THE EFFICACY OF URINE CYTOLOGY IN DIAGNOSIS OF URINARY BLADDER TUMORS AT MUHIMBILI NATIONAL HOSPITAL DAR ES SALAAM, TANZANIA.

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By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Medicine in Anatomical Pathology of the Muhimbili University of Health and Allied Sciences.

October 2019

CERTIFICATION

The undersigned certify that he has read and hereby recommends for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled ***"The efficacy of urine cytology in diagnosis of urinary bladder tumors at Muhimbili national hospital"*** in fulfilment of the requirements for the degree of Master of Medicine (Anatomical Pathology) of Muhimbili University of Health and Allied Sciences.

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I, **Gilbert Zacharia Nkya** declare that this **dissertation** in my own original work and it has not been presented and will not be presented to any other university for a similar or any other Degree Award.

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Table of Contents

CERTIFICATION I
DECLARATION AND COPY RIGHT II
AKNOWLEDGEMENT III
LIST OF TABLES I
DEDICATION II
LIST OF ABBREVIATIONS III
DEFINITION OF TERMS IV
ABSTRACT V
1.0 INTRODUCTION 1
1.1 BACKGROUND 1
1.2 PROBLEM STATEMENT 2
1.3 STUDY RATIONALE 3
1.4 CONCEPTUAL FRAMEWORK 4
1.5 HYPOTHESIS 5
1.6 RESEARCH QUESTIONS 5
1.7 OBJECTIVES 5
1.8 REVIEW OF LITERATURE 6
2.0 METHODOLOGY 9
2.1 STUDY DESIGN 9
2.2 STUDY AREA 9
2.3 STUDY POPULATION 9
2.4 INCLUSION AND EXCLUSION CRITERIA 9
2.5 SAMPLE SIZE ESTIMATION 9
2.6 DATA COLLECTION METHODS AND PROCEDURES 10
2.7 DATA PROCESSING AND ANALYSIS 11
2.8 ETHICAL CONSIDERATION 12
3.0 RESULTS 13
3.1 STUDY DEMOGRAPHICS. 13
3.2 CLINICOPATHOLOGICAL CHARACTERISTICS OF THE STUDY POPULATION 13
3.3 SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES 16
3.4 EFFECTIVENESS OF URINE CYTOLOGY IN DIAGNOSIS OF URINARY BLADDER TUMORS. 17
4.0 DISCUSSION 22
4.1 STUDY DEMOGRAPHICS 22

4.2 SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES 22
4.3 EFFECTIVENESS OF URINE CYTOLOGY IN DIAGNOSIS OF URINARY BLADDER TUMORS. 23
4.4 STUDY LIMITATIONS 23
5.0 CONCLUSION 25
6.0 RECOMMENDATIONS 25
7.0 DISSEMINATION OF RESULTS: 26
8.0 REFERENCES 27
9.0 APPENDICES 31

LIST OF TABLES

Table 1: Frequency of Urine cytology results in the study population (N=127)..... 14
Table 2: Categorization of urine cystoscopy diagnoses 15
Table 3: Sex distribution among histological diagnoses (N=88). 15
Table 4: Summary of sensitivity, specificity values. 16

DEDICATION

To my beloved wife Iraseni Ufoo Swai

To my parents Mr. & Mrs. Zacharia Simbaufoo Nkya

To my siblings Emmanuel, Gloria and Irene.

To Dr. Fred G. Minja, Arusha Lutheran Hospital

LIST OF ABBREVIATIONS

AUC	Atypical urothelial cells
CIS	Carcinoma in situ
DPX	Distyrene, Plasticizer and Xylene
EA-36	Eosin-azure
HCl	Hydrochloric acid
HGUC	High grade urothelial carcinoma
MNH	Muhimbili National Hospital
NHGUC	Negative for high-grade urothelial carcinoma
SCC	Squamous cell carcinoma
SHGUC	Suspicious for high-grade urothelial carcinoma

DEFINITION OF TERMS

Cytology: The medical and scientific study of cells. Cytopathology refers to a branch of pathology that deals with making diagnoses of diseases and conditions through the examination of cells from the body.

Urine cytology: A test to look for abnormal cells in urine.

Cystoscopy: Is a procedure to look inside the urinary bladder via the urethra using a thin instrument with a camera called a cystoscope.

Biopsy: Is a tissue specimen removed from a living body for microscopic examination of cells to discover the presence, cause or extent of a disease.

Sensitivity: Is the ability of a test to give a positive finding when the tested person truly has the disease under study.

Specificity: Is the ability of a test to give a negative finding when the tested person is truly free of the disease under study.

Positive predictive value: The probability that subjects with a positive screening test, truly have the disease.

Negative predictive value: The probability that subjects with a negative screening test truly don't have the disease.

ABSTRACT

Background: Bladder tumors are among the common cancers in the African population with an average prevalence of 30% in most regions. In Sub Sahara Africa the annual adjusted mortality rate is 2.8 whereas that of East Africa is 2.6. Currently bladder cystoscopy and biopsy are the gold standard for diagnosing bladder cancer, while urine cytology is used infrequently. Majority of patients in Tanzania cannot access cystoscopy and biopsy services timely, and the services are costly. Urine cytology therefore provides a convenient initial tool in the algorithm for early detection and diagnosing urinary bladder tumors as well as follow up for recurrences.

Most patients with bladder cancer present late with advanced disease because of poor accessibility and expensive cystoscopy and biopsy as diagnostic tools because majority of patients in Africa live in rural areas where there are no cystoscopy and biopsy services. Urine cytology allows early tumour detection and can be employed in screening programs and post management follow-up. Additionally, urine cytology is a non-invasive, cost efficient procedure, which can be used at all levels of the health care system.

Broad Objective: To determine the efficacy of urine cytology in diagnosing urinary bladder tumors at Muhimbili National Hospital (MNH), Tanzania.

Materials and Methods: A hospital based prospective study was conducted from June to December 2018, among patients attending the urology department at MNH, Dar es Salaam, Tanzania. A structured data-collecting instrument was used for collection of data. Urine samples collected from patients were processed and smeared on glass slides for cytological studies. Biopsies were collected and processed for histology. Bladder cystoscopy results were collected from patients' files. The obtained information was summarized using SPSS database version 23.0, followed by data analysis.

Results: Of 127 patients, only 88 patients were eligible for analysis. Urine cytology revealed a sensitivity of 90.5% and specificity of 92.0%. The positive and negative predictive values were 96.6% and 79.3% respectively ($p<0.05$). There was a moderate agreement between urine cytology results and their corresponding histological diagnosis, with Kappa coefficient (κ) of 0.78.

Conclusion: Urine cytology has an important role in early diagnosis of bladder tumors especially high-grade ones, in which it has good sensitivity. However, it is not meant to replace other techniques for bladder tumor diagnosis, but it remains one of the best ways to diagnose a variety of bladder tumors, especially high-grade ones.

1.0 INTRODUCTION

1.1 Background

Urine cytology is a method used for primary screening and diagnosis of urinary tract tumors. It targets early cellular changes in cells shed in urine, from different levels of the urinary tract of asymptomatic high-risk patients or symptomatic patients. Importantly it has been used over the years for the diagnosis of urothelial cancers with better results seen when used in combination with both cystoscopy and biopsy (1,2).

