNA molecular weight standard to guarantee consistent scoring between different gels and to determine the molecular weight of the band. Comparison between isolates were done by scoring the total bands of each isolates,the bands shared between two different isolates and the bands that are absent from one isolates and present in the other one.

The statistical analysis for this study was carried out using the version 18.0 of SPSS software package (SPSS,Chicago,IL) The PCR results will be stratified into high

(Ct<30),Moderate(30≤ Ct≥35), low (Ct>35).DNA load and negative (no amplification detected in 50 cycles). Proportional data were compared using chi-square test while data comparison was carried out using simple student t- test.

RESULT

3.1 Results

Table 1 shows a school/community related prevalence of urinary schistosomiasis out of 500 individual screened for *Schistosoma haematobium* infection 112 (%) were found to be infected. The highest prevalence by school/community was recorded in community primary and secondary school Obubra were 48(42.8%) while the least was in Christ the King Primary School Ogoja.

Table 2 is based on the prevalence of *Schistosomia haematobium*infection among subject by age and gender.the overall prevalence of 112(22.4%) was recorded among the 500 pupils from 5 school both in Cross-River State, South-South Nigeria were involved in the study. Three hundred and ten males 310(62%) and 190(38%) females of ages <5-16 years were involved in the study,Age related prevalence of urinary schistosomiasis in the infected community,

Considering the age 11-13year age group recorded the highest prevalence 54(48.2%) while the lowest was in the 5-7 year age group 10(8.9%). Table 4 shows intensity of haematuria and proteinura prevalence of schistosomia infection in the study area. Table 6 shows the distribution of light and heavy infections among the communities out of 112 infected with S .*haematobium* , 10(25%) of pupils from adim LGA had light infection and 34(77.3%) had heavy infection , 15(75%) had light and 5 (25%) heavy infection in pupils from OGOJO LGA while pupils from Obubra LGA had 7(14.6%) light infection and 41(85.4%) had heavy infection of S. *haematobium* ova.

TABLE 1

Demography of schools and pupils who participated in the study

LGA	School visited	Male	Female	Total
Adim	Apostolic Primary School	61	39	100
	Solomon memorial Secondary School Agwagwune	68	32	100
Ogoja	Christ the King Primary School	59	41	100
Obubra	Community Primary School Ochon Community Secondary School	62	38	100

LGA	School Visited	No. of males Examined	No. of females Examined	Total No. of Subjects Examined
Adim	Apostolic Pry. School	61	39	100
	Solomon Mem. Sec. School, Aguagwune	68	32	100
Ogoja	Christ the King Pry.Sch.	59	41	100
Obubra	Comm. Sce. Sch. ochon Comm Sec. School	62	38	100

TABLE 2
Distribution of *Schistosoma haematobium* infection according to age

Age (yrs)	No of examined	No infected (Gender)			% infection
			Male	Female	
5-7	50	10(8.9%	4(40)	6(60)	20%
8-10	100	18(16.0%)	1(61.7)	7(388)	18%
11-13	238	54(48.2%)	38(70.3)	16(296)	22.6%
14-16	112	30(26.8)	21(70)	9(30)	26.7%
Total	500	112	74	38	87.3%

Distribution of *Schistosoma haematobium* infection according to age

Age (yrs)	**No of examined**	**No infected**	**% infection**
5-7	50	10(8.9%	20%
8-10	100	18(16.0%)	18%
11-13	238	54(48.2%)	22.6%
14-16	112	30(26.8)	26.7%
Total	500	112	87.3%

Source of water supply related infection rate

The available source of water includes borehole, well, pond, stream and rain water. Based on questionnaire and responses, participants who use pond water as their source of water recorded the highest prevalence (28 %) the next highest was among those that use borehole water while those that use rain and stream water as their source of water recorded (20%) and 18% infection to *Schistosoma haematobuim* respectively.

