Urethelial Dysplasia and Desquamation Associated with Urinary Tract Infection: A Case-controlled Study

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Abstract This study explored use of urine cytology as an early first-line approach in the laboratory diagnosis of dysplasia associated with urinary tract infection. Fifty (50) patients attending the obstetrics and gynaecology clinic at Madonna University Teaching Hospital and 50 apparently healthy subjects were recruited for the study. Urine samples were cultured and analyzed using the dipstick and Giemsa methods. The result of this study revealed significant higher number of nitrite positive samples, bacteria and pus cells when female test subjects were compared with apparently healthy female subjects ($p < 0.05$). The frequency of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis* and *C. albicans* identified is 50.6, 27.3, 10.4 and 9.1 %, respectively while other isolated Coliforms amounted to 2.6%. Microscopy revealed marked cytolyses, desquamation and abnormal nuclear/cytoplasmic ratio. *S. aureus* and *E. coli* induced higher exfoliation and morphological change (72.7%) compared to other isolates. In conclusion, this study suggests that chronic bacterial infection could lead to accelerated desquamation of cells and urothelial carcinoma. More so, it revealed that females are at a higher risk of developing epithelial neoplasia following UTI when compared to the males. Thus, urine smears could be used as first line approach in the early diagnosis of urothelial malignancy.

Keywords Staphylococcus Aureus, Escherichia Coli, Desquamation, Urothelium, Dysplasia

[2,3]. In men, recurrent UTIs are also associated with chronic prostatitis, an infection of the prostate gland. Although UTIs are less common in men, they can cause more serious problems in men than in women [3,4]. Hospital-associated UTIs (frequently due to catheterization) involve a much broader range of pathogens including: *E. coli* (27%), *Klebsiella* (11%), *Pseudomonas* (11%), the fungal pathogen *Candida albicans* (9%), and *Enterococcus* (7%) among others [5]. When bacterial infection occur at the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). This study investigated the effect of bacterial activity on integrity of the urothelium.

2. Materials and Methods

2.1. Study Area

This study was carried out in Madonna University Teaching Hospital MUTH, Elele Rivers State, Nigeria. MUTH is located in the tropics of the southern part of Nigeria at the latitude of 5 27-1N and longitude of 6 55- 7 85E. The climate of the area is tropical with the mean daily temperature of 28 degree Celsius for most of the year. The annual rain fall in this region is between 217 and 240 cm. Its borders are located within four neighboring communities which include Isiokpo, Umuagwo, Ahoda, and Omoku [6].

2.2. Study Population

A total of 100 subjects within the age range of 20 to 50 years (50 test subjects and 50 apparently healthy subjects) attending the Out-patient clinic at Madonna University Teaching Hospital were recruited for this study. Patients (selected as the come) who presented symptoms (associated

1. Introduction

Urinary tract infection (UTI) is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria [1]. It occurs in women due to loss of protective vaginal flora, menopause and certain types of contraceptives, particularly diaphragms

with urinary tract infection) such as burning sensation during urinating, pain in the pelvic region, lower back pain, discomfort or pressure in the lower abdomen were recruited as they came. The control subjects were randomly selected to match the age of the test subjects. The mean age for the male test subjects (25), female test subjects (25), male control subjects (25), and female control subjects (25) were 37 years, 30 years, 33 years and 32 years, respectively. Pregnant women, menstruating females and diabetic subjects were excluded from the study.

2.3. Ethical Consideration

Ethical approval was obtained from the ethics committee of Medical Laboratory Science Department, Madonna University, Elele campus, Nigeria (MLS/D/11/179). The participants (subjects) were briefed on the aim and procedure of the study. They were reassured of confidentiality and the benefit of participating in the study. Verbal and written consents were obtained from each of the participants and the investigation carried out at no cost to the participant. Results of the investigations were made available to the patients at the end of the study.