Cytology provides the initial overview of the suspected lesion before more invasive and expensive techniques; such as urinary bladder cystoscopy and biopsy are employed therefore providing a timely diagnosis of urinary tract lesions especially tumors. The specificity of urinary cytology is higher than 90%, while the sensitivity for high-grade disease and Carcinoma in Situ (CIS) can be as high as 80% to 90% (3, 4). Studies have shown a low sensitivity of about 20-50% of urine cytology in detecting low-grade neoplasms such as benign papilloma or urothelial carcinoma (borderline) with low malignant potential (1). The observed low sensitivity has been attributed to the fact that tumor cells in low-grade neoplasms are more cohesive, leading to none or minimal shading of cells into the urine, but also the fact that low-grade tumor cells, which closely resemble normal cells could not be excluded. Urinary cytology, therefore, provides a useful non-invasive adjunct to cystoscopy and can also be used as a monitoring and follow-up tool in patients who have previously received treatment of various neoplastic conditions of the urinary tract, providing a cheap and convenient way to detect recurrences. Urine cytology is important in screening kidney transplant patients to identify infectious or inflammatory conditions, as well as acute renal allograft rejection. Urine cytology can also be used for these purposes as a low-cost non-invasive technique, performed at various healthcare levels (2, 4).

Programs for bladder cancer screening using urine cytology examination are not common in developing countries (5). The few programs known were conducted to screen individuals who were considered to be at high risk for bladder neoplasms, such as chronic cigarette smokers, industrial workers exposed to aromatic amines and individuals involved in agricultural activities with a high risk of schistosomiasis (2). It is known that

urine cytology has a high sensitivity for the non-papillary type of transitional cell carcinoma in situ, which is one of the precursor lesions for invasive bladder carcinoma (1, 2, 6). Therefore, screening programs of carefully selected individuals in high-risk population areas are essential, supported by the fact that the actual yield in screening programs of high-risk groups ranges from 10 to 70 per 1000 persons (2). Because of this, the promotion of early bladder cancer screening to detect and diagnose cancers before they become invasive is of crucial importance in the management of urinary bladder cancer (3).

1.2 Problem Statement

During the past ten years, an increasing incidence of urothelial carcinoma has been noted. Almost 75% of the initial diagnoses in Europe showed a five-year recurrence rate ranging from 31% to 78%, with a progression rate of 0.8% to 47% (4). This picture calls for regular monitoring and an integrated follow-up scheme in the detection of early tumor recurrences for effective early management.

Although urine cytology is an important cost-efficient and quick method of diagnosis of urinary tract conditions including tumors, in our settings the diagnosis of urinary tract tumors is mainly by bladder cystoscopy with few cases requiring biopsy. These techniques are invasive and expensive for the majority of patients in developing countries, including Tanzania. Because of this most patients come to the hospital late with advanced stage of the disease when no substantive treatment cannot be done but only palliation (7). Also, there are no established cytological services, which can offer better low cost but efficient and convenient screening strategy for early diagnosis. In addition, urinary bladder cystoscopy is also prone to missing flat bladder lesions (7). Despite the increasing frequency of urinary bladder diseases especially tumors (5), which may require early detection and initiation of treatment, most of patients with urinary bladder diseases live in rural areas where cystoscopy and biopsy services are not available. Even patients who live close to cystoscopy and biopsy services have low income and cannot afford to pay for services,, which contributes to the late stage disease.

Due to the above facts, it is therefore imperative to develop a diagnostic strategy, which is easily performed even in regional and rural settings where cystoscopy and biopsy

cannot be done to allow early diagnosis of urinary bladder tumors and other diseases. This study therefore, determined the efficacy of urine cytology as an affordable and accessible tool for early detection of bladder cancer at all levels of the health care system.

1.3 Study Rationale

Urine cytology remain one of the best ways to diagnose a variety of bladder lesions most importantly high grade ones (1). The combination of cystoscopy and urine cytology is considered to be the gold standard in the initial diagnosis of bladder cancer (8, 9). However, only bladder cystoscopy plus or minus biopsy is performed at MNH. Urine cytology can be readily done on voided urine, preserved and processed appropriately to allow screening of early disease.

This study provides a platform for the introduction of urine cytology as one of the tools for screening, diagnosing, monitoring and follow-up of patients with urinary bladder tumors at MNH and other levels of health care in the country. The results of the study will inform development of screening programs designed for high-risk patients, aiming at reducing late presentation with advanced disease stages (10), as it is the case now.

Urine cytology provides an affordable, quick diagnostic strategy, which majority of the population including those living in remote areas will have access. This strategy will be used for follow-up of patients diagnosed with bladder tumors at three to six months' intervals post-treatment, to determine response to treatment and detect recurrences early. This low-cost and quick diagnostic tool will lead to improved quality of care and significantly reduce mortality due to urinary bladder cancer.

1.4 Conceptual framework

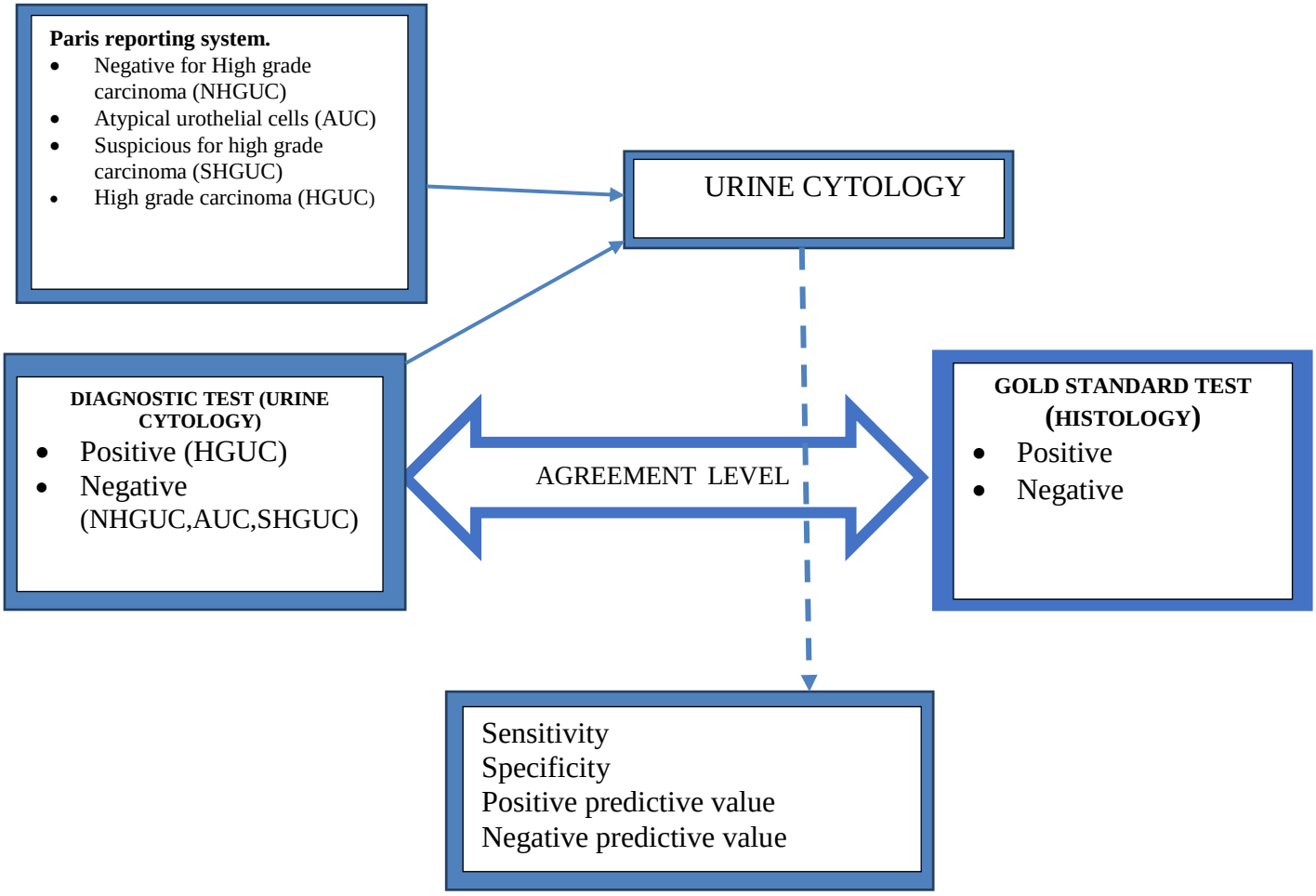


Figure 1: Diagram illustrating the conceptual framework for this study.

