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IL4, *IL13*, *GSTM1* and *T1* variants and susceptibility to Schistosomiasis and associated bladder pathologies in Eggua, Nigeria

[1]*Onile, O. S., [2]Awobode H. O., [3]Agunloye A. M., [4]Marquez-Duenas C., [4]Manning Cela R. G. and [5]*Anumudu C. I.

[1]Biotechnology Programme, Department of Biological Sciences, Elizade University, Ilara-Mokin, Nigeria; [2] Parasitology Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria; [3]Department of Radiology, University College Hospital, University of Ibadan, Nigeria, [4]Department of Molecular Biomedicine, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico D.F.; [5]Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Abstract

Failure of the human host to elicit adequate immune responses to the adult *Schistosoma haematobium* worm and continuous strong inflammatory responses to the eggs have been the main causes of bladder pathology in chronic Schistosomiasis. Identification of susceptibility biomarkers for schistosomiasis- associated bladder pathology is necessary in order to detect genetic factors responsible for the infection and spread of the disease. The aim of this study was to identify candidate-biomarkers for susceptibility to schistosomiasis and its associated pathologies. A total of 371 adult participants, comprising 130 males and 241 females from Eggua community, Ogun State, Nigeria, were randomly recruited into a cross sectional study from August 2012 to May 2014. They were screened for *S. haematobium* ova and bladder pathologies by microscopy and ultrasonography, respectively. Human host susceptibility to schistosomiasis and its associated bladder pathologies were determined by PCR genotyping of Interleukin (*IL4* and *IL13*) genes, and glutathione-S-transferase (*GSTT1* and *GSTM1*) genes. The overall prevalence of *S. haematobium* in the population was 29.3% (108/369). Bladder pathologies were observed in 32.3% (117/362) of the population. Polymorphisms in *IL 4-590* and *IL 13-1055* were observed in 24.1% and 9.3% schistosomiasis cases, respectively. The *IL 13-1055* polymorphism did not indicate susceptibility to schistosomiasis in males (OR 0.7, 95% CI 0.3-2.1) but a slight risk was found in females (OR 1.1, 95% CI 0.7-1.7). Participants with *GSTM1* and *GSTT1* polymorphisms expressed elevated risks of bladder pathologies (OR = 4.3, 95% CI 2.0 - 9.2 and OR = 4.2, 95% CI 1.5 – 12.0, respectively), with the pathology and schistosomiasis group having more GST polymorphisms than bladder pathologies.

Keywords: Polymorphisms, Cytokines, GST, schistosomiasis and pathologies
***Corresponding Author's Address:** Email: cianumudu@yahoo.com; olugbenga.onile@elizadeuniversity.edu.ng, Phone Number: +2348023590478; +2348133109389

Introduction

Not less than 700 million persons are globally estimated to be infected with schistosomiasis and at least 206.4 million people required preventive treatment in 2016 (Yassir et. al., 2017; WHO, 2018). Nigeria is one of the most severely affected countries in Africa, with 29 million cases as at 2010 (Adenowo et. al., 2015). Indeed, a recent meta-analysis of schistosomiasis prevalence studies in Nigeria shows that the prevalence in Nigeria varied

from 2-82.5% with a pooled prevalence of 34.7% (Abdulkadir et. al., 2017). Chronic morbidity during urinary schistosomiasis develops as a result of schistosome eggs that lodge in the bladder causing extensive tissue damage (Wilson et. al., 2007). The response to egg deposition could lead to calcification of the urinary bladder, infection, stone formation and mucosal proliferation (Zaghloul, 2012). Chronic infection with *Schistosoma haematobium* has been reported as a possible risk factor in the aetiology of bladder cancer (European Association of Urology, 2016; Onile et. al., 2016).

The intensity of schistosomiasis is reportedly influenced by a *Schistosoma mansoni* 1 (*SM1*) gene that is mapped to a region of chromosome 5 in the 5q31–q33 that codes for proteins that are associated with regulation of the Th2 response such as cytokines like Interleukin (IL)-3, IL-4, IL-5, IL-9, and IL-13 and Immunoglobulin (Ig) E (Dessein et. al*.,* 2001; Gatlin et. al*.,* 2009; Mbanefo et. al*.,* 2014). Gatlin et. al*.,* (2009) in a univariate analysis reported a significant correlation between resistance to reinfection with *Schistosoma mansoni* and the heterozygous (C/T) IL-13 -1055 genotype, any T allele in the Interferon (IFN)-c +874 genotype, and the heterozygous (C/T) in the IL-4 -590 genotype. Analysis of *S. haematobium* infection in Mali revealed that polymorphisms in the *IL13* gene promoter at −1055 and −591 were associated with the rate of *Schistosoma* infection. The IL-13 alleles −1055C and −591A were preferentially transmitted to children with 10% highest infection rate, whereas −1055T was associated with the lowest infection levels (Kouriba et. al., 2005).

It is thought that schistosomes redirect the early cell-mediated immunity in infection to cytokines that would favour establishment of the infection. *S. haematobium* infection-associated bladder damage is closely linked to immune reaction to the parasite deposited egg in the bladder which eventually induces chronic inflammation- related granulomatous injury. We propose that infected and re-infected persons are genetically directed to produce inappropriate cytokine responses that lead to the establishment of chronic infections.

