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Cytological Findings in Urine of Adult Residents of Urinary Schistosomiasis Endemic Community in Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author PCI designed the study, performed the statistical analysis, arranged and presented the manuscript. Authors CJA and VIE performed the literature search, collection of samples and parasitological screening, while author MIU performed the cytological analysis of the urine. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate urinary cytological abnormalities among adult residents of a urinary schistosomiasis endemic community.
Study Design: Ethical approval, consent from the village Head/villagers, parasitological survey and cytological analysis of urine.
Place and Duration of Study: Adim Community in Cross River State, Nigeria between May and November 2014
Methodology: Urine samples from 160 sex matched adults aged 18–85 years were examined using standard parasitological techniques for the presence of ova of *Schistosoma haematobium*. The urine smears were stained with Papanicolaou and Alcian Blue (PH 2.5) staining techniques and examined for cytological abnormalities and the presence of Hyaluronic acid respectively.
Results: 18 (11.3%) subjects were infected. Subjects in the age groups >35 – 45 years had the

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highest prevalence rate 9(20.5%) while those in the age group >75 – 85 years had the lowest prevalence rate 0(0%) and the difference was statistically significant (P = 0.359). The infection rate was higher among females 11(12.5%) than the males 7(9.7%) but the difference was not statistically significant (P = 0.580). Males had a higher mean egg count (18±25.7 egg/10 ml of urine) than the females (17±15.5 egg/10 ml of urine). Subjects in the age group >35–45 years had the highest level of abnormal epithelial cells 1(11.1%). Males had a higher prevalence of abnormal cells 3(42.9%) than the females 1(9.1%). There was a positive correlation between the occurrence of infection and abnormal epithelial cells (r = 0.5). 3(1.9%) of the subjects were positive for hyaluronic acid. Male subjects had a higher level of epithelial cells positive for hyaluronic acid 2(2.8%) than the female subjects 1(1.14%) but this was not statistically significant (p<0.05).

Conclusion: This study has reconfirmed the endemicity of urinary schistosomiasis and has also revealed the presence of abnormal epithelial cells as an indicator for bladder cancer in the urine of residents in Adim community.

Keywords: Urinary schistosomiasis; endemic community; cytology; bladder cancer.

1. INTRODUCTION

Urinary schistosomiasis is highly endemic in Adim community, a typical rural setting in Nigeria which lacked clean piped water and good sanitation with no effective integrated approach for the control of this infection. Various authors have reported different prevalences of urinary schistosomiasis in Adim; Ejezie et al. [1] reported 43.5%, Useh and Ejezie, [2] reported 50.4%, Inyang-Etoh et al. [3] reported 29.0% while Inyang-Etoh et al. [4] reported 38.5%. Urinary schistosomiasis has peak prevalence and intensity of early infection between the ages of 10 and 20 years and declines by the age of 65 years [5]. Adult *S. haematobium* commonly invade the venous plexus around the urinary bladder, the eggs released by adult worms cause chronic granulomatous inflammation in the mucosa and submucosa of the urinary bladder. Chronic granulomatous inflammation and irritation subsequently lead to the development of squamous metaplasia of the transitional epithelium and subsequent squamous cell carcinoma. Chronic granulomatous inflammation also leads to bladder fibrosis which causes urine stasis. These responses to egg deposition could lead to calcification of the urinary bladder, infection, stone formation and mucosal proliferation [6]. This work was an attempt to investigate urinary cytological abnormalities among adult residents of this urinary schistosomiasis endemic community.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Adim Community located in Biase Local Government Area in Cross River State, Nigeria. Adim is a typical rural community located 110 kilometers to the North of Calabar, the Cross River State capital. This community has an estimated population of 9, 612 people who are mainly peasant farmers. There is no pipe borne water in the community and the inhabitants depend mostly on three fresh water streams namely Ibeteuroma, Egboga and Ogamenah for their domestic, economic and recreational activities [1]. The area is situated in the tropical rain forest belt with an average annual rainfall of 1500-2000 centimeters. The principle crops grown by the farmers are rice *(Oryza sativa*), yam *(Discorea prachincilis*) and cassava (*Monilot utilissima*). Also they have 3 nursery schools, 2 primary schools, 1 secondary school and 1 health center in the community.