1.5 Hypothesis

The combination of urine cytology and urinary bladder cystoscopy and later on biopsy gives better results in the diagnosis and management of urinary bladder tumors compared to bladder cystoscopy and biopsy alone.

1.6 Research questions

What is the sensitivity and specificity of urine cytology at MNH?

What are the positive and negative predictive values for urine cytology at MNH?

How best can urine cytology be used in diagnosing and screening for bladder tumors with bladder biopsy as the gold standard?

1.7 Objectives

1.7.1 Broad Objective

To determine the efficacy of urine cytology in the diagnosis of urinary bladder tumors at MNH.

1.7.2 Specific Objectives

(i). To determine the sensitivity and specificity of urine cytology in diagnosis of urinary bladder tumors.

(ii). To determine the positive and negative predictive values of urine cytology in the diagnosis of urinary bladder tumors.

(iii). To assess the effectiveness urine cytology in the diagnosis of urinary bladder tumors compared to histology as the gold standard test.

1.8 Review of Literature

In 1945 Papanicolaou and Marshall introduced the use of urine cytology as a method for diagnosing bladder tumors. They exploited the fact that neoplastic lesions shed cells in the urine and cytological evaluation of urine samples was an initial method of diagnosing urinary bladder tumors, followed by cystoscopy and biopsy (2). Although attempts have been made to develop other diagnostic tools, cytology remains the best method of diagnosing various bladder lesions especially neoplastic ones (1).

Urinary bladder carcinoma is the 7^{th} most common cancer in men and the 17^{th} most common in women accounting for 3.2% of all cancers worldwide (11,12) it is one of the most prevalent (30.3%) cancer in Egypt and in most of the African countries such as Sudan, Kenya, Uganda, and Senegal (13). Cystoscopy (and biopsy) is the golden standard for diagnosing urothelial carcinoma of the urinary bladder. However, urine cytology is highly sensitive in the diagnosis of high-grade tumors of the bladder but less sensitive in low-grade tumors. The sensitivity of urine cytology range from 0-50% and significantly increase with tumor grade while the specificity of up to 99% have been observed (4,14,15). The positive and negative predictive values for urine cytology were found to be around 90% and 78% respectively (4).

The effectiveness of urine cytology in diagnosing urinary bladder tumors can be measured by calculating the degree of agreement when compared to the corresponding bladder histology results. The agreement of cytological and histological readings is determined by the Kappa coefficient (16). However, little research has been done both locally and in the rest of Africa leading to limited current literature on urine cytology as a diagnostic test.

A significant proportion (25%) of bladder cancer at first diagnosis is an invasive disease that is associated with a low 50% survival rate. In screening programs, cytology examination of urine is useful and has been applied in asymptomatic but high-risk patients for case finding, in evaluating and diagnosing patients with symptoms and in monitoring and follow-up of patients with known and treated disease (2,10). Urine cytology for transitional cell carcinoma (TCC) has been long used with the main technique being the cytomorphological study of cells shed in the urine. Despite the benefits of this

technique, cytopathologists still face challenges in studying the cells. Advancement in laboratory technology has made it possible to apply ancillary tests to urine cytology slides, making it a better and reliable diagnostic tool (6,17).

In order to get good results from urine cytology, specimen collection and handling, is of paramount importance (18). Difficulties in decision making are encountered when false negative results occur from failure of the tumor to shed cells in urine as in cases of low-grade lesions, but also false positive results from mimics of tumor cells during interpretation (8). In order to avoid false negatives and positives, for all screening programs, voided urine specimen is recommended for male patients and catheterized urine specimen for females. It is also recommended to collect the second voided urine in the morning (20) because the first morning urine has stagnant cells, which may have degenerated and hence leading to problems in interpretation. The medium amount of urine for sufficient cellularity is 25 ml (5). Bladder washing offers another option for sample collection; this includes irrigation of the urinary bladder with up to 50 ml of sterilized normal saline, followed by collection of the washings, processing and interpretation (1,5).

Fresh urine specimens, which are 1-12 hours old do not require fixation. If it is expected that it will take 12-24 hours before the specimen is received in the laboratory, refrigeration is advised, and if more than 24 hours' fixation with an equivalent volume of 50% to 70% ethanol to prevent further cellular degradation. Satisfactory diagnostic results can be guaranteed if specimens are kept fresh up to 48 hours prior processing with the best outcomes observed in tissues refrigerated in less than 4 hours (5,9).

Various techniques are used in the processing of specimen, and these include direct conventional smears, membrane filters, cytocentrifugation and Thin Prep, both with specific pros and cons. Direct smears have, however, proven to be more convenient to prepare. The major bottleneck is high cell loss and substandard cellular details. On the other hand, the best cytological information is obtained from membrane filter preparation (Thin Prep), but they also have challenges in preparation in terms of the technique and the need of more equipment for sample preparation. Cytocentrifuge preparations are widely used with minimal effects on the cytological details. Irrespective of the processing technique used all urine samples are stained with the Papanicolaou technique.

The remaining methods like Romanowsky dyes and air-dried materials have proven a failure in showing the important cytological details.

The Paris System working has provided a standardized platform for reporting cytological interpretation of urine samples. This brings about reproducibility and understanding to the urologists on the clinical implication of the results. The Paris System is the most consistently updated and has brought success in urine cytology as a diagnostic tool (22).

Ancillary studies such as ImmunoCyt/uCyt+ test have being developed over the years to develop the diagnostic ability of urine cytology (23). Another assay which has proven to be a success in the US is the UroVysion fluorescence in situ hybridization which detects the deletion of the 9p21 chromosomal region as well as amplification of chromosomes 3, 7 and 17 (24,25). Due to lack well-trained technicians ancillary tests are not performed on cytology cases. Also there are no enough funds to enable routine use of ancillary tests.

2.0 METHODOLOGY

2.1 Study design

This was a prospective study whereby all patients eligible for urinary bladder cystoscopy were investigated using urine cytology analysis.

2.2 Study area

The study was conducted at Muhimbili National Hospital (MNH) with 3000 beds, in the pathology and urology departments. Samples were collected from the Urology department where by about 60 patients are attended pre day, then sent to the MNH pathology laboratory where about a 1000 cytology samples are processed annually for processing, analysis and documentation.

2.3 Study population

The study population was patients who were seen at urology clinic and admitted at the urology department at MNH, who were booked for and scheduled for bladder cystoscopy from June to December 2018.