Decrease in the activity of the carcinogen-metabolizing enzyme glutathione-S-transferase (GST) in human bladder cancer tissues have been associated with *S. haematobium* infection (Sheweita et. al*.,* 2004). Somali et. al*.,* (2003) and Yajie et. al*.,* (2016) found that deficiencies in the *GSTT1* gene confer an increased risk of bladder cancer. The inhibition of GST activity may enhance the effect of many environmental carcinogens such as N-nitrosamines, thereby reducing the capacity of detoxifying many endogenous compounds in the bladder (Yajie et. al*.,* 2016).

In Nigeria, most studies have focused on the epidemiology of *S. haematobium* infection (Adenowo et. al*.,* 2015 and Abdulkadir et. al*.,* 2017), with limited information about the morbidity resulting from urinary schistosomiasis in adults (Nmorsi et. al*.,* 2007; Ekwunife et. al*.,* 2009; Onile et. al*.,* 2016) while information on schistosomiasis susceptibility and resistance factors are lacking. This study was aimed at genotyping polymorphisms in Interleukin (*IL-4* and *IL-13*) and GST (*M1* and *T1*) genes and determining their association with genetic susceptibility to schistosomiasis and its pathologies.

Materials and Methods

The study design, area, methods and participants have been described previously (Onile et. al*.,* 2016; Anumudu et. al., 2019). Briefly, a cross- sectional study was carried out from August 2012 to May 2014 in Eggua (Figure 1), a rural agrarian community where *S. haematobium* infections are prevalent. Children and participants with human immunodeficiency virus (HIV) were excluded from the study because the main objective was to determine the effect of chronic urinary schistosomiasis on the health of the adults within the community.

