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Cancer Genetic Markers Among School Children in Relation to Urogenital Schistosomiasis

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ABSTRACT

Chronic infection with urogenital schistosomiasis can lead to severe complications such as bladder cancer. Hence, this study determined the presence of Cancer Genetic Markers among School Children in relation to Urogenital Schistosomiasis. All 36 *S. haematobium* positive cases from an earlier study and randomly selected 156 negative samples were used. Immunogens tested for, included glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and fibroblast growth factor receptor (FGFR3). Mean optical density (OD) was 0.73 and 0.79 for GAPDH and FGFR3 respectively. A total of 21 (10.94%) participants, 16 females and 5 males were positive for GAPDH marker. Fifty (26.04%) participants were positive for FGFR3 via ELISA cutoff. Mean OD for *S. haematobium* positive individuals tested for GAPDH was 0.938, minimum value was 0.119 while maximum value was 4.507. For FGFR3 mean OD was 0.896, Maximum 2.882 and minimum 0.28, while females showed higher GAPDH OD. More females than males who were positive for *S. haematobium* were also positive for GAPDH (11.1%) and FGFR3 (25%). FGFR3 and GAPDH marker prevalence for Individuals Positive for *S. haematobium* is 25% and 16.7% respectively. The age group 11-15 years were the only group positive for GAPDH. There was a slight positive correlation between age and FGFR3 and age and GAPDH. The presence of these markers is an indicator that the children in the selected communities maybe at risk of developing bladder cancer in the future if the disease is not properly managed and controlled.

INTRODUCTION

Schistosomes are parasitic trematodes that cause the disease schistosomiasis; they are found in subtropical and tropical regions of the world including Nigeria (Ekwunife *et al*., 2004; Ndukwe *et al*., 2019). Anambra state, Nigeria, offer numerous favourable habitats for aquatic snails that serve as intermediate hosts to *Schistosoma* species (Ekwunife *et al*., 2004). Children have been identified to harbour the greatest number of worms leading to reduced growth, impaired memory and cognition and reduced school attendance (Crompton and Nesheim, 2002; Miguel and Kremer, 2004; Bundy *et al*., 2013). Also, cases of chronic infection with urogenital schistosomiasis can lead to adverse health outcomes including the development of urogenital cancer. (Ishida and Hsieh, 2018). There are certain genetic factors that could also play important role in the pathology of infection with schistosomes including the possibility of the development of urogenital cancer as a result of granuloma formation (Barosum, 2021). The general activity in an individual is controlled by the genetic expression of that individual Therefore information on the roles played by some genetic markers in relation to age of individuals predisposed to urogenital schistosomiasis will give vital information on the immune protective response to the parasite among group of people that are at risk to the development of severe disease pathology. Therefore, this study aimed to determined the presence of Cancer Genetic Markers among School Children in relation to Urogenital Schistosomiasis in Anambra North Senatorial District, Nigeria

LITERATURE REVIEW

Cancer is a disease of importance because it is a genetic disorder that can come about as a results genetic or epigenetic alterations in the somatic cells and is associated with abnormal cell growth, it could be invasive or non-invasive (Soria *et al*., 2019; Zhang *et al*., 2015). Cancer has been identified to be caused by a lot of factors including but not limited to tobacco smoking, infections like HIV, hepatitis b, Epstein-Barretc, poor diet, obesity, excessive consumption of alcohol, exposure to ionizing radiation, and gases, bladder inflammation due to microbial and parasitic infections, as well as some adverse side-effects of medications (Saini *et al*., 2020). There are different forms of cancer of which bladder cancer is inclusive.

Bladder cancer is a disease that can arise from various factors including infection with *Schistosoma haematobium* which has significant diagnostic, therapeutic and prognostic challenges (Kamat *et al*., 2013) cases of which squamous cell cancers of the urinary bladder were identified to be proportionately more common in populations with a high prevalence of *S. haematobium* infection and a high proportion of urinary bladder cancers (IARC, 2012). The estimated incidence of urinary bladder cancer has been related to the proportion of cancerous urinary bladder specimens which contains *S. haematobium* eggs or egg remnants (IARC, 2012). Also, the sex ratio of urinary bladder cancer cases show some variation although it corresponded to the relative involvement of men and women in agricultural work which has been identified as a risk factor for *S. haematobium* infection (IARC, 2012).