2.4 Inclusion and exclusion criteria

2.4.1 Inclusion criteria

The study included all patients seen at urology clinics and/or admitted for bladder cancer work out and eligible and scheduled for bladder cystoscopy.

2.4.2 Exclusion criteria

Excluded patients were those who had already started management for other bladder diseases or cancer and patients who did not consent for the study.

2.5 Sample size estimation

The estimated sample size for the study was 165 cases. This was based on the prevalence of urinary bladder cancer in Africa (30.3%) (11, 19) and a 7% precision.

The sample size (N) was calculated using the formula below:

$N= Z^{2} * (P(1-P)/\delta^{2})$

Z= Confidence interval (1.96)

P= Prevalence of urinary bladder cancer (30%)

δ= Precision (0.07)

N= 1.96^2 * ($0.3(1-0.3)/0.07^2$) = 165

2.6 Data collection methods and procedures

2.6.1 Data collection method

(a) Demographic information was obtained from patient's files and electronic records. Other collected variables included the date of collection and type of specimen (voided or catheterized).

(b) Urine samples were collected at a minimum volume of 30 ml from all patients who were scheduled for bladder cystoscopy before the procedure was performed. The volume of collected urine samples was immediately fixed in equal volumes of 70% ethanol and then sent to the pathology laboratory for analysis followed by cytological interpretation and reporting. The data collection sheet captured all information **(Appendix I).**

(c) At the laboratory, using the cytocentrifugation technique, 30 ml of urine were centrifuged for 10 minutes at 1200 rpm by using the Heraeus Labofuge 400 (Obsolete) 75008150 centrifuge machine (Made in Germany). The bottom layer was smeared directly on albumenized slides (to enhance cell adhesion to slides), then stained using the Papanicolaou technique as indicated in **Appendix II**. A total of three slides were prepared for each patient.

(d) The principal investigator and the supervising pathologists reviewed the slides.

(e) Bladder cystoscopy results for the correspondent patients were collected from the urology department and documented with other findings in the data collection sheet **(Appendix I).**

(f) For bladder lesions that required open biopsy, the specimens were collected and fixed as per MNH pathology laboratory protocol, then stained by H&E followed by histological interpretation **(Appendix III)**.

2.6.2 Cytological analysis and reporting

The slides were studied using a Leica DM 750 microscope and interpreted according to the 2016 Paris system for reporting urine cytology (27). For documentation and record keeping, pictures of the cytology and biopsy sections were taken using a 12-

megapixel Apple iPhone 7 rare camera, with the aid of the Vankey cell phone microscope adapter. Captured images were transferred to the principal investigator's laptop, which is protected by a password.

2.6.3 Data collection instrument

A data collection sheet was used to collect patients' information as indicated in **Appendix I**. The investigator provided special containers for urine collection together with albumenized slides for smear preparation. Other tools such as the centrifuge machine and microscopes were obtained from the pathology laboratory at MNH.

2.7 Data processing and analysis

2.7.1 Data processing

The obtained data was entered and analyzed using Statistical Package for Social Scientists (SPSS) version 23.0.

2.7.2 Data analysis

Data was transferred from data collection sheet and recorded into SPSS version 23 software and analyzed. Data was also summarized into proportion and frequency distribution tables.

Objective 1 and 2: Validation of urine cytology in diagnosis of urine bladder tumors was performed to determine the sensitivity, specificity, positive and negative predictive values. For urine cytology (High grade urothelial carcinoma) HGUC and (Squamous cell carcinoma) SCC were considered positive while (Negative for high grade urothelial carcinoma) NHGUC, (Atypical urothelial cells) AUC and (Suspicious for high grade urothelial carcinoma) SHGUC were regarded as negative.

Objective 3: Descriptive statistics with a 95% confidence interval were used to compare the proportion of positive bladder tumor cases between urine cytology and urinary bladder histology results. Bladder histological results were used as gold standard. The P value of <0.05 was considered statistically significant. The Cohen's Kappa (κ) coefficient was used to measure the agreement between the two tests. The agreement was classified into poor, fair, moderate, good and very good reflecting Kappa values <0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80, 0.81-1.0, respectively.

2.8 Ethical consideration

Ethical clearance was sought and obtained from Muhimbili University of Health and Allied Sciences (MUHAS) Institutional Review Board (IRB). Explanations were given to the participants about the research, its purpose, confidentiality, and safety. A written informed consent was read and willingly signed by the eligible participants (**Appendix IV).** Participation was voluntary, and the participants were informed on their freedom to withdraw from the study at any stage if they so desire without any consequence or prejudice.

3.0 RESULTS

3.1 Study demographics.

During the study period 127 patients were enrolled, 82 (64.6%) patients were males and 45 (35.4%) were females. Only 88 patients were eligible for comparison and calculation of sensitivity, specificity and positive and negative predictive values due to lack of histological biopsies to compare to cytology samples. The patients' ages ranged from 11 years to 88 years with mean age of 52.3 years and a median of 52 years. Forty-nine (49; 38.5%) of the patients were over 60 years, and 78 (61.4%) were below 60 years of age.

3.2 Clinicopathological characteristics of the study population

In urine cytology, 31(24.4%) of the patients were diagnosed as NHGUC, 20 (15.7%) as AUC, 9 (7.1%) as SHGUC, 58 (45.7%) as HGUC and 9 (7.1 %) of the study population were diagnosed as squamous cell carcinoma (SCC) by cytological examination **(Table 1, Figure 2).**

Bladder cystoscopy findings were grouped into inflammatory conditions 29 (22.8%), benign lesions 41 (32.3%) and bladder tumors 62 (48.8%). The commonest diagnosis among benign lesions category was urethral stricture 12 (9.4%) **(Table 2).**

A total of 39 (30.7%) patients had no tissue biopsies taken during cystoscopy procedures, the remaining 88 (69.3%) patients had tissue for histological diagnosis and therefore used for comparison groups in calculating sensitivity, specificity, positive and negative predictive values.

Histological diagnoses were categorized into urothelial carcinoma, squamous cell carcinoma, benign lesions and inflammatory conditions. Squamous cell carcinoma was the most frequent 34 (38.6%) histologically diagnosed malignancy followed by urothelial carcinoma 25 (28.4%). Males were over all mostly diagnosed to have malignancies by tissue histology technique with 14 (16%) diagnosed to have urothelial carcinoma and 19 (21.6%) diagnosed to have squamous cell carcinoma **(Figure 3).**

Table 1: Frequency of Urine cytology results in the study population (N=127)

Urine cytology results	Frequency (%)
High grade urothelial carcinoma	58 (45.7%)
Negative for high grade urothelial carcinoma	31 (24.4%)
Atypical urothelial cells	20 (15.7%)
Squamous cell carcinoma	9 (7.1%)
Suspicious for high grade urothelial carcinoma	9 (7.1%)