Using questionnaire

	Source of water	No of person
1	Borehole	120 (24%)
2	Pond	140 (28%)
3	Streams	100 (20%)
4	Rain water	80 (18%)
	5 other source (12%)	60

TABLE 3
Distribution of *Schistosoma haematobium* with reference to sex

Sex	Number examined	No infected	%infected
Male	310	74 (66.1%)	23.87%
Female	190	38(33.9%)	20%
Total	500	112(22.4%)	

Distribution of *Schistosoma haematobium*with reference to Gender

Gender	**Number examined**	**No infected**	**%infected**
Male	310	74 (66.1%)	23.87%
Female	190	38(33.9%)	20%
Total	500	112(22.4%)	

TABLE 4

Intensity/haematuria/proteinuria
The prevalence of schistosomahaematobium infection in the study communities

Communities	Number examined	Number of positive haematuria	Number (%) with schistosoma ova
Adim LGA	200	40(20%)	44(22%)
Ogoja LGA	100	15(15%)	20(20%)
Ebonyi LGA	200	43(21.5)	48(24%)
Total	500	98	112

Prevalence and Intensity
The prevalence of *Schistosoma haematobium* infection in the study communities examined

Communities	**Number examined**	**No. (%) Infected**	**Mean Ova Count**
Adim LGA	200	40(20%)	44(22%)
Ogoja LGA	100	15(15%)	20(20%)
Ebonyi LGA	200	43(21.5)	48(24%)
Total	500	98	112

TABLE 5a
Intensity of infection (Haematuria)

Sex	Number examine	Haematuria	Schistosoma ova %
Male	310 (62%)	58	74(23.7%)
Female	190 (38%)	40	38(14.7%)

TABLE 5b
Prevalence and Morbidity indicators

Sex	**Number examine**	**Haematuria**	**Schistosoma ova %**
Male	310 (62%)	58	74(23.7%)
Female	190 (38%)	40	38(14.7%)

TABLE 6a

The intensity of *Schistosoma haematobium* infection in positive urine samples in the communities

Communities (LGA)	Number of positive schistosoma ova	Light infection 1-49 eggs/10ml of urine	Heavy infection 50 eggs and more/ 10ml of urine
Adim	44 (39.2%)	10(25%)	34(77.3)
Ogoja LGA	20(17.8%)	15(75%)	5(25%)
Obubra	48(42.6)	7(14.6)	41(85.4)
Total	112	32	80

Table 6b
Distribution and intensity of infection in the three communities examined

Communities (LGA)	No. Examined	No. Infected	Light Infection 1 – 49 eggs/10ml Urine	Heavy Infection 50 eggs and more/10ml of urine
Adim	200	44 (39.2%)	10(25%)	34(77.3%)
Ogoja	100	20(17.8%)	15(75%)	5(25%)
Obubra	200	48(42.6%)	7(14.6%)	41(85.4%
Total	500	112	32	80

Table 7 shows number of infected and uninfected snails in the community. The highest number of snails infected 29 (18.2%) was in Obubra local government area and the lowest number of snail infected 7(4.4%) in Ogoja local government area.

Table 8 shows distribution of schistosoma intermediate host species in the communities. From the table below *B. globosusi*has the highest occurrence in the communities.

Table 9 describes the distribution and administration of the standard and enhanced drug regimes of praziquantel (PZQ) (40mg/kg and 60mg/kg) to the respondents according to their ages, weights and heights, to determine the number of tablets administered per dose, according to body weight as recommended by WHO (2002). The older children (11-15 years) were seen to receive higher doses (2.5 and 3.5 tablets respectively) for both drug regimes at 600mg per tablet of PZQ per body weight.

TABLE 7
Infected and uninfected snails as intermediate host in the study area

Communities (LGA)	Number of snails collected	Number of snails infected	Number of uninfected
Adim	55	13 (8.1%)	42(26.4%)
Ogoja	31	7(4.4%)	24(15%)

Obubra	73	29(18.2%)	44(27.6%)
Total	159	49	

Number of snailsintermediate host identified as Bulinus

Communities (LGA)	Number of snails collected as Bulinus	Number of snails Resembling Bulinus	Number of Snails Not resembling Bulinus
Adim	100	13 (8.1%)	42(26.4%)
Ogoja	100	7(4.4%)	24(15%)
Obubra	100	29(18.2%)	44(27.6%)
Total	300	49	110

TABLE 8
Distribution of schistosomic intermediate host species in the communities