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Adult worms in *Schistosoma haematobium* infection are seen to reside in the urinary system where they lay eggs that cause disease pathology. Studies reported in the International Agency for Research on Cancer (IARC) and other studies (Badawi *et al*., 1995; Mostafa *et al*., 1999; Mayer and Fried, 2007; Ekwunife *et al*., 2009) have supported an association between the occurrence of urinary bladder cancer and *S. haematobium* infection. Studies have shown that: the estimated incidence of urinary bladder cancer was higher in areas with a high prevalence of *S. haematobium* infection than in areas with a low prevalence (IARC, 2012).

Another study on the incidence of different histological types of bladder cancer in various racial groups living within the same geographic area of Kwazulu-Natal, South Africa, reported similar results: squamous cell carcinoma occurred in 53% of the African patients (who have a much higher risk of exposure and infestation to *S. haematobium* due to socioeconomic, cultural and educational factors), and in 2% of the Caucasian patients (Groeneveld *et al.* 1996). Also, Groeneveld *et al.* (1996) reported that eggs of *S. haematobium* were seen in microscopic sections of the bladder tumour in 85% of the patients with squamous cell carcinoma, and in 10% of the patients with transitional cell carcinoma with the mean age at presentation of African patients was at least 20 years younger than that of Caucasian patients.

Adult *Schistosoma haematobium* worms do not multiply in the host, but rather produce o♀spring that must exit the host to continue the parasite life cycle (Klion and Nutman, 2002). Helminth species such as *Schistosoma haematobium* have developed complex and redundant mechanisms which help them to maintain a chronic infection despite immune recognition by the host. These parasite are able to attain the chronic infection by adoption of certain strategies which include residing in anatomical locations that are relatively free to immune attack, molecular mimicry, shedding of antigenic surface proteins, and down- regulation of the host immune response to helminth antigens, thus, producing a state of parasite-specific immune tolerance (Brooks *et al*., 2010).

When an individual gets infected with a parasite or any foreign material, the immune system activates the humoral and cell mediated immune response (Thomas and Harn, 2004; Hokke and Yazdanbakhsh, 2005; Van Die and Cummings, 2006; Hokke *et al*., 2007; Mickum *et al*., 2014), this immune response can also be found in infection with Schistosomes.

Schistosomes are parasites that might not be cleared through the process of phagocytosis by host immune responses; therefore, in most cases the host immune system responds through inflammation and hypersensitivity. Immunoglobulin G (IgG), Eosinophils and immunoglobulin E (IgE) are activated to initiate inflammatory response in the site where the parasite is found. Infection with Schistosomes has been shown to elicit various immune responses such as the induced release of IL- 6, TNF, and IL1-*B* from monocytes (TeVelde *et al*., 1990), inhibition of Th17-development (Park *et al*., 2005) and trigger the alternative activation of macrophages with the help of IL-13 (Gea-Sorlí and Closa, 2009). Also, there is the suggestion that 70 % of bladder cancers involve a specific mutation in a particular gene called the telomerase reverse transcriptase (TERT) gene (Zhang and Zhang, 2015).

The TERT gene is involved in DNA protection, cellular aging processes, and cancer. There could be genetic mutations in some chromosomal genes, such as FGFR3, RB1, HRAS, TP53, TSC1, and others which may play certain roles in the formation of tumors in the urinary bladder (Zhang and Zhang, 2015). These genes play an important role in the regulation of gene mutations on p53 suppressor gene as was shown in a study which evaluated 18 different bladder tumors of which 11 (61 %) had genetic mutations of p53 gene (Zhang and Zhang, 2015). The p53 marker has also been associated with the most aggressive T1G3 cancers (Soria *et al*., 2019). HRAS is a proto-oncogene and has potential to cause cancer in several organs including the bladder. The TSC1 c. 1907 1908 del (E636fs) mutation in bladder cancer suggests that the location of the mutation is Exon 15 with frequency of TSC1 mutation of 11.7 %. The BAP1 mutations have shown that it contributes to BRCA pathway alterations in bladder cancer. The discoveries of more gene mutations and new biomarkers and polymerase chain reaction bioassays for gene mutations in bladder cancer need further research (Zhang and Zhang, 2015).

