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Cytological Evaluation of Urinary Samples among Vesicovaginal Fistula Patients in National Obstetrics Fistula Centre, Southeastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Vesicovaginal fistula (VVF) is a non-physiological epithelial fistulous tract between the bladder and the vagina, resulting in continuous urinary incontinence. VVF occurs most commonly after obstetrical and gynecological injury. This study is a descriptive cross-sectional study designed to carry out cytological evaluation of urine samples of vesicovaginal fistula patients in National Obstetrics Fistula Centre, Southeastern Nigeria. The voided and catheterized urine samples collected were stained using Papanicolaou and Diff-Quick staining techniques. A total number of 123 patients were involved in this study. The mean age of the patients was 36.66 (± 6.21) years. The patients were grouped according to age into 4 groups: 1 (0.8%) was ≤ 24 years, 59 (48.0%) were 25 to 34 years, 43 (35.0%) were 35 to 44 years and 20 (16.2%) were ≥ 45 years of age. The parity status of these cases was grand multipara 29 (23.6%), multipara 32 (37.4%), primipara 46 (37.4%) and nullipara 16 (13.0%). Majority of the patients 111 (90.2%) were from rural areas, 8

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(6.5%) from urban areas and 4 (3.3%) were from semi-urban areas. According to marital status, 91 (74.0%) were married, 16 (13.0%) were widowed, 5 (4.1%) were single and 11 (8.9%) were divorced. According to the period the patients had lived with fistula, majority of the patients 94 (76.4%) had lived less than one year with this problem, while 29 (23.6%) had suffered from VVF for 1-5 years. Cytological evaluation of the urine smears showed that a total of 92 (74.8%) urine samples had normal findings. The rest of the 31 (25.2%) urine samples showed a variety of findings. Out of these 31 samples, 16 (51.6%) showed inflammatory aggregates suggestive of urinary tract inflammation, 3 (9.7%) showed decoy cells suggestive of Polyoma virus infection, 7 (22.6%) cases showed perinuclei halo suggestive of *Trichomonas vaginalis* infection, 3 (9.7%) showed oval fat bodies suggestive of possible nephrotic syndrome, 8(25.8%) cases showed koilocytes, indicative of Human papilloma virus infections. Cases that showed presence of haematuria were 7 (22.6%). There was no case of tumor cells detected in all the urine samples examined. From the outcome of this study, it is important to take special note of the presence of the viral carcinogens: Polyoma virus and Human papilloma virus, since in theory and as reported in few case reports, malignancy of the urinary tract can lead to formation of vesicovaginal fistula.

Keywords: Cytological evaluation; urinary samples; vesicovaginal fistula; polyoma virus; human papilloma virus.

1. INTRODUCTION

Vesicovaginal fistula (VVF) is a non-physiological epithelialfistulous tract between the urinary bladder and the vagina, resulting in continuous urinary incontinence [1,2,3]. VVF, affects women physically, psychologically, emotionally, and economically [4]. Other than the physioanatomical effects of fistula, there are associated physical, mental, social and sexual effects on VVF patients [5]. The uncontrolled and involuntary urine passage results into major psychosocial problems for VVF patients. The associated foul odour leads to neglect and stigmatization by the members of their household and community. This can progress into low self-esteem on the part of the sufferers and in extreme cases, suicidal tendencies [6].

Prolonged obstructed labour is the most common cause of VVF in developing countries [5]. The non-obstetric causes of VVF include radiotherapy, gynaecological malignancies, gynaecological surgeries, retroperitoneal, vascular or pelvic surgery, urologic or gynecologic instrumentation, infectious and inflammatory diseases, sexual trauma, vaginal laser procedures, external violence, and vaginal foreign bodies [7,6].