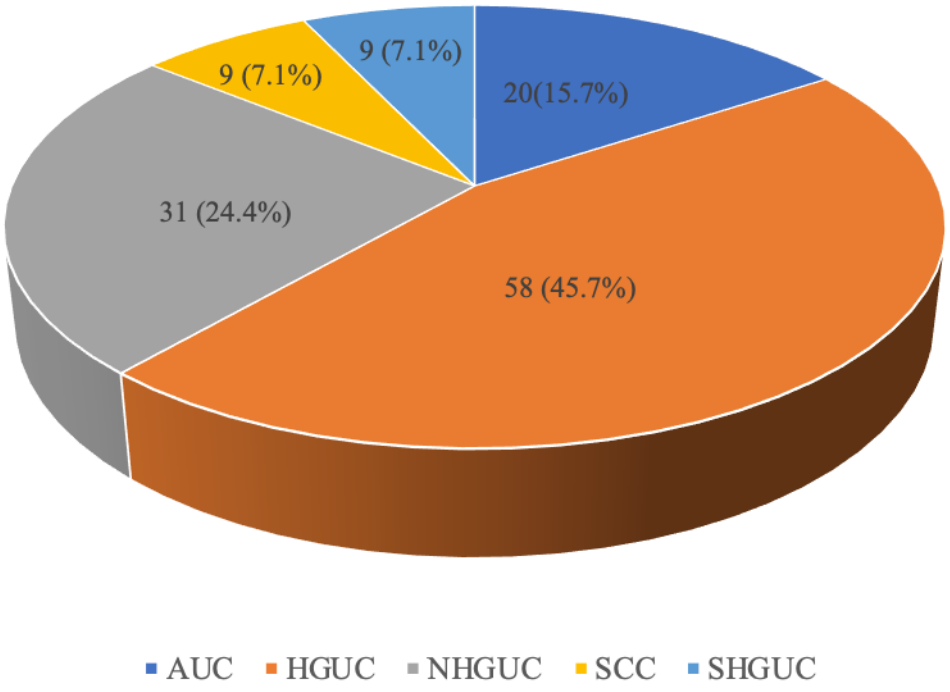


Figure 2: Percentages of Urine Cytology results

Table 2: Categorization of urine cystoscopy diagnoses

Bladder cystoscopy result categories	Frequencies (%) N=127
Inflammation (Cystitis)	**29 (22.8%)**
Benign Lesions	**37 (29.1%%)**
Cystolithiasis	5 (3.9%)
Nephrolithiasis	2 (1.6%)
Neurogenic bladder	3 (2.4%)
PUJO*	4 (3.1%)
PUV**	1 (0.8%)
Ureteric obstruction	5 (3.9%)
Ureteric stricture	1 (0.8%)
Ureterolithiasis	3 (2.4%)
Urethral stricture	13 (9.4%)
Tumors	**61 (48.8%)**

* Pelvi-ureteric junction obstruction

**Posterior urethral valve

Table 3: Sex distribution among histological diagnoses (N=88).

Gender	Urothelial Carcinoma	Squamous cell carcinoma	Benign Lesion	Inflammatory conditions	Total
Male	14 (25.5%)	19 (34.5%)	10 (18.2%)	12 (21.8%)	55 (100%)
Female	11 (33.3%)	15 (45.5%)	1 (3.0%)	6 (18.2%)	33 (100%)
Total	25 (28.4%)	34 (38.6%)	11 (12.5%)	18 (20.5%)	88 (100%)

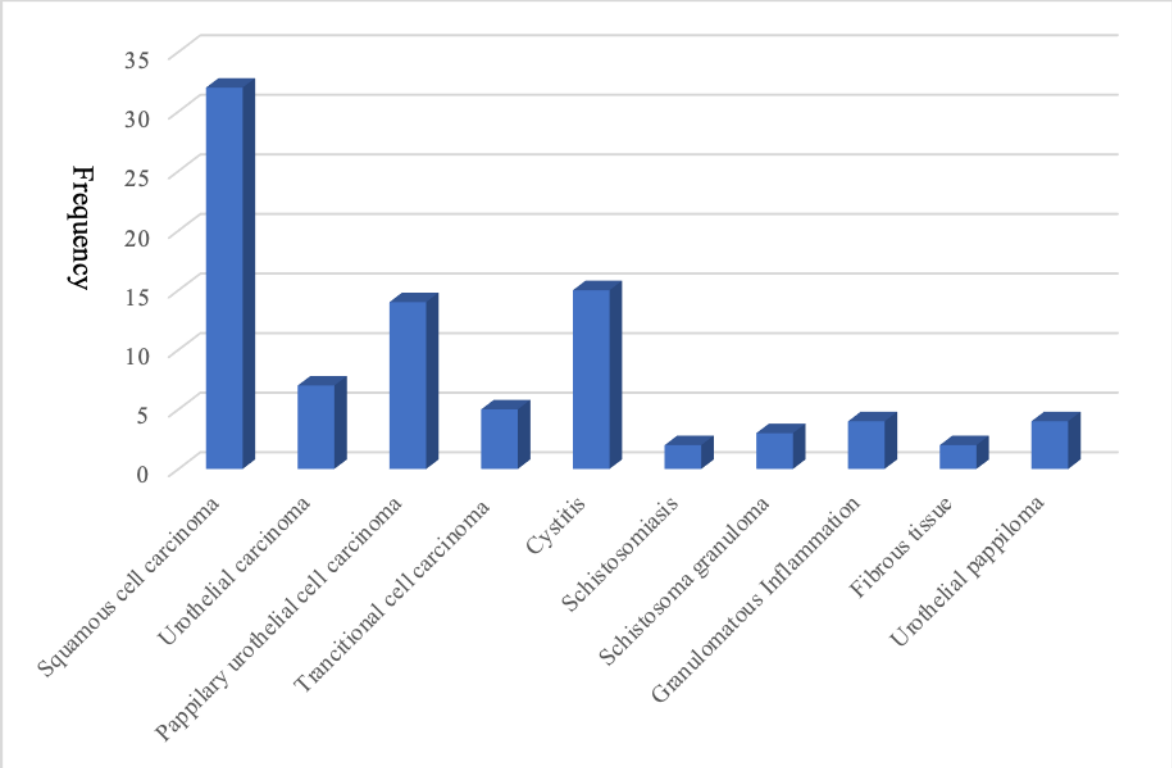


Figure 3: Frequencies of various histological diagnoses

3.3 Sensitivity, specificity, positive and negative predictive values

Results of sensitivity and specificity of urine cytology are summarized in **Table 4**. In a total of 88 patients urine cytology had a sensitivity of 90.5% and a specificity of 92.0% with a positive and negative predictive value of 96.6% and 79.3% respectively ($p<0.05$).

Table 4: Summary of sensitivity, specificity values.

	Histological Diagnosis			
Urine Cytology		**Positive**	**Negative**	**Total**
	Positive	57 (**90.5%**)	2 (8%)	59
	Negative	6 (9.5%)	23 (**92%**)	29
	Total	**63**	**25**	**88 (100%)**

3.4 Effectiveness of urine cytology in diagnosis of urinary bladder tumors.

The agreement between urine cytology as the diagnostic test and histological diagnosis which is the gold standard was substantial with a Kappa coefficient (κ) of 0.78 with a significant p value of <0.05. This was also supported morphologically as shown by the concordance of histologic and cytological findings by Figures 4a&b, Figure 5a&b, Figure 6a&b, Figure 7a&b and Figure 8a&b).