The regulation of the immune response in human schistosome infection determines the pathogenic response of the host. Also, an individual's genetic make-up is the template on which every of the organism's profile is written including the individual's response to diseases such as schistosomiasis. Chronic *Schistosoma* infections are usually seen to be established through the modulation of the host immune system (Waknine-Grinberg *et al*., 2010).

One of the complications of urogenital shistosomiasis is the formation of granuloma on the bladder walls, which is the main lesion found in schistosomiasis (Ekwunife *et al*., 2009) and is also a predisposing factor to the development of bladder cancer (Ishida and Hsieh, 2018). This bladder cancer is the worldwide 9th most common cancer (Siegel *et al*., 2015). Other pathologic effects due to *S. haematobium* include: irregularity of bladder wall, thickening of the bladder wall, massing of bladder wall, dilated kidney and bladder wall lesions (Ekwunife *et al*., 2009).

In non-invasive tumors, mutations had been found in the fibroblasts growth factor receptor 3 (FGFR3) where the presence of the FGFR3 mutation in urine is observed for low-grade tumors and are proposed to be associated with concomitant or future recurrence (Frantzi *et al*., 2012; Critelli *et al*., 2016). FGFR3 is a genetic marker that has been implicated in bladder cancer where some cases of multiple myeloma are seen to express both mutation and over-expression of FGFR3 (Akanksha and Sandhya, 2019).

Furthermore, there are also studies that has shown that there could be increased glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels in many human cancer types which has been correlated with possible reduction in survival (Altenberg and Greulich, 2004; Guo *et al*., 2013). There had also been report of deregulation of GAPDH in bladder cancer cases (Colell *et al*., 2009; Guo *et al*., 2013). GAPDH has been implicated to play certain roles in apoptosis (Colell *et al*., 2009) and there are suggestion that GAPDH participates in tumor progression and could serve as a new therapeutic target (Zhang *et al*., 2015).

MATERIALS AND METHODS

Ethical Approval: Ethical approval was obtained from ethics board of Nnamdi Azikiwe University Teaching Hospital (NAUTH) (NAUTH/CS/66/VOL.13/VER III/10/2020/07).

Study Site and Sample Collection: A cross-sectional study was earlier carried out in Anambra North Senatorial district of Anambra state for presence of urogenital schistosomiasis (the manuscript on the prevalence is presently under review in the Nigerian Journal of Parasitology). Anambra north senatorial district is made up of 7 local government (LGA) areas which are: Anambra East LGA, Anambra west LGA, Ayamelum LGA, Ogbaru LGA, Onitsha North LGA, Onitsha South LGA and Oyi LGA (MICTU/UNIZIK, 2019). Three LGAs were randomly selected. Informed consent from community heads, head teachers and parents were obtained before sample collection. Blood samples (2mls) were collected in EDTA bottles for further immunological studies.

A subset of the general study population was used for serology. Two plates of 96 wells was used for the assay. The blood samples collected in the EDTA bottles were spurned at 3000 revolution per minute for five minutes. The plasma was separated from the EDTA container into cryogenic vials. The separated plasma was stored in cryogenic vials for serology. Serology was done using Enzyme Linked Immunosorbent Assay (ELISA). This assay was done for both positive and negative samples. Immunogens tested for, included glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and fibroblast growth factor receptor (FGFR3). Age range of individuals tested was 6-18 years.