Therefore, the major risk factors for developing VVF are poverty, malnourishment, low literacy rate, early marriage and childbearing, harmful traditional practices like female genital mutilation and inadequate obstetric care [2]. In Nigeria, obstetric fistula reportedly occurs in 3.2 per 1000 birth and it is estimated that the annual occurrence of VVF is about 13,000 cases [8]. The prevalence of VVF seems underestimated since the data from which it is generated is usually more concerned with sufferers who present to the Hospital [9].

The outcome of cytological analysis of urine assists diagnosis and does provide treatment direction, prognosis, or predict disease recurrence (Chen et al., 2020). In comparison, because of the invasive nature of tissue biopsy, it is not conducive for long term monitoring of patients (Chen et al., 2020). Despite many attempts to develop tests with higher sensitivity and specificity, cytology remains one of the best ways to diagnose a variety of urinary bladder lesions [10]. In this work, cytological evaluation of urine was carried out. This would improve our understanding of the cytological features of urine in VVF in comparison to normal urine.

The study was done to carry out cytological evaluation of urine samples of vesicovaginal fistula patients in National Obstetrics Fistula Centre, Southeastern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This research was conducted at the National Obstetric Fistula Centre, Abakaliki, Ebonyi State, in the South Eastern region of Nigeria.

2.2 Study Population

The target population for this study consisted of patients admitted to the National Obstetric Fistula

Centre, Abakaliki, who had been evaluated and had undergone repair of vesicovaginal fistula or were awaiting treatment, and agreed to participate in the study. Patients who were visiting the centre for the first time and were not yet clinically diagnosed of having VVF were excluded from the study. Women with other causes of urinary incontinence, any co-morbidity and pregnant women were excluded as well.

2.3 Study Design

A descriptive cross sectional research was designed to cytologically examine the urine of patients diagnosed of vesicovaginal fistula. A total of one hundred and twenty-three (123) patients were recruited for the study. They were briefed on the objective of the research and after they all gave their consent, their demographic data were taken, using a questionnaire.

2.4 Sample Size Calculation

The sample size was calculated using the formula described by Sharma et al. [5]. The choice of this formula became necessary as there was no known previous cross sectional study reported in published literature which had utilised cytologically examined urine samples of VVF patients.

$$n = N / (1 + Nd^2)$$

Where:

n = number of sample
N = total population of patients (the centre treats an average of 177 VVF patients per year)
d = error margin (At 95% CI, and precision rate of 5%) = 0.05

$$n = 177/ (1 + 177(0.05)^2)$$
$$=177 / (1 + 177(0.0025))$$
$$= 177 (1 + 0.4425)$$
$$= 123$$

The sample size required for this study was therefore, 123 patients.

2.5 Sample Collection

For each patient a sterile screw cap universal container was provided. The urine samples of 92 of the patients were urinary catheter specimens while the remaining 31 were voided. Patients who provided voided urine were asked to pass out their urine specimens early in the morning first urine and then to drink some water. After that, they were asked to pass the first part of the urine into the toilet, then without stopping the flow of the urine, they should collect some into the given container. Catheterized urine samples were collected with the assistance of a clinician.

Early morning urine sample was not accepted because the exfoliated cells had undergone prolonged exposure to the acidic pH of the urine in the bladder. As a result, they might be abnormal, with features such as enlarged nuclei and washed out of the chromatin, mimicking malignant cells [11].

2.6 Sample Processing

The entire volume of the urine samples were centrifuged for 5 minutes at 3000rpm then the supernatant was discarded leaving the sediment. Meyer's egg albumin was applied on the slides as adhesive to ensure the urine sediments that would be smeared on them were not washed off during the subsequent staining procedures. The resulting sediment was then smeared on the slide using both thick smear and pull-apart methods. The samples were prepared in duplicates to be stained with Papanicolaou stain and Diff-Quick stain respectively. The smears prepared for Papanicolaou staining were fixed in 95% ethyl alcohol (V/V) for 15 minutes prior to the staining process.

2.7 Staining

2.7.1 PAP staining procedure

Papanicolaou staining procedures were carried out as described by Seddig et al. [11] with a few variations.