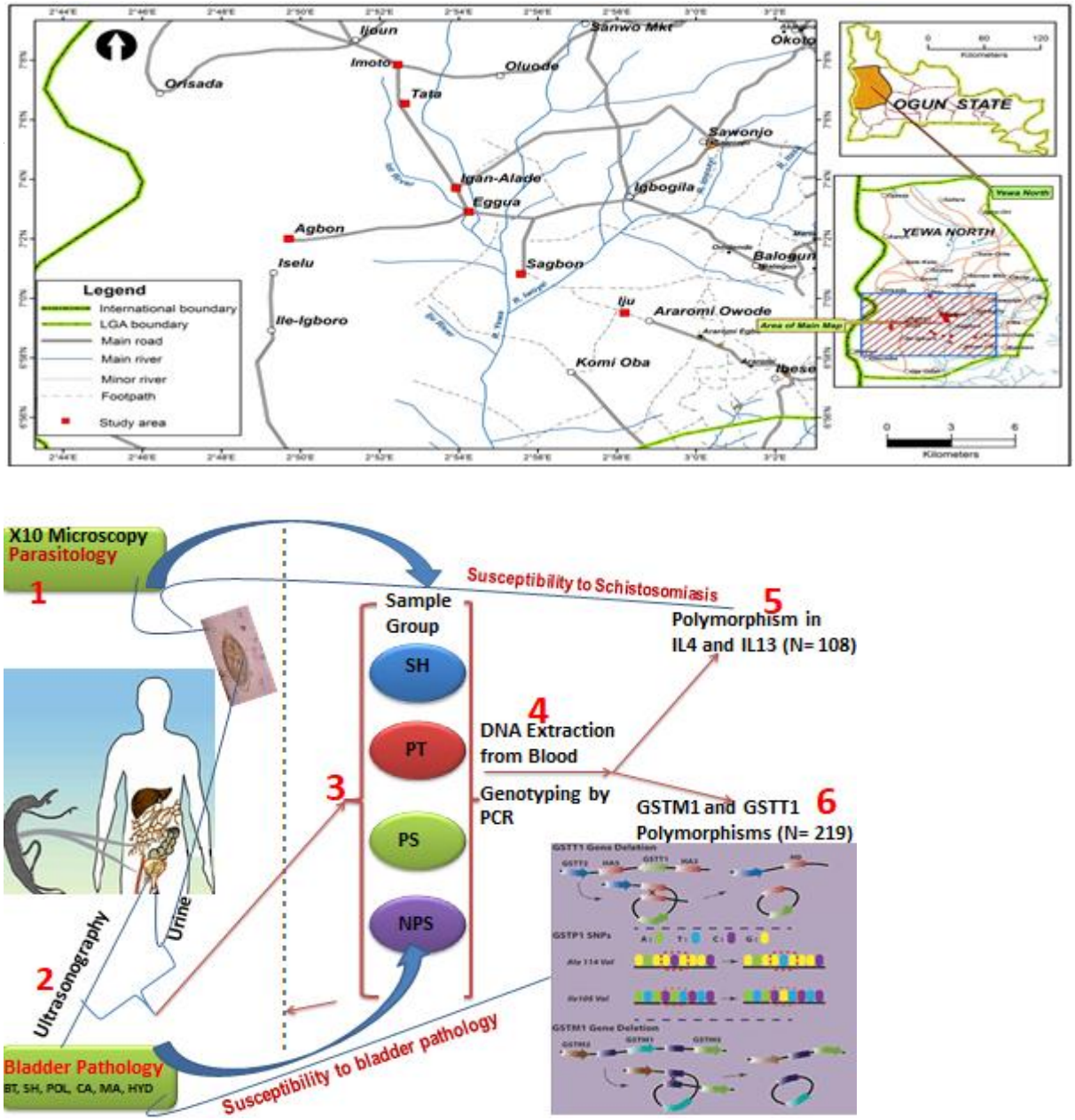

Figure 2: Polymorphisms in *IL4, IL13, GSTM1 and T1* in susceptibility to schistosomiasis and associated bladder pathologies. Abbreviations: SH- *S. haematobium* infected groups, PT- Bladder Pathology group, PS- group with combination of pathology and *S. haematobium* infection and NPS- No pathology and schistosomiasis (Control). GSTs- Glutathione-S-transferase, IL- cytokine Interleukin .

3. *Parasitology*

Study volunteers provided blood (2 mL) by venipuncture and urine (for egg count) specimens. The urine samples were collected between 10:00am and 2:00pm for maximum egg yield and were processed for parasitological examination and egg count (as previously described (Onile et. al., 2017; Adebayo et. al., 2017; Olayinka et. al., 2020). The urine sediment (obtained by centrifuging 10ml of sample at 5000 rpm for 5 minutes) was examined microscopically to identify *Schistosoma haematobium* ova characterised by the presence of a terminal spine. The eggs were counted and the intensity of infection classified as light if ≤50 eggs/10 mL of urine and heavy if >50 egg/10 mL urine were present

(Nmorsi et. al., 2007; Onile et. al., 2016). In addition to microscopy, detection of macro and microhaematuria (urinalysis) for schistosomiasis was also done as described in Onile et. al., (2017)

4. *Ultrasound and Pathology*

A blind ultrasound examination was carried out by a radiologist for each participant in the study and classification of bladder pathologies was as previously described (Onile et. al., 2016).

5. *DNA Extraction and Purification*

DNA was purified from the blood samples using Thermos Scientific GeneJET Whole Blood Genomic DNA purification kit (Lithuania), following the manufacturer's instructions. DNA concentration was measured by spectrophotometry. Aliquots (10 µL) of all samples was taken and subsequently adjusted to provide standard stock solutions of 20ng/µL. The *A*280/*A*260 ratio was estimated to provide an indication of the quality of the sample. Only samples that provided a yield of >20 ng/µL and *A*280/*A*260 ratio >1.8 and <1.95 were included for genotyping analysis.

1. *Genotyping for IL 4 and IL 13 Genes*

PCR for IL-13 and IL-4 Single nucleotide polymorhisms (SNPs) was performed for 108 samples from the participants that tested positive for urinary schistosomiasis by microscopy, using modifications from the original methods (references in Table 1), which enhanced optimal amplification.

PCR for IL-13 -1055 C/T was conducted in a 25 µl reaction mixture containing 100 ng DNA. Initial denaturation was performed at 95^{o}C for 3 min followed by 30 cycles of PCR with the following conditions: 95^{o}C for 30 sec, 62^{o}C for 30 sec for annealing, 72^{o}C for 1min for amplification, and a final extension at 72^{o}C for 3 min. This was done using the Hot Start Taq 2X Master Mix (M0496L, BioLabs, New England).

PCR for IL-13 -591 A/G was conducted in a 25 µL reaction mixture containing 100 ng DNA and was done using the Hot Start Taq 2X Master Mix (M0496L, BioLabs, New England). Initial denaturation was performed at 94^{o}C for 5 min followed by 30 cycles of PCR with the following conditions: 94^{o}C for 1 min, 61^{o}C for 45 sec for annealing, 72^{o}C for 45 sec for amplification, and a final extension at 72^{o}C for 3 min.

PCR for IL-13 +130 G/A was conducted in a 25 µL reaction mixture containing 100 ng DNA and the PCR reaction was done using the Hot Start Taq 2X Master Mix (M0496L, BioLabs, New England). Initial denaturation was performed at 94^{o}C for 5 min followed by 34 cycles of PCR with the following conditions: 94^{o}C for 1 min, 60^{o}C for 45 sec for annealing, 72^{o}C for 45 sec for amplification, and a final extension at 72^{o}C for 3 min.

PCR for IL-4 -590 C/T was conducted in a 25µL reaction mixture containing 100 ng DNA, using the Hot Start Taq 2X PCR Master Mix (M0496L, BioLabs, New England). Initial denaturation was performed at 95^{o}C for 5 min followed by 30 cycles of PCR with the following conditions: 94^{o}C for 30 sec, 59^{o}C for 30 sec for annealing, 72^{o}C for 30 sec for amplification, and a final extension at 72^{o}C for 3 min. 21 Purified PCR amplicons were further sent for sequencing (Fig. 3).