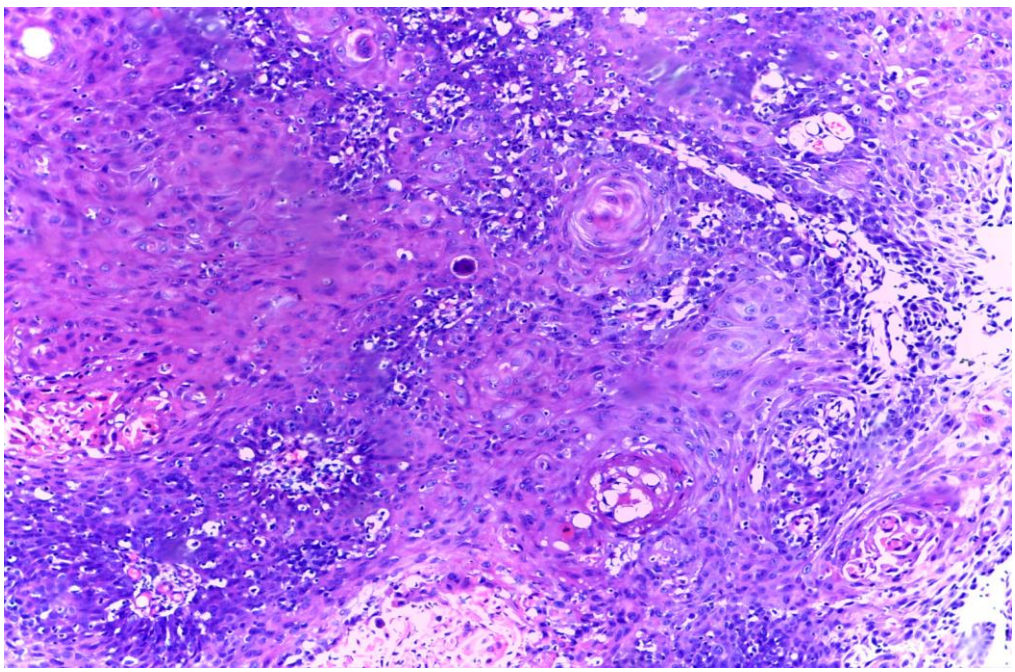


Figure 4a: Section of a tumor from a 52 years male. Squamous cell carcinoma with keratin pearl formation (H&E Stain x40)

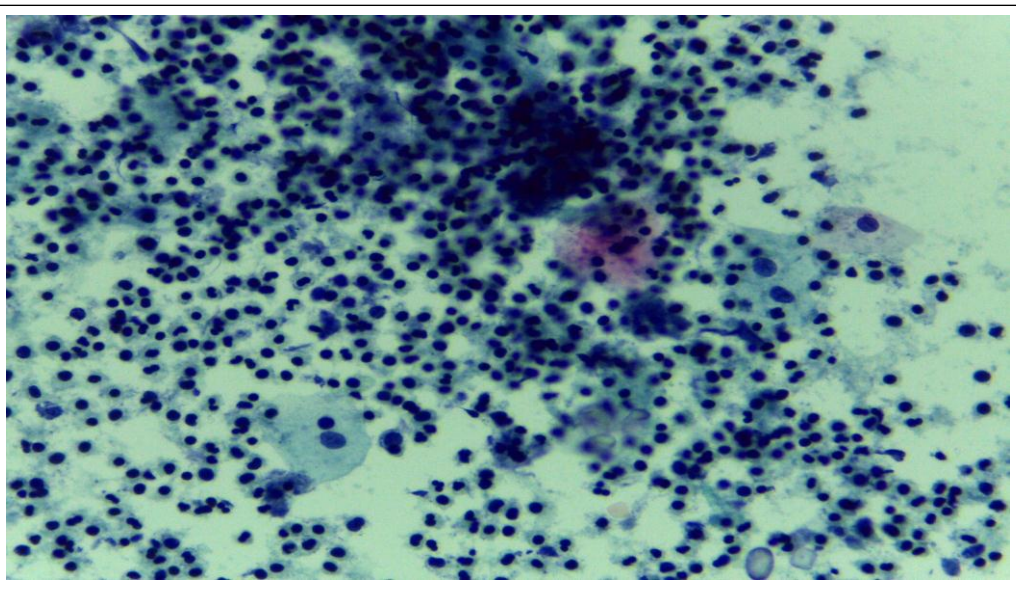


Figure 4b: Urine cytology smear of patient in Fig.4a. Atypical squamous cells with marked inflammation in the background (Papanicolaou's Stain x10).

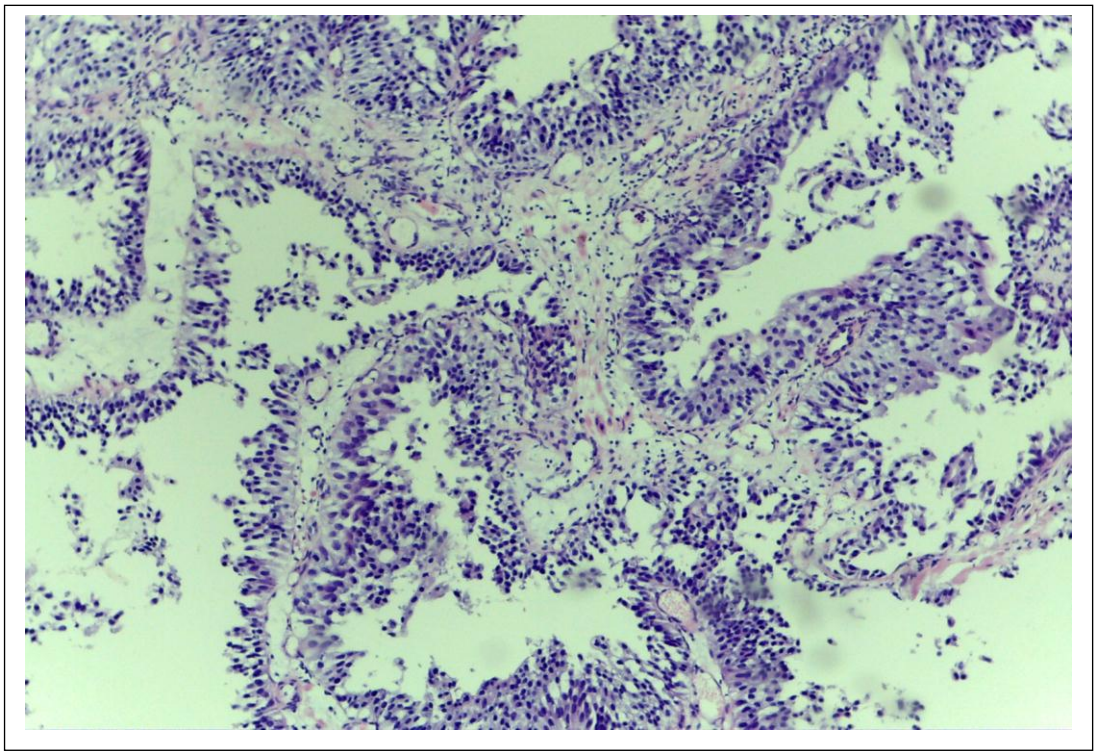


Figure 5a: Section from a 75 years Male, Papillary urothelial carcinoma, fibro vascular cores can well be appreciated from this section, lined by atypical urothelial cells (H&E Stain x10).