The smears were immersed in descending grade of ethanol from 95%, 80% to 60% (V/V) and then dipped five times each in two changes of distilled water. Then the smears were stained using Hematoxylin for 2 minutes, after that the smears were differentiated in 0.05% HCl solution by dipping twice. This was followed by bluing by leaving the smears in a bath of running water for 10 minutes, then immersed in 60%, two changes of 80% and 95% ethanol and stained with Orange G solution (OG-6) for 2 minutes. This was followed by washing in three changes of 95% alcohol for two minutes each, and then staining with Eosin Azure (EA-36) for 2 minutes and washed again in four changes of 95% alcohol for 2 minutes each. The smears were rinsed in three changes of 100% alcohol for 2 minutes each and blotted using a sterile filter paper to dry. Subsequently, the slides were

placed in three consecutive changes of Xylene for 5 minutes each, except in the last one where it was allowed till it cleared. They were mounted in DPX.

2.7.2 Diff-Quick staining procedure

The Diff-Quick staining procedure was carried out using the rapid Romanowsky stain (HD Supplies, UK). The reagents were made up of three solutions: a fixative solution containing Thiazine dye in methanol, solution B containing Eosin Y in phosphate buffer, and solution C containing Methylene Blue in phosphate buffer. The manufacturer's direction was followed as follows:

The solutions were dispensed into respective Coplin jars, and the slides were immersed into the fixative solution for 30 seconds. After that the slides were transferred without rinsing or drying to Solution B and stained for 30 seconds by slowly agitating the slide in the solution. Again, without rinsing, the slides were transferred to Solution C and stained for 30 seconds, after which they were rinsed briefly in buffered water (pH 6.8) and allowed to dry.

3. RESULTS

3.1 Photomicrograph of Urinary Samples from VVF Patients

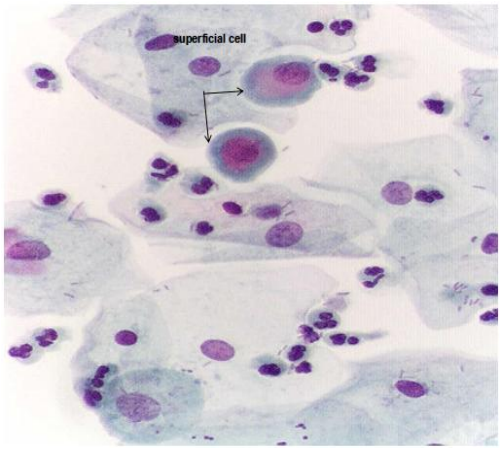

Plate 1a. (Mag. X40) is voided urine sediments from non vesicovaginal fistula stained with Papanicolaou stain; smear revealed normal urothelial cells (arrow) and superficial cells. The smear appeared normal

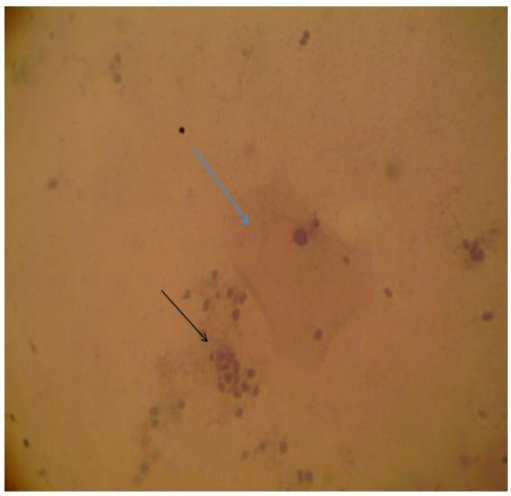

Plate 1b. (Mag. X40) is a voided urine smeared sediments of VVF patient stained with Papanicolaou; the smear demonstrated normal desquamated superficial cell (blue arrow), and inflammatory aggregates (black arrow)