2.4. Sample Collection and Handling

After proper cleansing and voiding instructions were given to the subjects, early-morning midstream clean-catch urine specimens were collected in sterile, dry, leak-proof, disposable, labeled universal containers and stored in a refrigerator (at 2°C to 8°C) until analyzed. Urine samples were cultured on Blood and MacConkey agar and allowed to stand in an incubator for 24 to 48hrs at 37°C, and identification carried out by the method described by Sood [7]. Urinalysis was carried out within 2 hours of sample collection using the method described by Strasinger and Di Lorenzo [8].

2.5. Giemsa Staining Technique

The urine samples were centrifuged at 1500 g for 5 minutes and smears made from the cell deposit on were fixed in 95% methanol. Smears were stained by Giemsa for method [9]. The stained smears were read and photomicrographs taken for documentation.

2.6. Statistical Analysis

The student t-test was carried out on the assessed parameters and microscopically counted desquamated cells using the statistical package for social science (SPSS; version 20). The results were expressed in mean $\pm$ SEM. Values are significant at $p < 0.05$ and 0.01 .

Figure 1. Photomicrograph of a smear with nests of desquamated squamous epithelial cells with clear cytoplasm (red arrow) and cluster of cells with pleomorphic nuclei (black arrows). The pleomorphic cells are appearing squamoid. This was observed in some of the female subjects Giemsa stained. X400

Figure 2. Photomicrograph with papillary lesion (arrow), almost indistinguishable from a small tissue biopsy and associated with low grade neoplasia of the upper urinary tract. This was observed in some of the male subjects. Giemsa stained. X200.

Figure 3. Photomicrograph of a smear with moderate number of markedly cytolysed epithelial cells (black arrows), cluster of enteric cells (green arrow) and an abnormal squamous epithelial cell with irregular cytoplasmic outline (red arrow). This was observed among the female subjects. Giemsa stained. X200

Figure 4. Photomicrograph with few cytolysed epithelial cells (black arrow) and heavy collections of short chain cocci bacteria. Giemsa stained. X 200.

Table 1. Effect of bacterial isolates on cell morphology and desquamation

Isolates	Average № of epithelial cells seen/hpf	Effect on cell morphology	№ of dysplasia cases (%)
<i>S. aureus</i>	26	Marked cytolysis with degenerated nuclei	7 (31.8)
<i>Streptococcus faecalis</i>	2	Marked cytolysis	1 (4.5)
<i>E. coli</i>	10	Marked cytolysis High nuclear/cytoplasmic ratio	3 (13.6)
<i>Coliform</i>	4	Marked cytolysis High nuclear/cytoplasmic ratio	1(4.5)
<i>S. aureus</i> + <i>E. coli</i>	36	Marked cytolysis	6 (27.3)
<i>C. Albican</i> + <i>Coliform</i>	5	Marked cytolysis High nuclear/cytoplasmic ratio	1 (4.5)
<i>S. aureus</i> + <i>Coliform</i>	30	Marked cytolysis High nuclear/cytoplasmic ratio	2 (9.1)
<i>Coliform</i> + <i>E. coli</i>	13	Marked cytolysis High nuclear/cytoplasmic ratio	1(4.5)
Total (%)			22 (100)

N=100, hpf= high power field, №=number

Table 2. Frequency of bacterial and candidal isolates in the test and control groups

No of subjects	Groups	№ of isolates					
		<i>S. aureus</i>	<i>S. faecalis</i>	<i>E. coli</i>	<i>Coliform</i>	<i>C. albican</i>	
25	Control subject (males)	1	0	1	0	0	
25	Control subjects (Females)	4	1	3	2	1	
25	Test Subjects (males)	20	1	7	1	0	
25	Test Subjects (females)	14	0	10	5	6	
50	Control subjects (M+F)	5	1	4	2	1	
50	Test subjects (M+F)	34	1	17	6	6	
Total № of isolates		39	2	21	8	7	
Frequency of isolates (%)		50.6	2.6	27.3	10.4	9.1	

In table 2 above, higher number of microbial isolates was observed among the test group when compared with the control group, except for *S. faecalis*.