The Human FGFR3 ELISA Kit and the Anti-GAPDH monoclonal Antibody by MyBiosource.com was used for this study. The assay procedures were carried out following manufacturer's instructions. Procedure for the coating of plate for the FGFR3 and Anti-GAPDH monoclonal Antibody was done following the protocol by Thermo Fisher Scientific Inc. (2010). Optical density (OD) of plate was read at 450nm using an ELISA plate reader. Wash buffer was prepared using 0.05% tween 20 and Phosphate buffer saline (PBS). The markers, HRP conjugated anti-Rabbit antigen, blocking buffer, Substrate and Stop solution were all provided by the manufacturer. Data was entered and cleaned in Microsoft excel. Data was analyzed using Microsoft Excel and IBM SPSS 20 software. McNemar's test and Inter rater reliability tests were tested for all ELISA tests. The Fisher exact tests and chi-square were used to compare *S. haematobium* infection rates between age and sex. The Spearman's and Pearson correlation coefficient was used to determine relationships.

RESULTS AND DISCUSSION

In the said study, a total of 396 children were sampled and urine microscopy done for *Schistosoma haematobium* detection. From this, 36 children were positive for schistosomiasis. All 36 *S. haematobium* positive consisting of 26 females and 10 males and randomly selected 156 negative samples were used for the study. The study subset included a total of 192 samples, 108 females and 84 males. Absorbance cutoff points were determined as 1.0 and 1.1 for GAPDH and FGFR3 respectively (Figure 1, Figure 2). Mean optical density was 0.73 and 0.79 for GAPDH and FGFR3 respectively. The result from the *S. haematobium* 36 positive cases showed a Mean OD for GAPDH as 0.938, minimum value was 0.119 while maximum value was 4.507. For FGFR3 mean OD was 0.896, Maximum 2.882 and minimum 0.28, while females showed higher GAPDH OD (Table 1).

This study identified that there are some school children that are positive for the cancer genetic markers GAPDH and FGFR3 in the study area. Also, in the case of individuals that are positive for *S. haematobium* eggs, it was also noted that some of the participants are positive for the cancer genetic markers. More positivity rate was noted for FGFR3 than GAPDH. A study on S. mansoni has sown that there is an indication that the expression of the glycolytic enzyme GAPDH is as a result of the parasite activity (Pirovich *et al*., 2020). GAPDH has been implicated as an immune marker that is essential for cancer cells by influencing cancer cell fate and may be a critical regulator of cancer cell functions and hence a marker of cancer cell progression and prognosis (Zhang *et al*., 2015). FGFR3 has also been implicated in the development of different forms of cancer and could also serve as cancer prognostic marker (Akanksha and Sandhya, 2019).

This basically implies that among this study group, there is a risk of possible progression of bladder cancer development if infection with urogenital schistosomiais is not well managed. This should be an issue of public health importance since this implies potential risk of bladder cancer development because, the positivity for these markers identifies that there is a potential risk of them developing bladder cancer in the future because, these markers could serve as cancer prognostic markers (Akanksha and Sandhya, 2019; Zhang *et al*., 2015).

A total of 21 (10.94%) participants were positive for

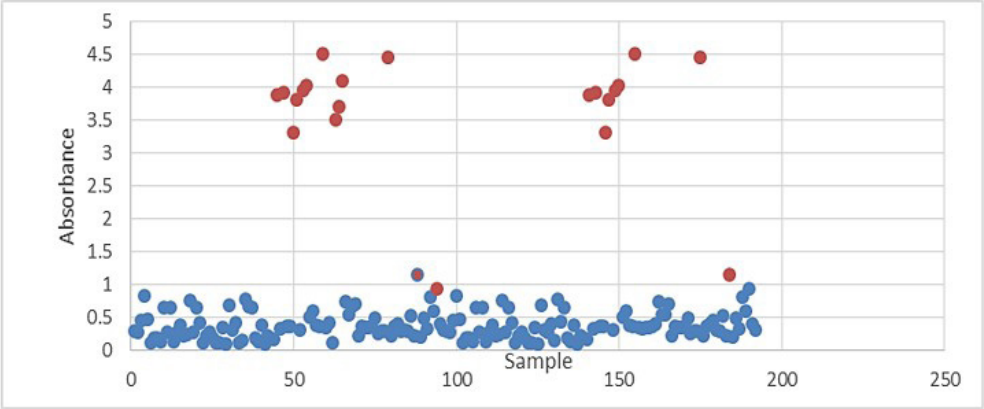

Figure 1: Scatter plot of GAPDH ELISA detection results.