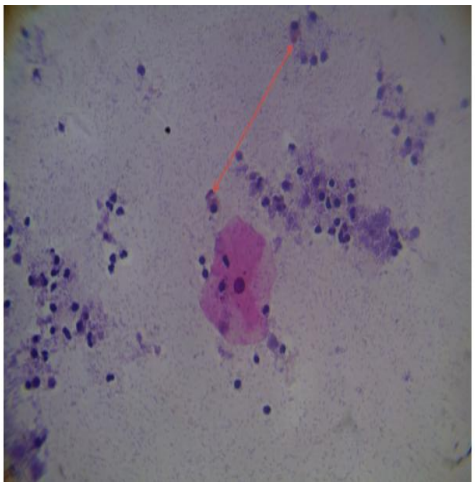

Plate 1c. (Mag. X40) is a voided urine smeared sediments of VVF patient stained with Papanicolaou; the smear showed desquamated superficial cell, and decoy cells with eccentric nuclei (red double arrow)

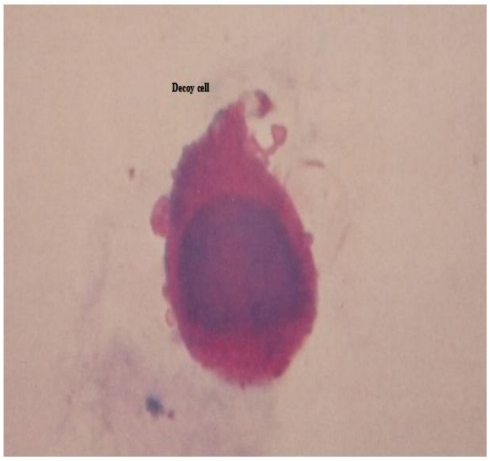

Plate 1d. (Mag. X100) is a voided urine smeared sediments of VVF patient stained with Papanicolaou; the smear showed decoy cell

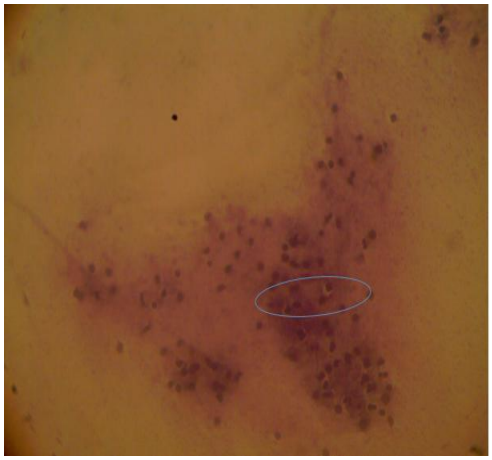

Plate 1e. (Mag. X40) is a voided urine smeared sediments of VVF patient stained with Papanicolaou; the smear showed perinuclei halo (oval) on superficial cells, there is a uniformity in all the halos; suggestive of *Trichomonas vaginalis* contamination from the vagina

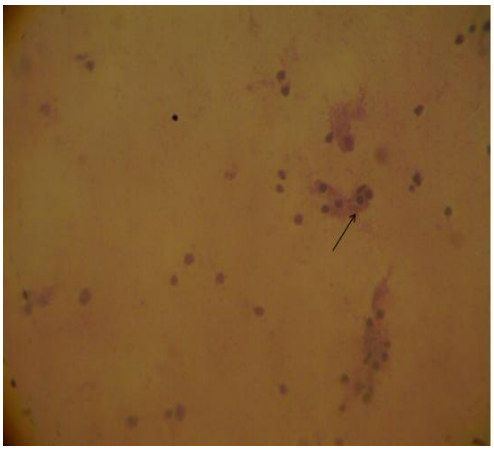

Plate 1f. (Mag. X40) is a voided urine smeared sediments of VVF patient stained with Papanicolaou; the smear showed perinuclei halo (arrow) on superficial cells; suggestive of *Trichomonas vaginalis* contaminant from the vagina