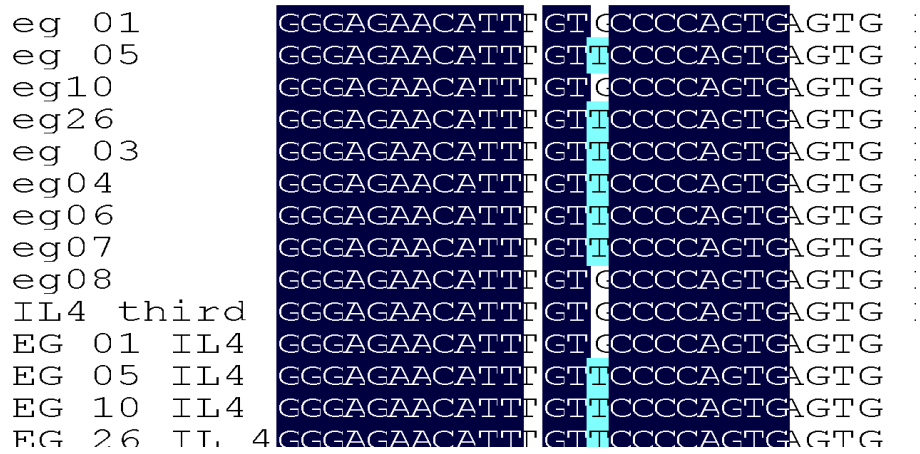

Figure 3: Identifying polymorphisms in IL4 509 T/C sequence, sense strand from Eggua

6. *GSTM1 and GSTT1 Genotyping*

A total of 219 samples were genotyped for GST polymorphisms; these included 118 urinary tract pathology and 101 control cases. The control cases were randomly selected from among participants. The genotypes *GSTM1-null* and *GSTT1-null,* produced no GSTM1 and GSTT1 protein and consequently completely lack GSTM1 and GSTT1 enzymatic activity (Matic et. al., 2016).

1. *Genotyping for GSTM1 Polymorphism*

This reaction was used to distinguish between GSTM1-active and GSTM1-null individuals. Two primers (G1 and G2) were used to amplify GSTM1 complementary DNA sequences (Brockmoller et. al., 2000). The G1 and G2 amplified a 500- base pair (bp) product specific for the GSTM1 gene. The presence of a GSTM1-null polymorphism was concluded from the absence of the specific 500-base pair fragment. 5µL DNA was amplified in a final volume of 25µL amplification reaction of 30 cycles, using the Hot Start Taq 2X Master Mix (M0496L, BioLabs, New England) according to the manufacturer's instructions.

2. *Genotyping for GSTT1 Polymorphism*

The 25 µL volume amplification reaction for *GSTT1* gene was done in a duplex PCR assay with a Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers used as internal control, for determination of GSTT1 and GSTT1-null genotype. A 5 µL DNA template was amplified in a final volume of 25 µL amplification reaction of 30 cycles, using the Hot Start Taq 2X Master Mix (M0496L, BioLabs, New England) according to the manufacturer's instructions.

Table 1: The sets of primers used to genotype for *IL 4* and *IL 13* polymorphisms in the study

S/N	Primer Name	Primer Sequence	Reference
1	IL13 -1055 C/T	Forward 5′-ATGCCTTGTGAGGAGGGTCAC-3′ Reverse 5′-CCAGTCTCTGCAGGATCAACC- 3′	
2	IL13 -591 A/T	Forward 5′-CCAGCCTGGCCCAGTTAAGAGTTT-3′ Reverse 5′-CTAATTCCTCCTTGGCCCCACT- 3′	Saric et. Al., 2008
3	IL13 +130 G/A	Forward 5′- TGGCGTTCTACTCACGTGCT-3′ Reverse 5′-CAGCACAGGCTGAGGTCTAA- 3′	
4	IL4 -590 C/T	Forward 5′- ACTAGGCCTCACCTGATACG-3′ Reverse 5′- GTTGTAATGCAGTCCTCCTG-3′	Gatlin et. al., 2009

5	GSTM1	G1: 5′-CTGCCCTACTTGATTGATGGG- 3′ G2: 5′-CTGGATTGTAGCAGATCATGC -3′	Brockmoller et. al., 2000
6	GSTT1	Forward: 5′- TCT GCC GCC CGA AAC CTT- 3′ Reverse: 5′- ACG TCC TCT TGT CCC CCA TTC- 3′	Matic et. al., 2016
7	GAPDH	Forward: 5′- CAA AGC TTG TGC CCA GAC TGT- 3′ Reverse: 5′- CGC CCA ATA CGA CCA AAT CT- 3′	23

Statistical Analysis

Statistical analysis was done using SPSS version 20.0 to determine the χ^2 and Odds ratio with P value set at <0.05.

2. Results

1. *Prevalence of urinary schistosomiasis and bladder pathology*

A total of 371 participants (130 males and 241 females) aged 30-90 years were recruited for the study. While 369 were eventually screened for *S. haematobium* infection and 362 for bladder pathologies, some of the volunteers were excluded from the study using the exclusion criteria (Table 2). The mean age of the participants was 48.6 ±0.6 years. The overall prevalence of *S. haematobium* in the sampled population was 29.3% (108/369), 42 (11.4%) in males and 66 (17.9%) in females (Table 2). The Eggua community had the highest prevalence of infection 58 (16.0%), while Ibeku 9 (2.5%) had the least prevalence of infection (Table 2). Bladder pathologies were observed in 32.3% (117/362) of sampled population (Table 2).