Table 3. Mean comparison of urinalysis and urine microscopy between female test and control subjects

Parameters	Control Mean±SEM	Test Mean±SEM	P-Value	T-Value
Protein	0.16±0.07	0.32±0.12	0.279	1.095
No of Nitrite Pos	0.04±0.040	0.28±0.09	0.020*	2.400
pH	6.12±0.14	6.10±0.12	0.918	0.103
Pus cells	3.32±0.97	8.92±1.70	0.006*	2.853
Epithelial cells	1.52±0.19	1.68±0.13	0.503	0.675
Red blood cells	0.32±0.16	0.42±0.20	0.678	0.420
Yeast	0.00±0.00	0.20±0.12	0.090	1.732
Bacteria	0.36±0.14	1.00±0.20	0.014*	2.551

P-value is significant at $p < 0.05$. * N=50, n=25, Mean age= 30 years, Chi-square analysis, Pos=Positive

In table 3 above, significant increase in the number of nitrite positive samples, pus cells and bacteria counts were observed in female test subjects when compared with the female control subjects ($P < 0.05$). However, insignificant increase in the number of epithelial cells, yeast, pH and protein were also observed in the samples of the test subjects when compared with that of the control subjects ($p > 0.05$).

Table 4. Mean comparison of urinalysis and urine microscopy between male test and control subjects

Parameters	Control Mean±SEM	Test Mean±SEM	P-Value	T-Value
Protein	0.40±0.14	0.24±0.13	0.413	0.825
No of Nitrite Pos	0.00±0.00	0.08±0.05	0.155	1.445
pH	6.06±0.18	6.20±0.14	0.551	0.600
Pus Cells	2.96±0.46	7.64±2.48	0.070	1.850
Epithelial Cells	1.08±0.14	1.24±0.16	0.466	0.735
Red Blood Cells	0.60±0.24	0.78±0.30	0.588	0.549
Yeast	0.00±0.00	0.00±0.00	0.000	0.000
Bacteria	0.16±0.09	0.44±0.17	0.164	1.414

P-value is significant at $p < 0.05$. * means significant difference when compared to control

In table 4 above, insignificant higher nitrite, pH, pus cells, epithelial cells and bacteria count were observed in the male test subjects when compared with that of the apparently healthy male subjects ($p > 0.05$). Also, insignificant higher amount of protein were found among the apparently healthy subjects when compared with test subject ($p > 0.05$).

Table 5. Mean comparison of urinalysis and urine microscopy between test and control subjects

Parameters	Control Mean±SEM	Test Mean±SEM	P-Value	T-Value
Protein	0.20±0.07	0.28±0.09	0.499	0.678
No of Nitrite pos	0.06±0.03	0.18±0.05	0.066	1.860
PH	6.16±0.10	6.15±0.09	0.943	0.072
Pus cells	5.48±1.35	8.28±1.49	0.168	1.388
Epithelial cells	1.38±0.12	1.46±0.11	0.638	0.473
Red blood cells	0.46±0.15	0.60±0.18	0.455	0.754
Yeast	0.00±0.00	0.10±0.05	0.093	1.698
Bacteria	0.40±0.11	0.72±0.14	0.076	1.793

P-value is significant at $p < 0.05$. N=100, n=50

In table 5 above, insignificant higher number of nitrite positive samples, pus cells, protein, epithelial cells, yeast and bacteria count were observed in the (overall) test subjects when compared with the apparently healthy subjects ($P > 0.05$). Insignificant higher pH was also observed in the samples of the apparently healthy subjects when compared with that of the test subjects ($p > 0.05$).

3. Discussion

The renal pelvis, the ureters, bladder and the urethra are lined by a highly specialized and unique epithelium "the urothelium" also known as transitional epithelium [10]. The presence of a centrally located rather than eccentrically placed nucleus, and supravital staining, can aid in their identification [8]. Urinary tract infection occurs as a result of interaction between an uropathogen and the urothelium [8]. The presence of nitrite and higher pus cells (than normal) in urine (due to bacterial and fungal infection; tables 1 and 2) among the test subjects (tables 3-5) are indicative of host and uropathogen interactions.