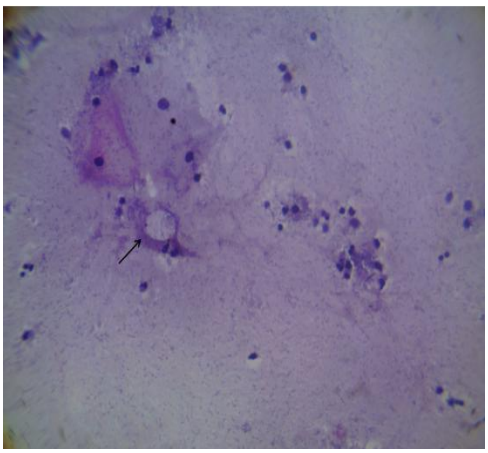

Plate 1g. (Mag. X40) is a catheterized urine smeared sediments of VVF patient stained with quick diff; the smear shown Fat body (arrow) and superficial cells

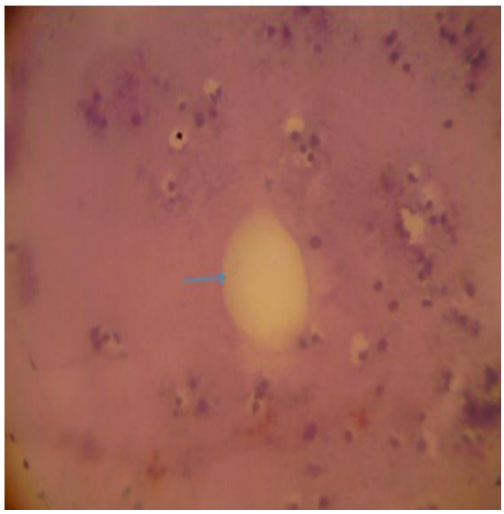

Plate 1h. (Mag. X40) is catheterized urine smeared sediments of VVF patient stained with Papanicolaou stain; the smear showed oval Fat body (arrow) and superficial cells

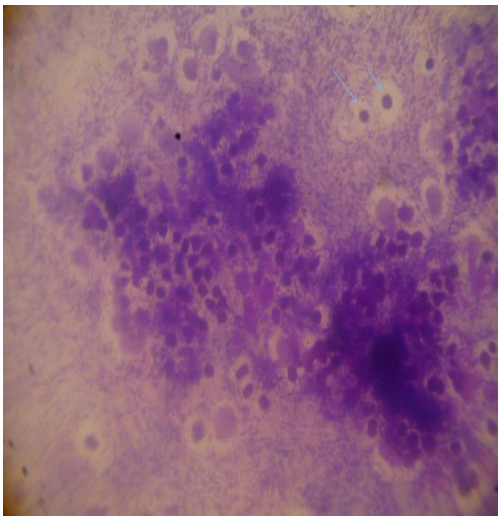

Plate 1i. (Mag. X40) is a catheterized urine smeared sediments of VVF patient stained with Diff Quick stain; the smear showed koilocytes with ground glass appearance (blue arrow) with nuclei polymorphism suggestive of human papilloma virus infected cells

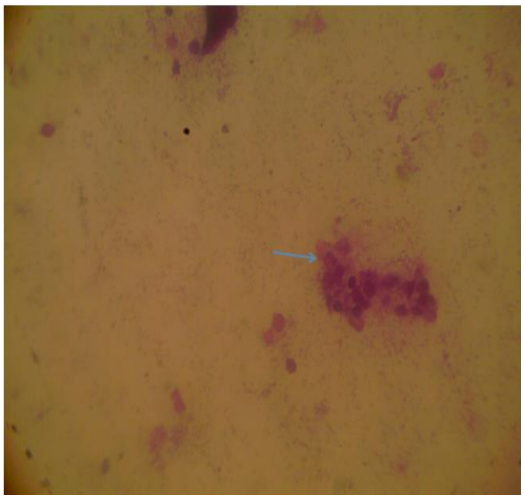

Plate 1j. (Mag. X40) is a catheterized urine smeared sediments of VVF patient stained with Diff Quick stain; the smear shown red blood cells cast (blue arrow)