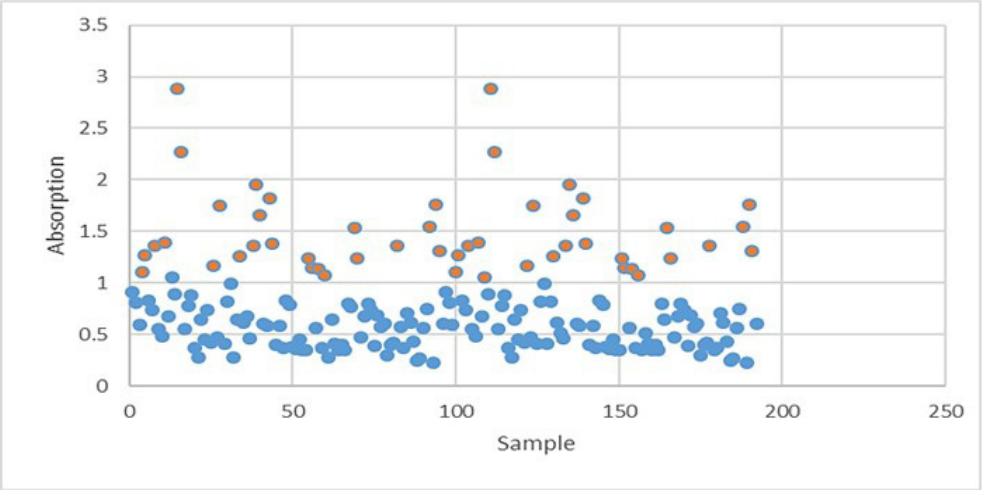

Figure 2: Scatter plot of FGFR3 ELISA detection results.

Table 1: OD and Prevalence of GAPDH and FGFR3 by Sex

	GAPDH		**FGFR3**	
	Female	Male	Female	Male
Mean OD	0.876	0.557	0.822	0.733
Max OD	4.448	4.507	2.26	2.882
Min OD	0.14	0.119	0.248	0.225
No. Negative (%)	92 (85.2)	79 (94)	82 (75.9)	60 (71.4)
No. Positive (%)	16 (14.8)	5 (6.0)	26 (24.1)	24 (28.6)
Total	108	84	108	84

P>0.05

GAPDH while 50 (26.04%) participants were positive for FGFR3 via ELISA cutoff (Table 2). Age range of individuals tested was 6-18 years. There was a slight positive correlation between age and GAPDH though this was not statistically significant (r= 0.27, p>0.005). There was also a slight positive correlation between age and FGFR3 (r= 0.27, p>0.005). *S. haematobium* positive individuals in the age group 11-15 years were the only group positive for GAPDH while for FGFR3 positivity was noticed across all age groups (Table 3). This study was also able to show that there was a weak positive correlation between age and GAPDH and age and FGFR3 for the general assayed population though this was not statistically significant. Individuals from the assayed samples in the age group 11-15 years had a slightly higher GAPDH OD though those in the age group 6-10 years had more positive individuals for the assayed marker. On the other hand, individuals assayed for FGFR3 had more positive cases for those in age group 11-15 years. Furthermore, in individuals that are positive for *S. haematobium* there was no relationship between age and the presence of the markers. Though it was noted that six (6) individuals in the age group 11-15 years who were positive for *S. haematobium* eggs were also positive for GAPDH while for FGFR3 there were positive cases across the different age group represented in the study. This may imply that the risk of the development of

urogenital cancer increases with age as it had been noted that age is the greatest single risk factor for developing urogenital cancer (Shariat *et al.*, 2019). This could also be an indication that the expression of these markers is more represented with an increase in years of school children in the sampled communities. A study by Ahmad *et al* (2018) identified that older age patients tend to show increased frequency of FGFR3 mutations.