The higher bacteria count observed among the female test subjects when compared with that of the males (tables 2 and 3) is consistent with the reports of Beerepoot et al. [11] who stated that women are more prone to UTIs than men due to the fact that their urethra are much shorter and closer to the anus. According to Madersbacher et al. [12] most bacteria enter the urinary tract from the fecal reservoir via ascent through the urethra into the bladder, hence the isolated *S. faecalis* in this study (tables 1 and 2). Bacteria adhesion close to Teichoic acids on host cells is mediated by glycoprotein fibronectin [13]. Adhesion of bacteria to the urothelium induces oedema and ulcer with increased urothelial desquamation [14] as a result of inflammatory response [1].

Universally, chronic inflammatory conditions are believed to create the favourable microenvironment for malignancy initiation through a cascade of events. Examples include the link between: gastroesophageal reflux disease and esophageal cancer, atrophic gastritis and stomach cancer and inflammatory bowel diseases and colon cancer [15]. The chronic inflammatory due to infection and irritation may encourage genomic lesions and tumor initiation. An efficient system through which immune cells and enzymes attack microbial infection is the synthesis of free radicals such as reactive oxygen intermediated (ROI), hydroxyl radical, superoxide and reactive nitrogen intermediates (RNI), nitric oxide and peroxynitrite. Interestingly, ROI and RNI result in oxidative damage and nitration of DNA bases which increases the risk of DNA mutations [16]. Mutagen induced DNA damage or oncogenic activation may either initiate cell DNA repair or death of initiated cells. In the event of enormous cell death as a result of repeated exposure to microbial infection or tissue trauma, lost cells must be replaced by undifferentiated reserve cells such as tissue stem cells through proliferation [17,18]. Concurrent DNA damage and cell division in chronic inflammation, in a bid to maintain homeostasis, could lead to cancer because cells that are dividing are more susceptible to mutations caused by DNA damage [19].

Escherichia coli have been reported to cause cancer when the gut is inflamed. It does that by producing a DNA damaging protein known as colibactin [20]. More so, *S. aureus* DNA have been identified in biopsies from actinic keratosis and squamous cell carcinoma, which suggests that it plays a role in the carcinogenesis processes [21]. Higher

desquamation of cell associated with *S. aureus* and *E. coli* infection observed in this study (table 1) could be adduced to the bacterial adhesion molecule. The observed pleomorphic cells and higher nuclear/cytoplasmic ratio (figures 1 and 3, respectively) could be due to bacterial and candidal DNA integration into the human genome [22]. Cytoplasmic vacuolation (clear cell; figure 1) is characteristic of a lesion which is usually associated with cystitis [23] while the papillary lesion (figure 2) is associated with pyelonephritis. Lesions are generally associated with vascular damage which results in blood loss. The higher red blood cell count observed among the test subjects (tables 3-5) may be due to damaging bacterial activities on the urothelium which are also associated with some types of malignancy. Figures 2 and 3 suggest that females are at a higher risk of developing epithelial malignancy following UTI when compared to males while males suffer more upper urinary tract neoplastic lesion compared to females. The reason for this is still unclear but could be associated with the anatomy of urinary system.

4. Conclusions

The findings of this study suggest that chronic urinary tract infection could affect the integrity of the urothelium and may lead to malignancy if left untreated. Thus, urine smears can be used as an early first-line approach in the early diagnosis of bacterial induced urothelial malignancy.

Acknowledgements

Special thanks are to the management and staff of Medical Laboratory Services, Madonna University Teaching Hospital for their technical support and for allowing us use of their facility.

Author Contributions

JOO and **BOJ** designed the experiments and carried out the cytological analysis, **KO** carried out the microbiology investigations; **JOO** analyzed the data and wrote the paper.

Conflict of Interests

Authors declared no conflict of interest.

Research Funding

Authors did not receive any funding from any organization or institution for this study.

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