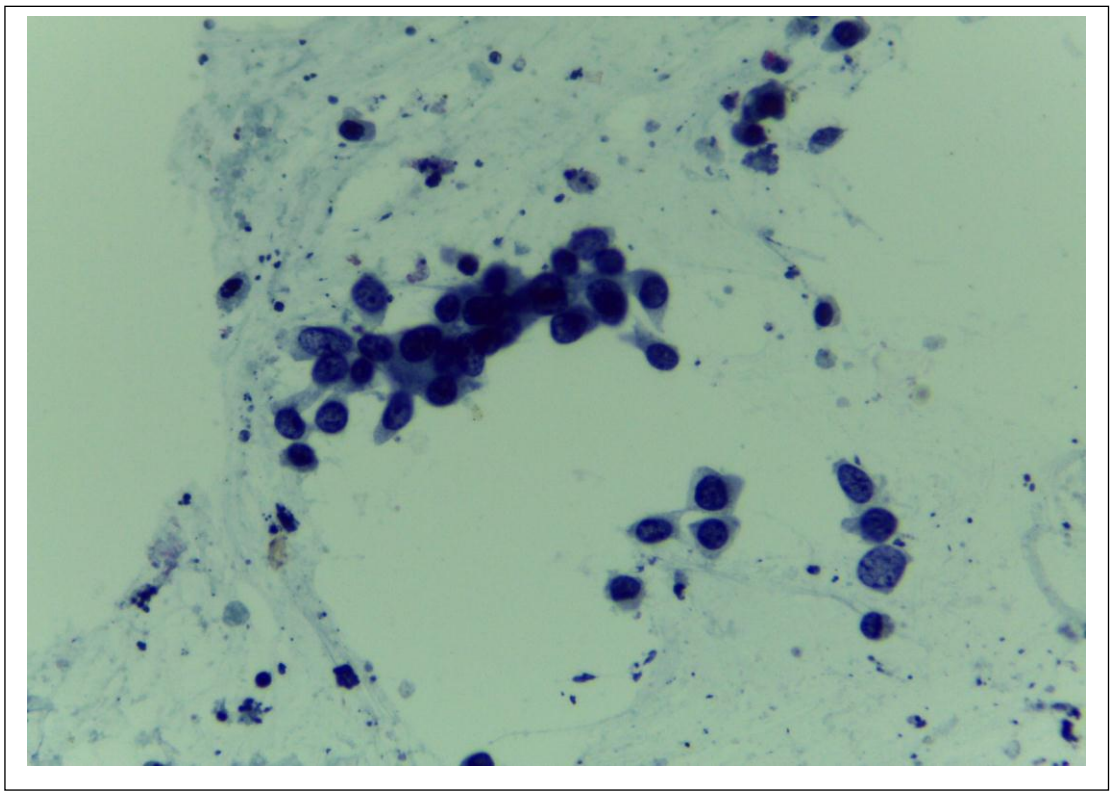


Figure 5b: Urine cytology smear of patient in Fig 5a. A cluster of atypical urothelial cells. (Papanicolaou's Stain x40).

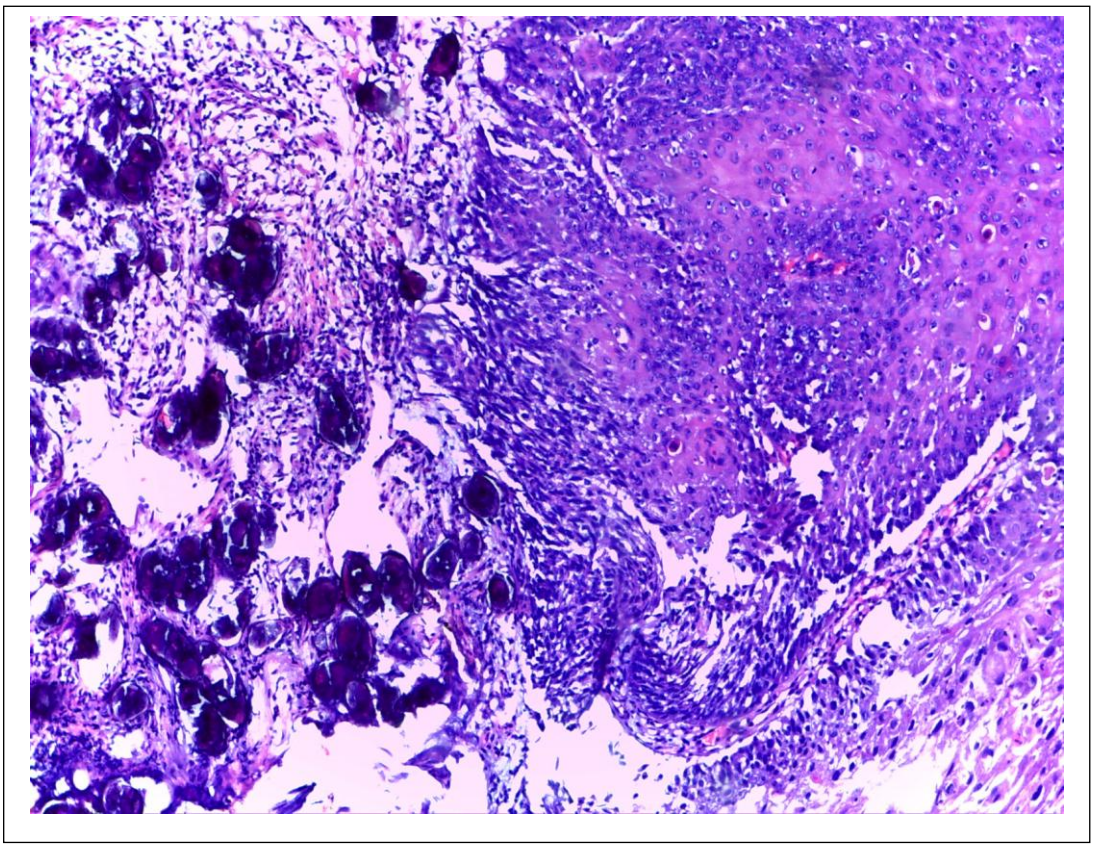


Figure 6a: Section form a 48 years old Female. Squamous cell carcinoma with numerous Schistosoma ova. (H&E Stain x40).

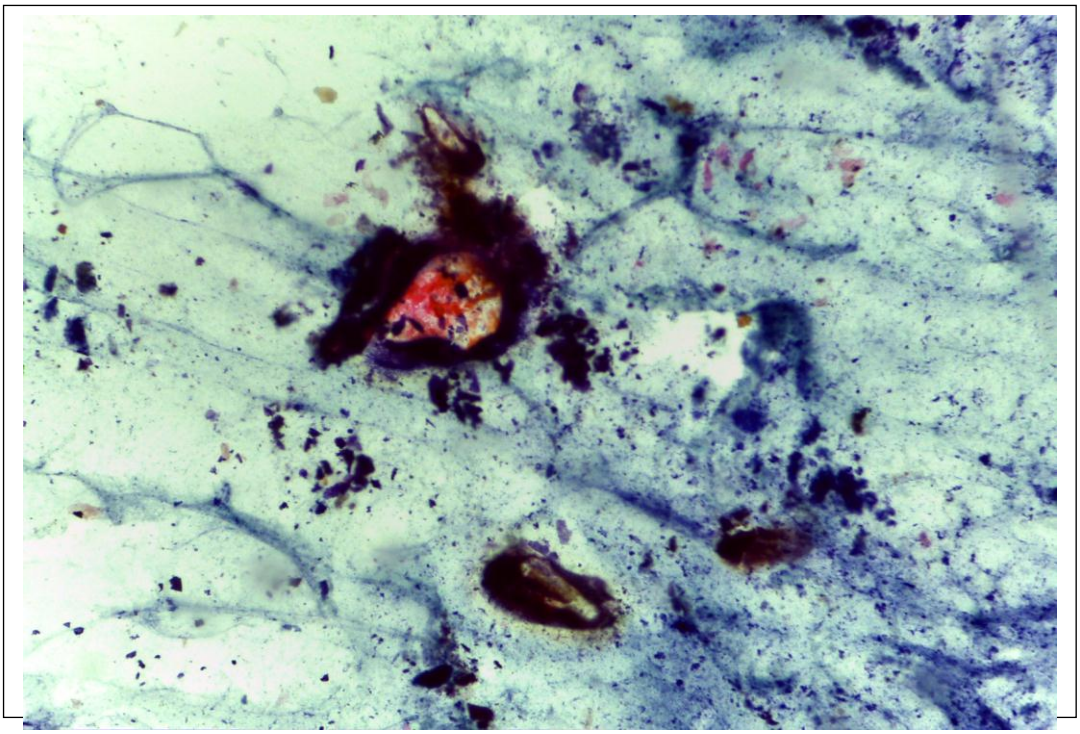


Figure 6b: Schistosoma ova are well demonstrated in a urine cytology smear from the patient in Fig 6a (Papanicolaou's Stain x40).