Table 2: Prevalence of Schistosomiasis among the settlements in Eggua

	***S. haematobium* Infection**					
Villages	**Positive N (%)**	**Negative N (%)**	**Total N (%)**	**Prevalence/ villages (%)**	**Bladder Pathology**	
Eggua	58 (16.0)	149 (41.2)	207 (57.2)	28.0	Mild	109 (30.1)
Iganalade	10 (2.8)	27 (7.5)	37 (10.2)	27.0	Severe	8 (2.21)
Agbon-Ojodu	31(8.6)	56 (15.5)	87 (24.0)	35.6	**Total**	117 (32.3)
Ibeku	9 (2.5)	18 (5.0)	27 (7.5)	33.3		
Tata	0(0.0)	4 (1.1)	4 (1.1)	0		
Total	108 (29.8)	254 (70.2)	362 (100.0)			
Infection Intensity/ Gender	**Light**	**Heavy**	**Male**	**Female**		
	28 (7.5)	80 (21.8)	42 (11.4)	66 (17.9)		

2.

3. *Cytokine gene polymorphisms and genetic susceptibility to schistosomiasis*

None of the *S. haematobium* infection cases was amplified for *IL 13 +130* and *IL 13 -591* gene polymorphisms while genetic polymorphisms in *IL 4 -590* and *IL 13 -1055* were found in some of the infected participants. *IL 4 -590* and *IL 13 -1055* were amplified and found to have 200bp and 230bp, respectively. Majority (4 (40%) *IL13-1055* and 11 (42.3%) *IL4-590*) of the cytokine polymorphisms clustered around Eggua central among the understudied settlements (Table 3).

Table 3: Distribution of cytokine polymorphisms across settlements in Eggua

Villages	IL 13 -1055 C/T			IL 4 -590 C/T		
	Present N (%)	Absent N (%)	Total N (%)	Present N (%)	Absent N (%)	Total N (%)
Eggua Central	4 (40.0)	25 (25.5)	29 (26.9)	11 (42.3)	18 (22.0)	29 (26.9)
Iganalade	2 (20.0)	17 (17.3)	19 (17.6)	4 (15.4)	15 (18.3)	19 (17.6)
Agbon Ojodu	3 (30.0)	21 (21.4)	24 (22.2)	3 (11.5)	21 (25.6)	24 (22.2)
Ibeku	1 (10.0)	18 (18.4)	19 (17.6)	1 (3.8)	18 (22.0)	19 (17.6)
Tata	0 (0.0)	17 (17.3)	17 (15.7)	7 (26.9)	10 (12.2)	17 (15.7)
Total	10 (100.0)	98 (100.0)	108 (100.0)	26 (100.0)	82 (100.0)	108 (100.0)

Only 10 samples from the infected participants (9.3%) amplified for *IL 13 -1055* polymorphism when analyzed for genetic susceptibility to schistosomiasis, and this showed no risk of infection in males 3 (7.5%) (OR 0.7, 95% CI 0.3-2.1) and slight risk in females 7 (10.3%) (OR 1.1, 95% CI 0.7-1.7). Among 26 (24.1%) *S. haematobium*- infected participants with *IL 4 -590* polymorphism , a slight risk of infection was found both in male 10 (25%) (OR 1.05, 95% CI 0.5-01.8) and female 16 (23.5%) (OR 1.05; 95% CI 0.5-01.8) participants (Table 4).

Table 4: Cytokines as susceptibility risk factors for schistosomiasis after stratification by gender

Cytokines			**GENDER** Male N (%)	Female N (%)	Total N (%)
IL 4 χ^2 = 0.03, df= 1, *P*= 1.0					
S. haematobium Positive	IL 4 -590 C/T	Present	10 (25.0)	16 (23.5)	26 (24.1)
		Absent	30 (75.0)	52 (76.5)	82 (75.9)
		Total	40 (100.0)	68 (100.0)	108 (100.0)
		OR (95% CI)	**1.05 (0.5-1.8)**	**0.97 (0.7-1.4)**	
IL 13 χ^2 = 0.23, df= 1, *P*= 0.74					
S. haematobium	IL 13 -1055 C/T	Present	3 (7.5)	7 (10.3)	10 (9.3)
		Absent	37 (92.5)	61 (89.7)	98 (90.7)
		OR (95% CI)	**0.7 (0.3-2.1)**	**1.1 (0.7-1.7)**	

4. *GST gene polymorphisms in susceptibility to schistosomiasis- associated bladder pathologies*

Amplification of GSTM1 and GSTT1 genes yielded 500bp and 400bp fragments, respectively, among the sampled population. The GSTMI amplicons were found in 36 (30.8%) pathology cases and 7 (7.1%) control samples while GSTT1 was found in 20 (17.1%) pathology cases and 4 (4.1%) control samples. Most of the participants with bladder pathologies had GSTM1 81 (69.2%) and GSTT1 97 (82.9) null genotypes, respectively. These revealed an elevated risk of bladder pathologies for participants carrying either the GSTM1 (OR= 4.3, 95% CI 2.0-9.2) or GSTT1 (OR= 4.2, 95% CI 1.5-12) polymorphic null genotype when compared to those with the corresponding genotype. However there were wide confidence intervals (Table 5).