2.2 Subjects and Ethical Consideration

The targeted populations were adults between 18 and 85 years. The consent and ethical approval were sought and obtained from the school authority and the ethical committee of the Cross River State Ministry of Health. The Onun of Adim (village head) was briefed on the significance of the study and the level of involvement of the communities before its commencement. The procedures, significant benefits, and the unharmful nature of the study were also explained to all the people in the community. English language was used as means of communication. An interpreter from the community was used where subjects could not speak or understand English language.

2.3 Study Design

The under listed sequence of activities were followed-

- Visit to Adim and the village head
- Notification and briefing of the village head about the study and seeking the consent of villagers or community members who will be involved in the study. Inform consent which explains the detail procedure of the study and questionnaire was given to them to sign or thumb print.
- Parasitological survey to ascertain the current schistosomiasis situation in the village.
- Cytological analysis of the urine of the adult residents of the community

2.4 Collection of Urine Samples

Two clean universal containers for collection of urine sample were issued to each subject selected for the study. The samples were collected between 10.00 am and 12.00 pm when maximum egg excretion occurs in the urinary bladder [7]. The people were allowed to do a little exercise in order to get a maximum egg shield. Each bottle was labeled according to the code number of the subjects.

2.5 Parasitological Examination

2.5.1 Examination of urine for haematuria and proteinuria

A report on the appearance of the urine, that is color whether clear, cloudy, presence or absence of visible blood were observed and recorded accordingly. Heamaturia was detected soon after collection of urine sample using dipstick (Ames: Bayer Diagnostic Brussels, Belgium) [8]. It was carefully dipped into the bottle containing the urine for 5 seconds. The resulting change in color of the strip was compared with the manufacturer's colour chart to estimate the amount of blood in the urine. The same methods were also used for the examination of proteinuria. Haematuria was reported as 5-10 ery/µl (+), 50ery/µl (++), 250ery/µl (+++). Proteinuria was reported as 10 mg protein/dl indicating trace proteinuria 30 mg/µl(+), 100 mg/µl(++), and 500 mg/µl(+++) [8].

2.5.2 Examination of urine for *Schistosoma haematobium* ova

Ten millitre (10 ml) of the urine sample was transferred into a universal container holding 5ml of 1% aqueous solution of carbol fuchsin for staining and preservation of ova [1]. The specimens were preserved this way until the time of filtration. The modified filtration system for the detection of ova of *Schistosoma haematobium* was adopted for the study as describe elsewhere by [2].

2.6 Cytological Examination of Urine

Twenty milliliters (20 ml) of urine from each subject was placed in a plastic tube containing 5 ml of fixative (95% in ethyl alcohol). The samples were centrifuged and smears of each sediment were prepared and made. The dried smears were stained with Papanicolaou stain and Alcian Blue (PH 2.5) staining technique. The smears were graded, without the knowledge of the subjects clinical or infection status, as positive, negative or suspicious for squamous cell metaplasia, atypia and for inflammation [9]. Normal epithelial cells were characterized by basal or superficial squamous cells with cyanophilic cytoplasms and central round nuclei having fine chromatin. The abnormal epithelial cells were characterized by multinucleated squamous cells or single nucleated squamous cells having orangophilic cytoplasm, hyperkeratosis, hyperchromatic and pyknotic nuclei with coarse chromatin.

2.7 Sample Collection Procedures

Household survey method was used in selecting the different households for the study. A simple random sampling technique was used to select three (3) subjects per household. The three subjects each selected from 54 households gave a total of 162 but two (2) subjects opted out after selection leaving a total of 160 samples.

2.8 Data Analysis

Statistical analysis was performed using a commercial statistical package: SPSS version 16.0 for windows and Microsoft Excel Tool Pak (SPSS for Windows: SPSS Benedeux, Gorinchem, Netherlands). The Chi-squared (X^2) test was used to test for the difference in prevalence of infection and to determine the cytological level of bladder cancer in gender. Correlation between infection, proteinuria and haematuria was analyzed using the Pearson Correlation Coefficient.