Communities (LGA)	Number of snails collected	Number of snails infected	Bulinus species
Adim	55	13	10 (B. *globosus*) 3 (B. *truncatus*)
Ogoja	31	7	5 (B. *globosus*) 2 (B. *truncatus*)
Obubra	73	29	22 (B. *globosus*) 7 (B. *truncatus*)
Total	159	49	49

TABLE 9a

Demography of pupils by treatment groups

Treatment	Body weight	Pre treatment	Post treatment (no cured)
5 -7	20-25kg	10	-
8 -10	25 -30kg	18	-
11- 13	35-40kg	54	5
14 -16	40 -45kg	30	3

TABLE 9b

Demography of pupils by Age groups

Age group	No. Examine	No. Infected	No. treated	No. Cured	No. not cured
5 - 7	50	10 (20)	10	10(100%)	0
8 – 10	100	18 (38)	14	14 (100%)	0
11 – 13	238	54 (22.7)	50	45 (90%)	5 (10)
14 – 16	112	30 (26.7)	29	26 (89.60%)	3 (10.34)
Total	**500**	**112**	**103**	**95**	**8**

Table 10

Demography of pupils Treatment by Gender

Gender	No. Examine	No. Infected	No.Treated	No.Cured	No. Not cured
Male	310	70	65	55	10
Female	190	38	38	40	3
Total	**500**	**108**	**103**	**95**	**13**

TABLE 11

Demography of pupils by communities

Communities (LGA)	No. Treated	No.(%) Cured	No.(%) Not cured
Adim	44(3)	35(93.18)	9(6.82)
Ogoja	20(2)	15(90)	5(10)
Obubra	48(3)	46(93.75)	2(6.25)
Total	**112**	**96(85.7)**	**16(14.3)**

TABLE 12

Demography of pupils by Body weight

Body weight (kg)	No. Treated	No. (%) Cured	No.(%) (Not cured)
20 – 25	10	10 (100)	0
25 – 30	18	18 (100)	0
35 – 40	54	49(90.74)	5(9.26)
45 – 45	30	27(90)	3(10)
Total	**112**	**96**	**16(14.3)**

Molecular characterization of *S.haematobium*and *S. mansonni*strains:
The electrophoregram displaced in Figure 1 showed three different test samples obtained from Adim, Ogoja, and Obubra in polyacrylamide gel using 1500 bp DNA molecularmarker. Monomorphic bands were visible for all test samples from Adim, Ogoja and Obubra polymorphic band marker 200bp, while polymorphic bands were also visible at 1000bp for Adim and Obubra test sample(Figure 1). The 1000bp test sample bands corresponds to the molecular size of *S. haematobium* obtained from Adim and Obubra samples only while 200 bp reveals the corresponding molecular size of *S. mansonni* obtained from all test samples across all locations. (figure 1) using the inter simple sequence repeat markers.

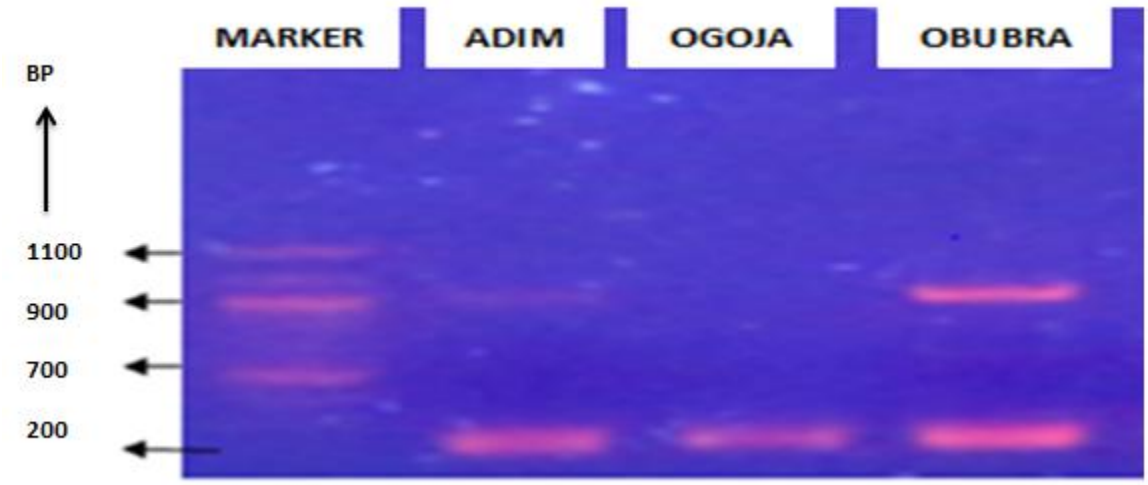


Figure 1: Electrophoregram showing molecular size of samples and control with DNA ladder.