Table 1 shows the demographic data of the study participants. A total number of 123 patients participated in this research. The mean age of patients was 36.66 (± 6.21) years. The youngest patient was 23 years old and oldest was 49 years old. The patients were grouped according to age into 4 groups: 1(0.8%) was ≤ 24 years, 59(48.0%) were 25 to 34 years, 43(35.0%) were 35 to 44 years and 20(16.2%) were ≥ 45 years of age. The parity status of these cases was grand multipara 29(23.6%), multipara 32(37.4%), primipara 46(37.4%) and nullipara 16(13.0%). Majority of the patients 111(90.2%) were from rural area, 8(6.5%) were from urban area and 4(3.3%) were from semi-urban area. According to marital status 91(74.0%) were married, 16(13.0%) were widowed, 5(4.1%) were single and 11(8.9%) were divorced. According to period lived with fistula, majority of the patients 94(76.4%) had lived less than one year with this problem, while 29(23.6%) had suffered from VVF for 1-5 years.

Cytological evaluation of the urine smears revealed different cytological features including normal desquamated superficial cells and inflammatory aggregates, decoy cells, perinuclei halo, oval fat bodies, koilocytes indicative of Human papilloma virus infection and red blood cell cast.

Table 2 shows the result of cytological evaluation of the urine smears. A total of 92(74.8%) urine samples had normal findings. The rest of the 31(25.2%) urine samples showed a variety of findings. Out of these 31 samples, 16(51.6%) showed inflammatory aggregates suggestive of urinary tract inflammation, 3(9.7%) showed decoy cells suggestive of Polyoma virus infection, 7(22.6%) cases showed perinuclei halo suggestive of *Trichomonas vaginalis* infection, 3(9.7%) showed oval fat bodies suggestive of possible nephrotic syndrome, 8(25.8%) cases showed koilocytes, indicative of human papilloma virus changes. Cases that showed presence of haematuria were 7(22.6%). There was no case of tumor cells detected in all the urine samples examined.

4. DISCUSSION

This study evaluated the cytomorphology of urine samples of VVF patients at National Obstetrics Fistula Centre, Abakaliki, using Papanicolaou and Quick Diff cytology staining techniques.

The presence of inflammatory aggregates is frequently related to infections of the urinary tract [12]. VVF can also present significant inflammation around the fistula tract [7].
Polyoma virus was detected in 3(9.7%) of the 31 samples that showed non-tumoral pathologies. The key feature for the presence of Polyomavirus is the presence of typical decoy cells, unique cytomorphological feature [11]. This virus produces nuclear lysis, transforming the cellular

Table 1. Demographic data of study participants

Variables	No of subjects (n = 123)	Percentage (%)
Age in groups		
≤ 24	1	0.8
25 – 34	59	48.0
35 – 44	43	35.0
≥ 45	20	16.2
Parity		
Nullipara	16	13.0
Primpara	46	37.4
Multipara	32	26.0
Grand multipara	29	23.6
Marital status		
Single	5	4.1
Married	91	74.0
Widowed	16	13.0
Divorced	11	8.9
Residence		
Urban	8	6.5
Rural	111	90.2
Semi-urban	4	3.3
Period lived with fistula		
< 1 year	94	76.4
1 – 5 years	29	23.6
6 – 10 years	0	0
> 10 years	0	0

Table 2. Cytomorphological findings in the urine samples with pathologies

Cytomorphological features	No of samples (n = 31)	Percentage (%)
Inflammatory aggregates	16	51.6
Decoy cells	3	9.7
Perinuclei halo	7	22.6
Oval fat body cells	3	9.7
Koilocytes	8	25.8
Red blood cells	7	22.6

nuclei into amorphous hyperchromatic masses that can occupy the totality of the nuclei or be arranged as peripheral or central clumps (decoy cells). Polyomaviruses can be activated, either without apparent cause or following immunodepression due to chemotherapy, transplants, or acquired immunodeficiency syndrome (AIDS) [12]. Even though most literature report that decoy cells are indicative of Polyomavirus, Kimura and Hayashi [13] noted that decoy cells can be found in other urinary viral infections. They suggested the use of imunostaining when decoy cells are seen in order to clarify the infective agent.