3. RESULTS

A total of 160 urine samples were examined for ova of *S. haematobium.* The urine smears were

stained with Papanicolaou and Alcian Blue (PH 2.5) for cytological abnormality in the urine. Of the 160 samples examined, 18(11.3%) subjects were found to be infected with *Schistosoma haematobium*. Table 1 shows the distribution of *S. haematobium* infection, haematuria and proteinuria according to age group of subjects examined. Subjects in the age group >35 - 45 years had the highest prevalence rate 9 (20.5%) and the highest ova/egg count 17(19.0) while subjects in the age group >75 – 85 years had the lowest prevalence rate 0(0%). There was no statistically significant difference in the prevalence of infection according to age groups (X^2 = 6.625; df (6) P = 0.359). Subject in the age group 18 – 25 years had the highest rate of haematuria 4(19.1%) while the lowest rate 0(0%) occurred among subjects in the age group >75 – 85 years. There was a positive correlation between the presence of infection and haematuria. (r = 0.7). Also subject in the age group >55 – 65 years had the highest prevalence of proteinuria 5(33.3) while subject in the age group >75 – 85 years had the lowest rate 0(0%). There was a positive correlation between the presence of infection and proteinuria (r = 0.6).

The distribution of *S. haematobium* infection, haematuria and proteinuria according to gender of subjects examined is shown in Table 2. Female subjects had a higher rate of infection 11(12.5%) than the males 7(9.7%). Also female subjects had a lower rate of haematuria 11(12.5%) than the males 15(20.8%). There was no statistical significant difference in infection according to gender of subjects examined (P = 0.580). Table 3 shows the distribution of abnormal epithelial cells according to age group of subjects examined. 4(2.5%) had abnormal epithelial cells. Subjects in the age group >35 – 45 years had the highest level of abnormal cells 1(11.1%). While subjects in the age group >65 – 76 years and >75 – 86 years had the lowest prevalence rate 0(0%). There was a positive correlation between the presence of infection and abnormal epithelial cells (r = 0.5).Subjects in the age group >55-65 years had the highest number of abnormal epithelial cells positive for hyaluronic acid 1(6.7%) while those in the age group 18-25 years and >45-55years, >65-75 years and also 75 years and above had no abnormal epithelial cells positive for hyaluronic acid. There was no statistically significant difference in the presence of abnormal epithelial cells positive for hyaluronic acid according to age group of subjects examined (X^2 =2.76; df(5); p>0.05). The distribution of abnormal epithelial cells as an indicator for bladder cancer arranged according to gender is shown on Table 4. Males had a higher prevalence rate of abnormal cells 3(4.2%) than the females 1(1.1%). Male subjects had a higher number of abnormal epithelial cells positive for hyaluronic acid 2(2.8%) than female 1(1.1%). There was no statistically significant difference in the presence of abnormal cells positive for hyaluronic acid according to gender of subjects examined (X^2=0.81; df(1); p>0.05).

4. DISCUSSION

Urinary schistosomiasis is endemic in Adim community, Nigeria [1-3]. The control of infection in this community is at the household level and is dependent on the socio-economic status and literacy level of the residents [2]. Several researchers have administered different treatment regimens of artesunate/or praziquantal on children in this community [1,3,4] but the infection still persist. The prevalence of 18 (11.3%) observed in this study can be compared with that of [10] who had 5.7% in Odau and Obedum in Port-Harcourt, Rivers State, 10% in Abriba, Abia State [11], 18.7% in Jos, Plateau State [12] and in other African countries, 5.6% in Bandiagara in the North-earthen, Mali [13] 10.4% in Blantyre district, Malawi [14] 16.0% in Niamey, Niger [15]. But this prevalence of 11.3% observed amongst adult in this study area is low compared to that observed amongst children by several authors in the same study area [1-4] 26% in part of Bauchi State [16] and 24.0% in Niamey, Niger [17].

In this work a prevalence of 11.3% infection was observed. Subjects in the age group >35 – 45 years had the highest prevalence rate and egg count of 19% and 17±1.0 respectively. This observation agrees with those of [12,18,19]. This could be explained by [19] who postulated that water related activities is proportional to the risks of acquiring more infection and harboring more worms.

A gradual decrease in the prevalence rate among subject with age between >65 – 75 year and >75 – 85 years were observed. The reason could be due to the fact that subjects in this age group have less water contact activities.

The results of this study indicate a low level of urinary cytological abnormalities among residents of this urinary schistosomiasis endemic community. Among 160 subjects examined, 4 (2.5%) had cytological abnormalities (Table 3).