Molecular Characterization of *S.haematobium*and *S. mansonni*strains:
Inter simple sequence repeats of PCR test performed for the collected urine samples using ISSR test reveals 73% study subjects had a polymorphism for UPA02 and UPA13 primers, while primer UPA13 showed 24% polymorphism. Total number of polymorphic bands were 2 each for primers UPA02 and UPA13 primers while UPA12 showed only one polymorphic band. Major allele frequencies (MAF) were 0.53 for each of UPA02 and UPA 13 primers but showed 0.71 frequency with UPA12 primer. Allele frequencies (AF) also varied slightly among the primers used. UPA02 and UPA 13 had allele frequencies of 8 each while UPA12 had 4 allele frequencies (Table 13).Nei's genetic diversity indices for the primers revealed variations among the different

primers. UPA02 and UPA13 Nei's gene diversity of 0.64 each while primer UPA12 showed gene diversity of 0.28. Results of polymorphic information content showed that primers UPA02 and UPA13 discriminately revealed a PIC of 0.68 while UPA12 discriminated 0.28 PIC.

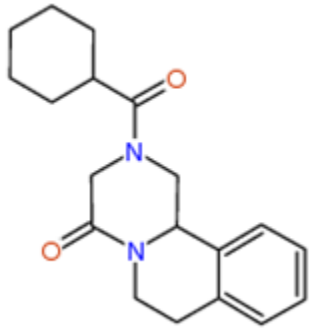
Table 13: Genetic diversity indices and characteristics of*Schistosoma haematobium* in the study area.

Primers	NPB	MAF	AF	Nei's Gene Diversity	PIC	% Polymorphism
UPA02	**2.00**	**0.53**	**8**	**0.64**	**0.68**	**73.00**
UPA12	**1.00**	**0.71**	**4**	**0.28**	**0.32**	**24.00**
UPA13	**2.00**	**0.53**	**8**	**0.64**	**0.68**	**73.00**
Average	**1.67**	**0.59**	**3.67**	**0.76**	**0.53**	**76.67**

NPB = No. of polymorphic bands; MAF = Major allele frequency; AF= Allele frequency; PIC = Polymorphic information content; UPA = Universal primers for ISSR

Praziquantel –DRA 1 Protein/gene interactions in *Schistosoma mansonni* proteins (Smp)

Praziquantel (Biltricode) is an antihelmintic used in humans and animals for the treatment of tapeworms and flukes. Specifically, it is effective against schistosoma.