Table 5: Association between *GSTT1* and *GSTM1* polymorphisms and bladder pathology risk among the participants

Genotype	Pathology				Pathology Intensity			
	Cases N (%)	Control N (%)	OR[b] 95%CI[a]	*P* 2 tailed	Mild N (%)	Severe N (%)	OR[b] 95%CI[a]	*P* 2 tailed
GSTM1								
Active	36 (30.8)	7 (7.1)	1.00ref	0.001	34 (30.2)	2 (30.0)	1.00ref	1.00
Null	81 (69.2)	91 (92.9)	4.3 (2.0-9.2)		74 (69.8)	6 (70.0)	1.0 (0.4-2.7)	
GSTT1								
Active	20 (17.1)	4 (4.1)	1.00ref	0.002	18 (16.5)	2 (20.0)	1.00ref	0.63
Null	97 (82.9)	94 (95.9)	4.2 (1.5-12)		91 (83.5)	6 (80.0)	0.8 (0.2-3.1)	
Combined Genotype								
Both Active	7 (6.0)	0 (0)	1.00ref	0.1	7 (6.6)	0 (0)	1.00ref	0.49
Either Active	42 (35.9)	11 (11.2)	3.4 (1.9-6.2)		36 (34.0)	4 (50.0)	0.7 (0.4-1.4)	
Both Null	68 (58.1)	87 (88.8)	0.6 (0.6-0.8)		63 (59.4)	4 (50.0)	1.2 (0.7-2.4)	

[a]Confidence Interval, [b]odds ratio, ref-Reference

The risks associated with combined activities of the *GSTM1* and *GSTT1* genes were also examined and a higher risk of having bladder pathology was found in participants with one of either of the active genes (OR= 3.4, 95% CI 1.9-6.2) compared to those having both null genotypes (OR= 0.6, 95% CI 0.6-0.8). There was no distinctive risk found in having a high intensity of pathology with any of the GST genotypes when the combined variant and active genotype were considered, but there was a slight risk of mild bladder pathology in the participants in the presence of both inactive variants (both null genotype) when compared to those with either one alone (OR= 1.2, 95% CI 0.7-2.4).

Association of schistosomiasis and smoking in the development of bladder pathology among the participants was examined within the genotypes. It showed that the *S. haematobium*- infected participants had more variant GSTM1 73.1% (OR= 1.7, 95% CI 1.0-3) and GSTT1 85.9% (OR= 1.5, 95% CI 0.7-3.1) null polymorphisms, revealing a slight risk of bladder pathology (Table 6). The case was different among smokers with null genotype for the GSTM1 and GSTT1 genotypes with higher risk of bladder pathology (OR= 3, 95% CI 1-7, *P*= 0.05; OR= 4, 95% CI 1-11, *P*= 0.006 respectively).

There was a slight risk of bladder pathology among the smokers and *S. haematobium*-infected participants with GSTT1 and GSTM1 null polymorphisms (Supplementary Table 1).

Supplementary Table 1: Relative risk estimates of bladder pathology associated with smoking and *S. haematobium* infection after stratification by genotype

Risk Factors	*GSTs* Genotype	Pathology Status Cases N (%)	Control N (%)	[b]OR (95% [a]CI)	P value (2 Tailed)
Cigarette Smoking					
GSTT1					
Smokers	Active	6 (40.0)	0 (0)	1.0ref	0.4
	Null	9 (60.0)	2 (100)	1.2 (0.9-1.6)	
Non Smokers	Active	14 (13.7)	4 (4.2)	1.0ref	0.02
	Null	88 (86.3)	92 (95.8)	1.6 (1.1-2.1)	
GSTM1					
Smokers	Active	7 (46.7)	0(0.0)	1.0ref	0.48

	Null	8 (53.3)	2 (100)	1.3 (0.9-1.7)	
Non-smokers	Active	29 (28.4)	7 (7.3)	1.0ref	0.001
	Null	73 (71.6)	89 (92.7)	1.8	
***S. haematobium* Infection**					
GSTT1					
Positive	Active	10 (16.4)	1 (5.9)	1.0ref	0.43
	Null	51 (83.6)	16 (94.1)	1.2 (0.9-1.5)	
Negative	Active	10 (18.2)	3 (3.7)	1.0ref	0.007
	Null	45 (81.8)	78 (96.3)	2.1 (1.4-3.1)	
GSTM1					
Positive	Active	20 (32.8)	1 (5.9)	1.0ref	0.02
	Null	41 (67.2)	16 (94.1)	7.8 (0.9-63.1)	
Negative	Active	15 (27.3)	6 (7.4)	1.0ref	0.002
	Null	40 (72.7)	75 (92.6)	4.6 (1.7-13.0)	