Males were more infected 3(4.2%) compared to females 1(1.1%). The abnormal epithelial cells occurred between the age range of 18-25 and >55-65 years. This is similar to findings by [15] who reported these cytological abnormalities amongst age group 18-25years and 56-65 years. This suggests an age-dependent epithelial cell change associated with *Schistosoma haematobium* infection. The cytological abnormalities were characterized by squamous metaplasia and atypia. Hodder et al. [20] had reported similar cytological abnormalities.

The presence of squamous metaplastic cells associated with *Schistosoma haematobium* infection has been found to be a predisposing factor to the occurrence of squamous cell carcinoma of the bladder [21,22]. Squamous metaplasia is an epithelial change that occurs when the bladder epithelium is mechanically initiated with chronic inflammatory cells which cause some changed that can lead to cancer [21]. These may include the production of endogenous oxygen radicals which may include gene mutation [23,24] or may activate procarcinogens to their carcinogenic forms [21].

Table 1. The distribution of *S. haematobium* infection, haematuria and proteinuria according to age group of subjects examined

Age group (years)	No. examined	No. (%) with infection	Mean egg count /10ml/urine	No. (%) with haematuria	No. (%) with proteinuria
18 – 25	21	2 (9.5)	4±2.0	5 (23.8)	4 (19.1)
>25 – 35	46	2 (4.3)	4±2.0	5 (10.9)	4 (8.7)
>35 – 45	44	9 (20.5)	17±19.0	6 (13.6)	4 (9.1)
>45 – 55	21	2 (9.5)	2±1.0	4 (19.0)	1 (4.8)
>55 – 65	15	2 (13.3)	2±1.0	5 (33.3)	2 (13.3)
>65 – 75	9	1 (11.1)	1±1.0	1 (11.1)	1 (11.1)
>75 – 85	4	0 (0)	0±0	0 (0)	0 (0)
Total	160	18 (11.3)	30±17.0	26 (16.3)	16 (10.0)

Table 2. The distribution of *S. haematobium* infection, haematuria and proteinuria according to gender of subjects examined

Gender	No. examined	No. (%) with infection	Mean egg count /10ml/urine	No. (%) with haematuria	No. (%) with proteinuria
Males	72	7 (9.7)	18±25.7	15 (20.8)	9 (12.5)
Females	88	11(12.5)	17±15.5	11 (12.5)	7 (8.0)
Total	160	18 (11.3)	30±17.0	26 (16.3)	16 (10.0)

Table 3. The distribution of abnormal epithelial cells according to age groups of subject examined

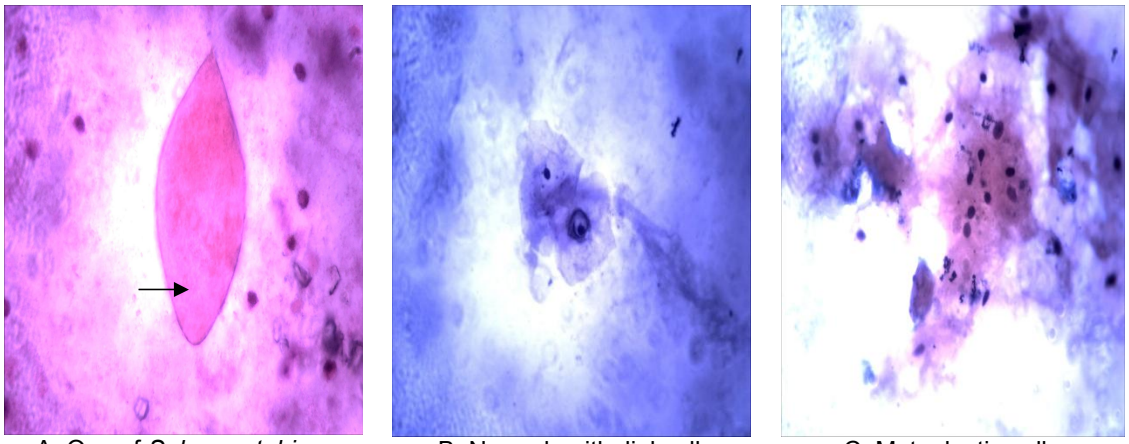
Age group	No. examined	No. (%) with infection	No. (%) with abnormal epithelial cells	No (%) positive to hyaluronic acid
18 – 25	21	2 (9.5)	1 (5.0)	0(0.0)
>25 – 35	46	2 (4.3)	1 (5.0)	1(2.2)
>35 – 45	44	9 (20.5)	1 (11.1)	1(2.3)
>45 – 55	21	2 (9.5)	0 (0)	0(0)
>55 – 65	15	2 (13.3)	1 (5.0)	1(6.7)
>65 – 75	9	1 (11.1)	0 (0)	0(0.0)
>75 – 85	4	0 (0)	0 (0)	0(0.0)
Total	160	18(11.3)	4 (2.5)	3(1.9)