Chemical structure of praziquantel

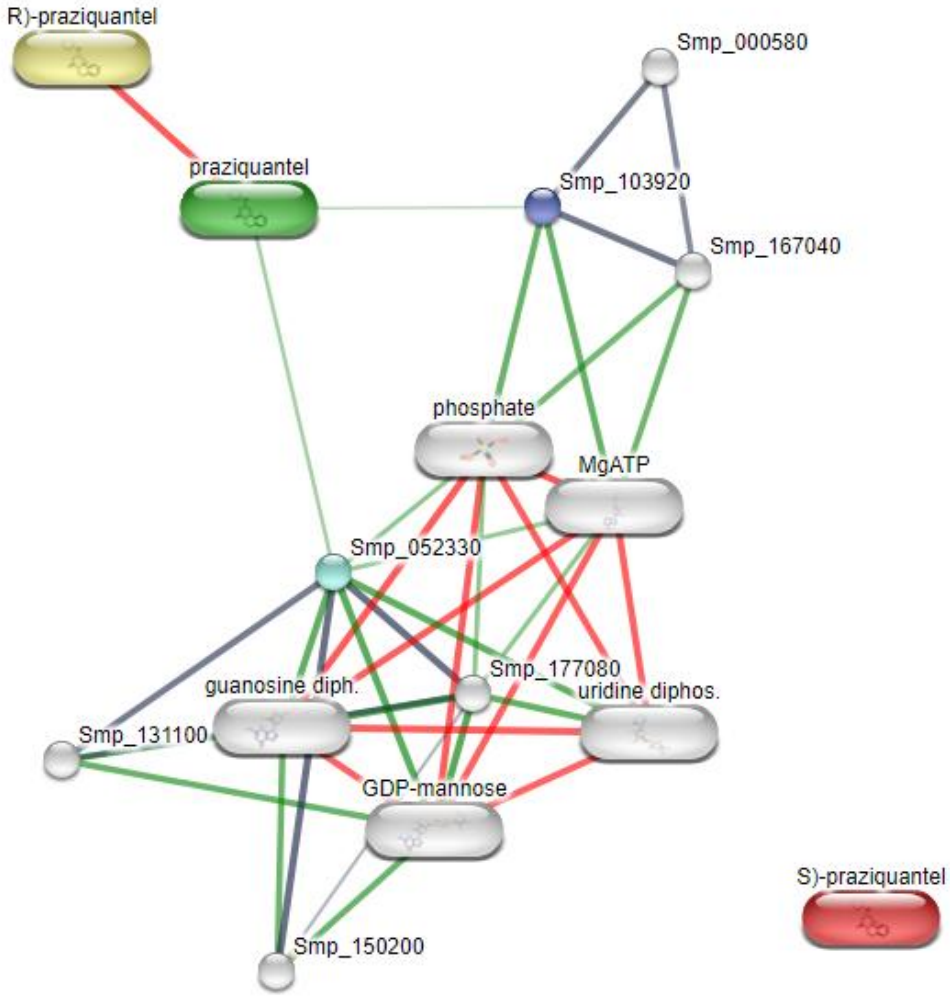


Image 2: gene interactions

S. haematobium DRA 1 gene oncology

Protein –protein network statistics
Number of functional nodes =7
Number of functional edges =8
Average nodal degree = 229
Clustering coefficient 0.905
Expected number of edges = 7
Protein –protein interaction enrichment p-value = 0.415

Molecular characteristics of DRA 1 genes

1. N-Glycan biosynthesis gene, 4 sets involved showing false discovery rate of 0.0000409
2. Glycosyltransferases group 1 gene, 2 sets involved showing false discovery rate of 0.0193
3. Glycosyltransferase family 1 gene, 2 sets involved and showing a false discovery rate of 0.0354

4.2 Discussion

Schistosomiasis affects approximately 200 million people, mainly in rural areas of developing countries, with an estimated 79 million people at risk of the disease [24]. Both *S. haematobium*and*S. mansoni*are present in Sudan. Schistosomiasis is found in many different areas in Sudan especially in Gezira, Rahad, Kenana and other irrigation schemes [25]; [26].The dynamics of the transmission are necessarily complicated and subject to considerable variation due to many factors influencing the common environment, the behavioural patterns of the definitive host and the bionomic of the intermediate host.

This study was selected local villages in three Local Government Areas of Cross River State Nigeria. The villages are characterized by insufficient safe water supply especially during the dry season. *S. haematobium* started increasing in the last decade in the area [27]. Schistosomiasis affects males more than females, since males are more exposed to the water supply than females.

In this study 51% of study subjects were found to haveabnormal pathological conditions including 85.9%(49) abnormal wall thickness 56.8 %(29) multiple nodularity and 3.9% (2) one polyps and 1.9% (1) wall bladder calcifications. When compared to a similar study from Zambia [28] more irregular bladder wall were the most frequently diagnosed abnormality in 3.4% of children. Study from Nigeria [29] reported71% abnormal pathological conditions; abnormal wall thickness (55.8%) while in our study 56.8%,irregular bladder wall (27.9%), masses (23.3%), false polyps 2 (4.7%) compare with 5.8% in this study.

These studies reported similar findings that most abnormal urinary bladder wall recorded in children below15 years of age. In this study there was no correlation between ultrasound abnormal urinary tract findings and intensity of infection (P=0.21).This is contrary to the study from Nigeria [29] that reported abnormal pathology slightly more common in the study subjects with heavy infection than those with light infection.