[a]Confidence Interval, [b]odds ratio, ref- Reference

These risks were significant among non-smokers with GSTT1 (OR= 1.6, 95% CI 1.1-2.1, *P*= 0.02) and GSTM1 (OR= 1.8, 95% CI 1.4-2.2, *P*= 0.001) null genotypes in the population studied. Participants with or without *S. haematobium* infection having the null GSTT1 and GSTM1 genotype also had a relatively small elevation in risk while some differences in the magnitude of risk associated with *S. haematobium* infection were apparent between the variants of the *GSTM1* genotype (OR 7.8, 95%CI 0.9-63.1, *P*= 0.02). Using multivariate analysis, it is evident in this study that bladder pathology has a statistically significant association with Schistosoma infection (F (1,205) =33.04; p=0.001; partial η^2=0.14), GSTM1 polymorphisms (F (1,205) =13.32; p=0.001; partial η^2=0.06) and cigarette smoking (F (1,205) =5.79; p=0.01; partial η^2=0.03). there was also statistically significant difference in bladder pathology and the studied risk factors (F (5,201) =11.42; p=0.001; Wilk's A= 0.779, partial η^2=0.06) (Supplementary Table 2).

Supplementary Table 2: Multivariate analysis showing the relationship between bladder pathology and gene polymorphisms among the participants

Source	**Risk Factors**	**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**	**Noncent. Parameter**
Bladder Pathology	*Schistosoma* Infection	6.637	1	6.637	33.035	0.000	0.139	33.035
	GSTM1 polymorphisms	1.775	1	1.775	13.324	0.000	0.061	13.324
	GSTT polymorphisms	0.318	1	0.318	4.259	0.040	0.020	4.259
	Combine Genotype for GSTs	3.594	1	3.594	20.846	0.000	0.092	20.846
	Cigarette Smoking	0.334	1	0.334	5.787	0.017	0.027	5.787
	Effect	**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**	**Partial Eta Squared**	**Noncent. Parameter**
Bladder Pathology	Wilks' Lambda	0.779	11.419[b]	5.000	201.000	0.000	0.221	57.094

3.

4. DISCUSSION

1. *Infection and Pathology*

The overall prevalence (29.3%) in this study was higher than several reported cases in Nigeria (Nmorsi et. al., 2007; Dawet et. al., 2012; Ugochukwu et. al., 2013; Olayinka et. al., 2020). This could further explain the continuous and long-time exposure to infection and possibly contribute to *S. haematobium* subtle morbidity.

The consistently higher frequency of light intensity of *S. haematobium* infection observed in this study as previously seen in Onile et. al., (2016) could be explained by some possible level of gradual development of acquired protected immunity by adults in this community due to chronic exposure to schistosomiasis (Barbosa et. al., 2006). Also, according to the WHO Expert Committee (WHO, 2002), prevalence and intensity of infection have been directly related to the patterns of variation with age with a reported decline in adults, an assertion supported by studies in Nigerian populations (Pukuma and Musa, 2007; Dawaki et. al., 2016). Pearce and MacDonald, (2002) also reported an obvious pattern of age-dependent intensity of infection where those who are below the age of puberty carry the most parasites, and those in older age groups are generally less heavily infected.

The association between *S. haematobium* infection and the presence of urinary tract abnormalities was consistent in our studies and similar to other previous reports (Nmorsi et. al., 2007; Ekwunife et. al., 2009; Onile et. al., 2016; Serieye et. al., 1996). Also, *S. haematobium* infection has been associated with a two to tenfold increase in the risk of bladder squamous cell carcinoma, as well as being a potential cause of kidney damage (Driguez et. al., 2016). In fact, in some of the regions where *S. haematobium* is endemic, bladder cancer has been marked as the most common cancer in men and the second in women, just behind breast cancer, and accounts for as much as 30% of all cancer cases (Botelho et. al., 2010). A meta-analysis of the estimated disease burden showed that morbidity and mortality attributed to schistosomiasis increases with DALYs (disability-adjusted life years) which had risen to about ~20% increase in the past 20 years (Driguez et. al., 2016; Murray et. al., 2013). A retrospective review of clinical records of bladder cancer cases in Sokoto, Nigeria between 1999 and 2004 showed a 4.7 fold rise in the number of bladder cancer cases, with squamous cell carcinoma composed of 65.1% of histologically verified cases and 50% of the squamous cell carcinoma showed evidence of chronic urinary schistosomiasis (Mungadi and Malami, 2007).