Table 4. The distribution of abnormal epithelial cells according to gender of subjects examined

Gender	No. examined	No. (%) with infection	No. (%) with abnormal epithelial cells	No. (%) positive to hyaluronic acid
Males	72	7 (9.7)	3 (4.2)	2(2.8)
Females	88	11 (12.5)	1 (1.1)	1(1.14)
Total	160	18 (11.3)	4 (2.5)	3(1.9)

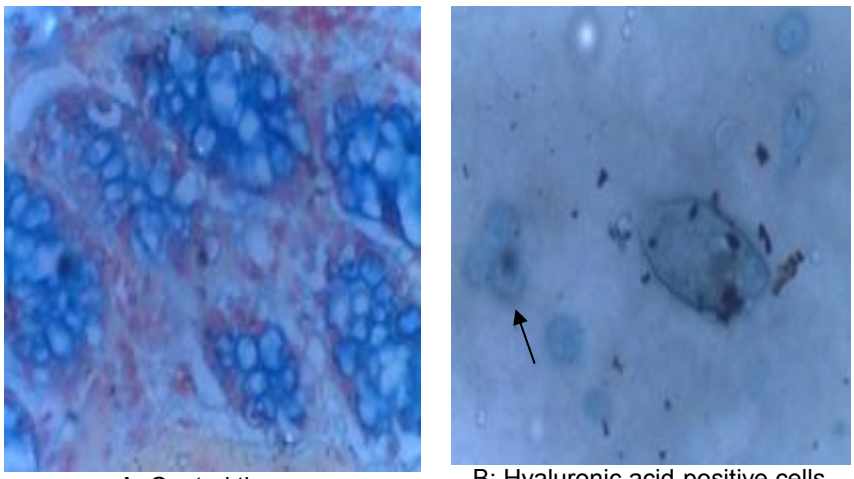
This study also stained the urine epithelial cells for hyaluronic acid. A prevalence rate of 3(1.9%) was observed. Males had 2(2.8%) and females 1(1.1%). Hyaluronic acid staining has been associated with many tumour cells [25,26] including bladder cancer. Hyaluronic acid, a glycosaminoglycan is often expressed by tumour epithelial cells. It aids tumour cell adhesion and angiogenesis [27]. Studies have showed that hyaluronic is a promising marker for the detection of bladder cancers [27]. The staining of hyaluronic acid in the epithelial cells further confirms that, the positive cases may have progressed to or developed into bladder cancer [25].

The demonstration of squamous metaplastic epithelial cells stained with Papanicolaou staining technique is shown in plat 1 while that of Hyaluronic acid-positive cells is shown in plat 2.



A: Ova of *S. haematobium* B: Normal epithelial cell C: Metaplastic cells

Plate 1. Cytological findings in urine. A shows ova of *Schistosoma haematobium* (arrow) stained with dilute carbol fuschin 400X magnification. B is normal epithelial cell and C shows squamous metaplastic epithelial cells in urine stained with Papanicolaou stain 100X magnification. Normal epithelial cells were characterized by basal or superficial squamous cells with cyanophilic cytoplasms and central round nuclei having fine chromatin. The abnormal epithelial cells were characterized by multinucleated squamous cells or single nucleated squamous cells having orangophilic cytoplasms, hyperkeratosis, hyperchromatic and pyknotic nuclei with coarse chromatin



A: Control tissue B: Hyaluronic acid-positive cells

Plate 2: Photomicrograph of A- Control tissue. The glands of small intestine tissue stained for hyaluronic acid. B-arrow shows bladder epithelial cells stained positive for hyaluronic acid with ova of *S. haematobium* in the background. H&E 100X

5. CONCLUSION

This study has established that residents of this community are predisposed to the development of bladder cancer. Hence, for proper management of the affected residents, control strategy should be put in place for parasitological and cytological screening of urine of residents of this urinary schistosomiasis endemic community for proper management of the disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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