Another study conducted in southern Nigeria identified about 6.7% and 1.7% of the patients had the right pelvis and left pelvis of their kidney moderately dilated, respectively [30];in contrast to this study where no kidney abnormalities were found probably due to duration of infection in the study participants.

Schistosomiasis diagnosis by PCR results recorded 72.3% (60) while schistosomiasis diagnosis by other methods revealed 54.2% (45), this may be due to presence of a small number of eggs that are difficult to diagnose by the microscope. Many studies confirmed the sensitivity of PCR to detect egg of *S.haematobium* in urine to be more sensitive than in other techniques.

A study performed in Ghana using PCR methods for diagnosis reported sensitivity 100% and specificity at89% [31], in contrast using PCR in this study, sensitivity was 100% and specificity was 60.5%. It is well known that sensitivity of PCR to diagnosis *S.haematobium* is more sensitive than filtration; this was indicated by 15 study subject in this study found negative by filtration while they were positive by PCR. But the study from northern Senegal showed significant correlation with microscopic egg counts both for *S. mansoni*in stool and *S.haematobium* in urine. They found that *Schistosoma* detection rate of PCR (84.1%) was similar to that of microscopy performed on duplicate stool samples (79.5%)[32], that may be dueto high intensity of infection that made the sensitivity of filtration similar to PCR.

Differences in alleles were recorded among the 37 variable bands. In this study, only three variable bands were recorded, and polymorphic region was between 700 and 1100 bp. The small number of variation alleles in this study refer to the samples collection from children infected with schistosomiasis which transmitted to them from the same study area, while [13] study

samples were collected from infected students coming from 203 different regions infected with *Schistosoma*, this can increase the chances of finding more different polymorphic.

ISSR primers have been used in several studies to examine the genetic diversity among populations of schistosomes in snails [5]. Studies done by [5] at different sites in Bamako, on cercariae isolated from different snails and 47 different genotypes were recorded from 414 schistosome individuals.

Six universal primers (UPA01, UPA02, UPA10,UPA12, UPA13, UPA19,) were used but only three primers showed distinctiveness and discrimination (UPA02, UPA12, UPA13) were selected and used for the study. Primer UPA02 revealed two polymorphic bands in marker 1000bp, while in our study other variability in marker between 200 and 1000bp was noted. That means the findings show more different genotype when more than three primers are used or by increasing the study population that increases the chances of finding other genetic differences. In this study 46% of the subjects were positive by PCR with normal pathology by ultrasound. Most of these subjects belong to type UPA02, that may refer that the genotype is not aggressive enough to cause severe disease.

Infection intensity have great role in diagnosis *S.haematobium* and genetic differences may lead to some strains being innately more immunogenic or fecund than others, these differences may play important role in severity features. There are few studies that found the relation between severity and genetic diversity [33]. PCR was used to characterize the extent of urinary tract pathology of infected children with the genetic markers to examine the relationship between genetic diversity of *S. haematobium* and clinical outcome. It was found that parasite heterogeneity did differ slightly; allelic frequencies at two loci differed significantly between the mild and severe groups.

This may be due to *S. haematobium* infections differ under controlled circumstances, where factors such as exposure to parasites, infection intensity, type of parasite strains were found to significantly influenced infection rate.

Conclusion:

The rate of Schistosomiasis prevalence and molecular characterization on *S.haematobium*have reveal that the inter simple sequence repeats makers were able to discriminate among *Schistosoma haematobium* in the study locations and have shown that S. haematobium along side*S. mansonni*are responsible for the high prevalence of the disease in the endemic areas evaluated. The DRA -1 protein gene in S. haematobium among the endemic areas was found to possess a molecular size of 1000 bp and also accounted for the high prevalence of the disease in Adim and obubra study areas. The study also revealed that S. mansonniDRA-1 gene were also responsible for the disease across the three study areas with same molecular size of 200 bp and were also responsible for the high prevalence of the disease in Adim and Obubra areas playing a significant role the severity of the disease in the state.Difference in genotypes of *Sschistosoma*species in the study areamay play a major role in severity features. However**,** molecular characterization of strains was to afford the resaerchers the opportunity to identify the type of strain in the area which may facilitate prevention and treatment.

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