Mean FGFR3 and GAPDH OD was higher in females than males. GAPDH mean OD for females was 0.876 while male was 0.557. FGFR3 mean OD for females was 0.866 and males was 0.733. Prevalence of GAPDH showed that 16 (14.8%) of the 108 females were positive while 5 (6.0%) of 84 males were positive for the marker. Also, 26 (24.1%) females and 24 (28.6%) males were positive for FGFR3 (Table 1). More females positive for *S. haematobium* were also positive for GAPDH (11.1%) and FGFR3 (25%) (Table 4). FGFR3 and GAPDH marker prevalence for Individuals Positive for *S. haematobium* is 25% and 16.7% respectively. In this study, female participants showed higher mean GAPDH and FGFR3 OD than male participants. Also, for those positive for *S. haematobium* there were more females positive for the marker than males. This occurrence may be attributed to the fact that they are the most exposed group since they carry out day to day activities in potentially infected *S. haematobium* water bodies and this could make them more likely to present with the markers. This is in line with other studies on FGFR3 gene mutation studies that identified that female patients tend to show increased frequency of FGFR3 mutations (Beukers *et al.*, 2017; Ahmad *et al.*, 2018). In contrast, another study had reported that the presentation of this marker is more common in male than female (Akanksha and Sandhya, 2019). However, this study noted that there is no relationship between sex and the presence of the markers in participants that are positive for *S. haematobium*. Moreover, the International Agency for Research on Cancer has stated that bladder cancer could be associated with the profession of an individual and is more related to Agricultural farmer (IARC, 2012) because of their contact with infected water.

Table 2: Prevalence of GAPDH and FGFR3 in various age group

	GAPDH			**FGFR3**	
Age (Years)	**Total**	**Positive**	**Prevalence (%)**	**Positive**	**Prevalence (%)**
0-5	0	0	0	0	0
6-10	72	12	16.67	16	22.22
11-15	83	6	7.32	24	29.27
16-20	38	3	7.89	10	26.32
Total	**192**	**21**	**10.94**	**50**	**26.04**

P>0.05

Table 3: GAPDH and FGFR3 Prevalence for Individuals Positive for *S. haematobium* in various age group

	GAPDH			**FGFR3**	
Age (Years)	**Total**	**Positive**	**Prevalence (%)**	**Positive**	**Prevalence (%)**
0-5	0	0	0	0	0.00
6-10	9	0	0	5	13.89
11-15	18	6	16.67	5	13.89
16-20	9	0	0	3	8.33
Total	**36**	**6**	**16.67**	**13**	**36.11**

Table 4: GAPDH and FGFR3 Prevalence for Individuals Positive for *S. haematobium* in various age group

	GAPDH			**FGFR3**		
	Overall	**Female**	**Male**	**Overall**	**Female**	**Male**
Average OD	0.956	1.155	0.694	0.896	1.018	0.782
Maximum OD	4.507	4.027	4.507	2.882	2.882	2.26
Minimum OD	0.119	0.229	0.119	0.28	0.364	0.28
Prevalence (%)	6(16.7)	4(11.1)	2(5.6)	13(36.1)	9(25)	4(11.1)

CONCLUSIONS

The study has produced evidence showing that there are children that are positive for the cancer genetic markers FGFR3 and GAPDH with more females being positive for the markers. The presence of these markers are an indicator that children in the selected communities maybe at risk of developing bladder cancer in the future

if the disease is not properly managed and controlled. Also, there is need for more studies on these identified immune markers which may serve as prognostic markers for schistosomiasis progression or urogenital cancer development in at risk population.

This study has potential limitations. This study does not have age representation for children 0-5 years. Though, this has no direct impact on the result of the findings for the other age groups represented. However, further research can be done to include the age group 0-5years to ascertain the representation of the cancer genetic markers of the group. Also, determining the marker concentration using a standard curve will help elucidate more on the relationship between concentration and the different categories assayed in this study.

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