Genetic Susceptibility to Schistosomiasis

The presence of polymorphisms in cytokine genes *IL 4-590 C/T* and *IL13-1055 C/T* among the *S. haematobium*- infected participants in this study was similar to findings from some previous studies (Kouriba et. al., 2005; Gatlin et. al., 2009; He et. al., 2008). Gatlin et. al., (2009) had reported more resistance to infection among men with a combination of *IL-13 21055* C/T and *IL-4 2590* C/T genotypes when compared to those seen with the sum of the separate effects of *IL-13 21055* C/T and *IL-4 2590* C/T on resistance. Other studies have shown a marked increase in the plasma levels of IL-5 and IL-13 in individuals identified as being resistant to schistosome infection (Leenstra et. al., 2006). Therefore, to understand the role of this heterozygous cytokine as a susceptibility or resistance factor, further post- treatment follow-up study will be required among the infected participants to establish the role of these cytokines as has been done in other similar studies (Cameron et. al., 2006; Gatlin et. al., 2009). Gatlin et. al., (2009) had reported that individuals with polymorphisms at positions *IL-13 -1055* and *IL-4 -590* are more likely to require fewer reinfections and treatments to become resistant to reinfection than individuals who are homozygous at either position. Another study in Mali revealed an association between a single-nucleotide polymorphism in the *STAT6* gene at 12q13.3 and intensity of infection by *S. haematobium*; this polymorphism had an additive effect with *IL13 −1055* (He et. al., 2008).

Other analyses of *S. haematobium* infection in Mali revealed that in chromosomal region 5q31-q33, polymorphisms in the *IL13* gene promoter

at position *−1055* and *−591* were associated with the infection rate: alleles −1055C and −591A were preferentially transmitted to children with 10% highest infection rate, whereas *−1055T* associated with the lowest infection levels (Kouriba et. al., 2005). *IL4 −590T* allele has been associated with high IgE production, thereby having increased resistance to infection (Russell et. al., 2015).

Evidence has shown that imbalance in activation and detoxification by detoxifying enzymes (*GSTs*) due to gene polymorphisms may influence an increase in bladder cancer risk due to accumulation of carcinogen metabolites (McGrath et. al., 2006; Ying et. al., 2016; Jobaida et. al., 2016; Yajie et. al., 2016). Common polymorphisms occur in almost all members of GSTs (Ying et. al., 2016) and several types of allelic variations have been observed (Djukic et. al., 2013; Yajie et. al., 2016; Matic et. al., 2016) which include *GSTM1* and *GSTT1* class deletion polymorphism (*GSTM1-null* and *GSTT1-null*). The null genotypes produce no GSTM1 and GSTT1 protein and consequently completely lack GSTM1 and GSTT1 enzymatic activity (Djukic et. al., 2013).

In this study, *GSTM1* and *GSTT1* null polymorphisms were shown to significantly increase the risk of structural bladder pathology. This agrees with Arnaldo et. al., (2000), Aktas et. al., (2001), Cengiz et. al., (2007), Yajie et. al., (2016) and Jobaida et. al., (2016) who also observed a risk of bladder cancer with *GSTM1* and *GSTT1* null polymorphisms. Okkels et. al., (1996) and Arnaldo et. al., (2000) reported that the association of *GSTM1* null genotype with bladder tumour was more apparent in a group with less aggressive tumours, as we also observed in this study. This could further support the presence of *GSTM1* null genotype among the urinary tract pathology cases (an indicator of early stage of possible progression to bladder cancer). Among the bladder pathology cases, the distribution of the polymorphisms was relatively similar to the control group with slightly higher *GSTM1* null genotype in the control cases. This is similar but considerably higher than what was reported in several other studies (Okkels et. al., 1996; Arnaldo *et. al.,*2000; Jobaida et. al., 2016). The role of *GSTT1* null genotype in bladder cancer risk remains unresolved. Several studies suggested an increased risk (Moore et. al., 2004; Yajie et. al., 2016; Jobaida et. al., 2016; Ying et. al., 2016), but others suggested no risks or low risks (Karagas et. al., 2005; McGrath et. al., 2006; Matic et. al., 2016). In the present study, an elevated risk of bladder cancer was found among the *S. haematobium* - infected participants; and this was more in smokers with null *GSTM1* and *GSTT1* polymorphisms. This finding is similar to those observed by Moore et. al., (2004) and Yu et. al., (2017) where elevated risk to bladder cancer was only seen in smokers with the *GSTT1* null polymorphism and seems to buttress the observation that smoking is the most important risk factor for susceptibility to bladder cancer.

2. Conclusion

The prevalence of urinary schistosomiasis among adults is relatively high in the study area when compared with other areas in Nigeria. Individuals with bladder pathologies could have heavy or light intensity of schistosomiasis or have no existing infection at all. However, long term exposure to schistosomiasis is necessary for the development of bladder pathology which may eventually advance to cancer. *IL 13-1055* polymorphisms did not indicate susceptibility to schistosomiasis in males, but a slight risk was found in females. *GSTM1* and *GSTT1* polymorphisms were associated with elevated risk of bladder pathology with the pathology and schistosomiasis group having more GST polymorphisms than the group with only bladder pathology.

Limitation of the study

In order to elucidate properly the role of GST (*GSTM1* and *GSTT1*) polymorphisms in susceptibility to urinary schistosomiasis associated bladder pathology, it is important to conduct a repeat of this study with a much larger sample size. Also, molecularly characterized schistosomiasis negative control samples will be required to further establishe the relationship (susceptibility/resistance) between the cytokines (*IL-13 -1055* and *IL-4 -590*) and urinary schistosomiasis.

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The authors declare that they have no conflicts of interest.

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