The presence of perinuclei halo was indicative of *T. vaginalis*, an extracellular protozoan parasite that infects the vagina [14]. *T. vaginalis* infection is said to play a role in the development of cervical neoplasia [15]. The finding of T. vaginal may seem not have a direct connection to VVF; however, the presence of this parasite triggers inflammatory processes in the vaginal epithelial cells which may consequently lead to precancerous [14].

Human papilloma virus (HPV) induces the transformation of cells and is associated with malignancies such as cervical cancer. Siddig et al. [11] emphasized that care should be taken when evaluating HPV, because of the difficulty in differentiating whether koilocytic cells are from the urinary tract or the uterine cervix.

There was haematuria in 7(22.6%) of the samples with abnormal cytological findings. Among possible causes of haematuria the most probable in this study is the VVF. Anyaeze [16] enumerated bleeding as one of the manifestations of VVF. Most bladder cancers are also linked to haematuria [17], and it serves as one of the common manifestations that prompts cytology examination [11].

There was no tumor cell detected in the urine samples evaluated. However, a negative cytology doesn't always exclude malignancy. Urine cytology is considered a noninvasive, cost-effective technique for diagnosing a wide range of abnormalities including infections and malignancies. Researchers are divided on the usefulness of urine cytology to detect urinary tumor. Siddappa et al. [17] enumerated the inconsistencies with using urine cytology as a diagnostic tool for urinary malignancy. According to their report, diagnostic inaccuracies in urine cytology arise from the fact that urine is not conducive for cells, as they undergo degenerative changes which can make diagnosis difficult. Another twist in urine cytology is that pleomorphic cells with enlarged hyperchromatic nuclei with prominent nucleoli can be benign, whereas cancer cells can be composed of nearly normal looking monomorphic cells with benign nuclei. Reactive changes emanating from inflammation and infections can increase the number of false negative diagnosis in urine cytology specimen. Siddappa et al. [17] stated that urine cytology was not a perfect tool for the detection of bladder tumors, and is more reliable in diagnosing a high grade tumor.

Despite the false positive and negative results have been reported, McIntire et al. [18] rates urine cytology and microscopy as the gold standard for determination of cell morphology. Fojecki et al. [10] was also of the same opinion, stating that cytology remains one of the best ways to diagnose bladder lesions. Other studies [19,20-23] have also supported the use of urine cytology as gold standard for detecting urothelial cancers. The false-positive results from urine cytology may be attributed to the presence of viral infections, such as polyoma virus [17] False negative results have been linked to the sampling method used, the number of the samples obtained from the patient and the volume of the urine being processed [18]. In this present study, two urine samples (voided and catheterized) were taken from each of the patients, and this may probably decrease the possibilities of a false negative result.

5. CONCLUSION

All the urine samples evaluated were negative for tumor cells. However, some showed a diversity of cytological abnormalities. From the outcome of this study, it is important to note that, while no tumoral pathology was identified, viral and other pathologies were found. Worthy of note is the presence of the viral pathologic agents: Polyoma virus and Human papilloma virus which are known predisposing factors for malignancy. These agents deserve to be given closer diagnostic attention since in theory and as reported in few case reports, malignancy of the urinary tract can lead to formation of vesicovaginal fistula. It can therefore be concluded from this study that there was no incidence of urinary tract malignancy among vesicovaginal fistula patients.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

A letter of introduction was submitted to the medical director requesting for permission to obtain the patients information and urine samples in line with research ethical best practice in maintaining patients' confidentiality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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