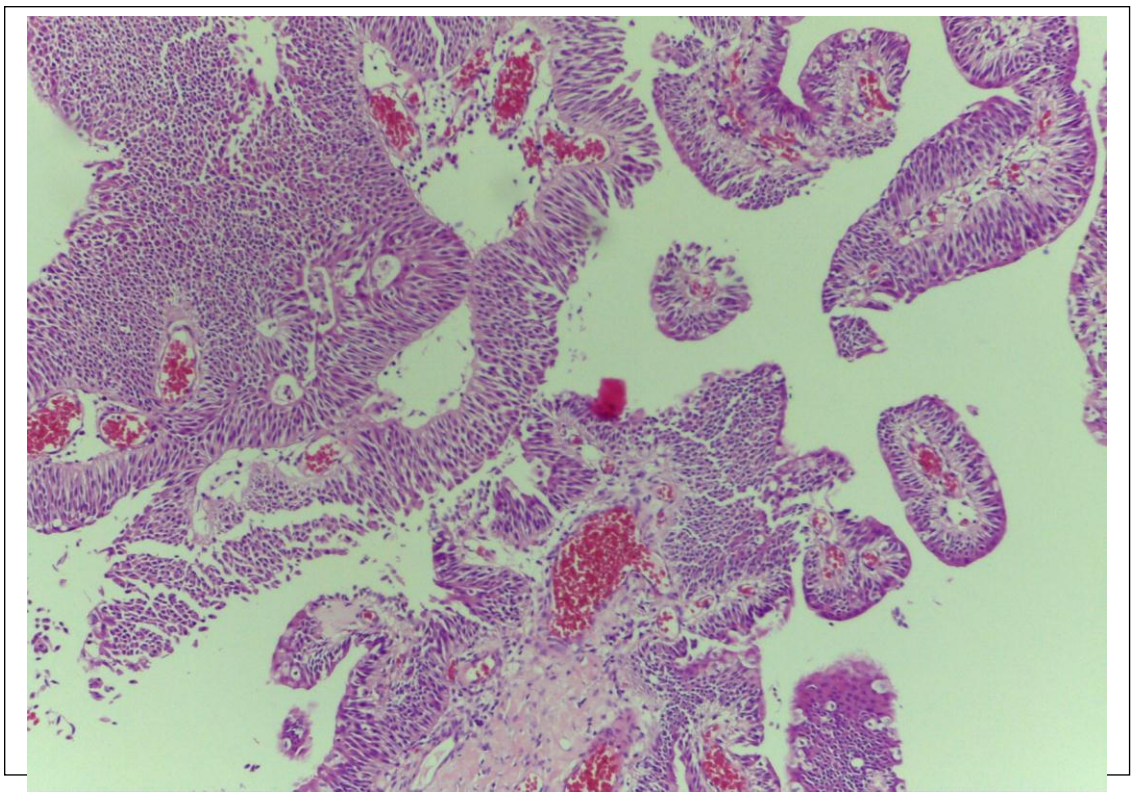


Figure 7a: Section from 67 years Male. Papillary urothelial carcinoma (H&E Stain x10).

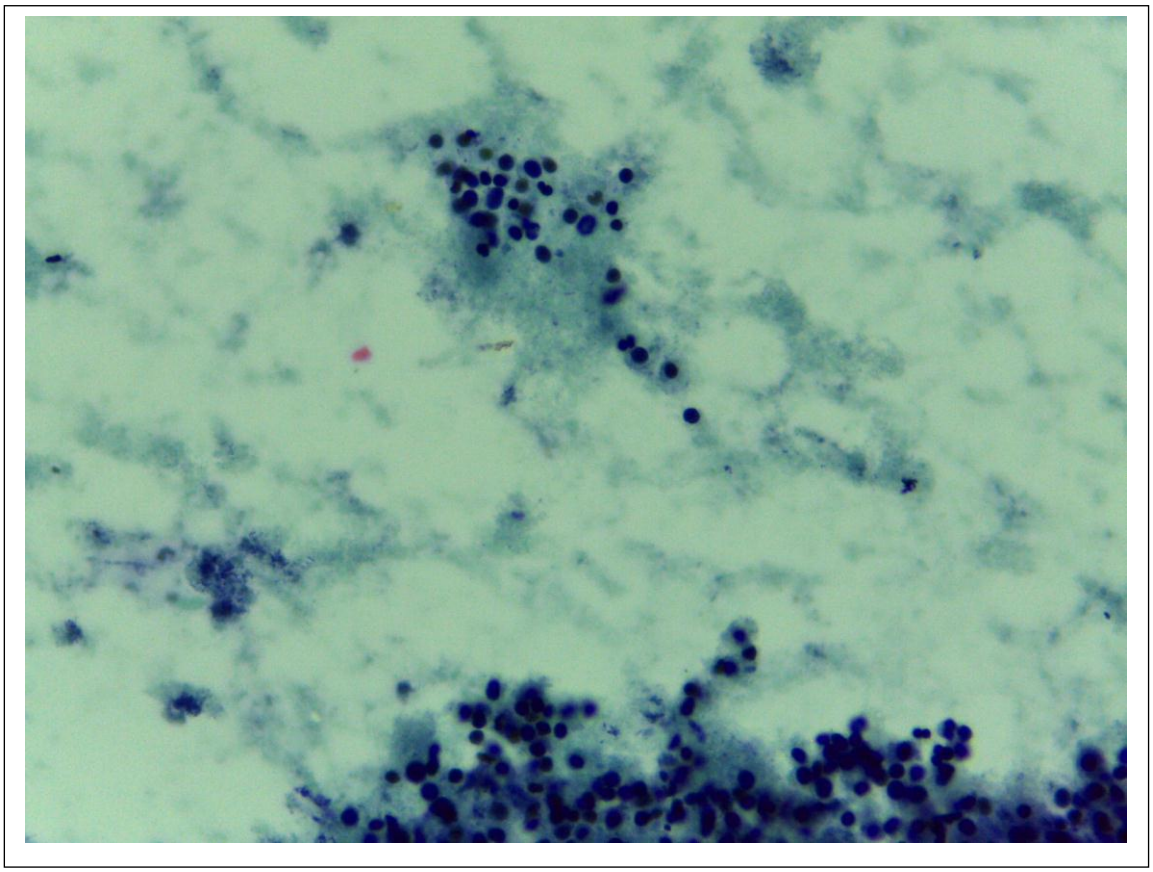


Figure 7b: Smear from the patient in Figure 7a, displaying atypical urothelial cells admixed with inflammatory cells (Papanicolaou's Stain x10).

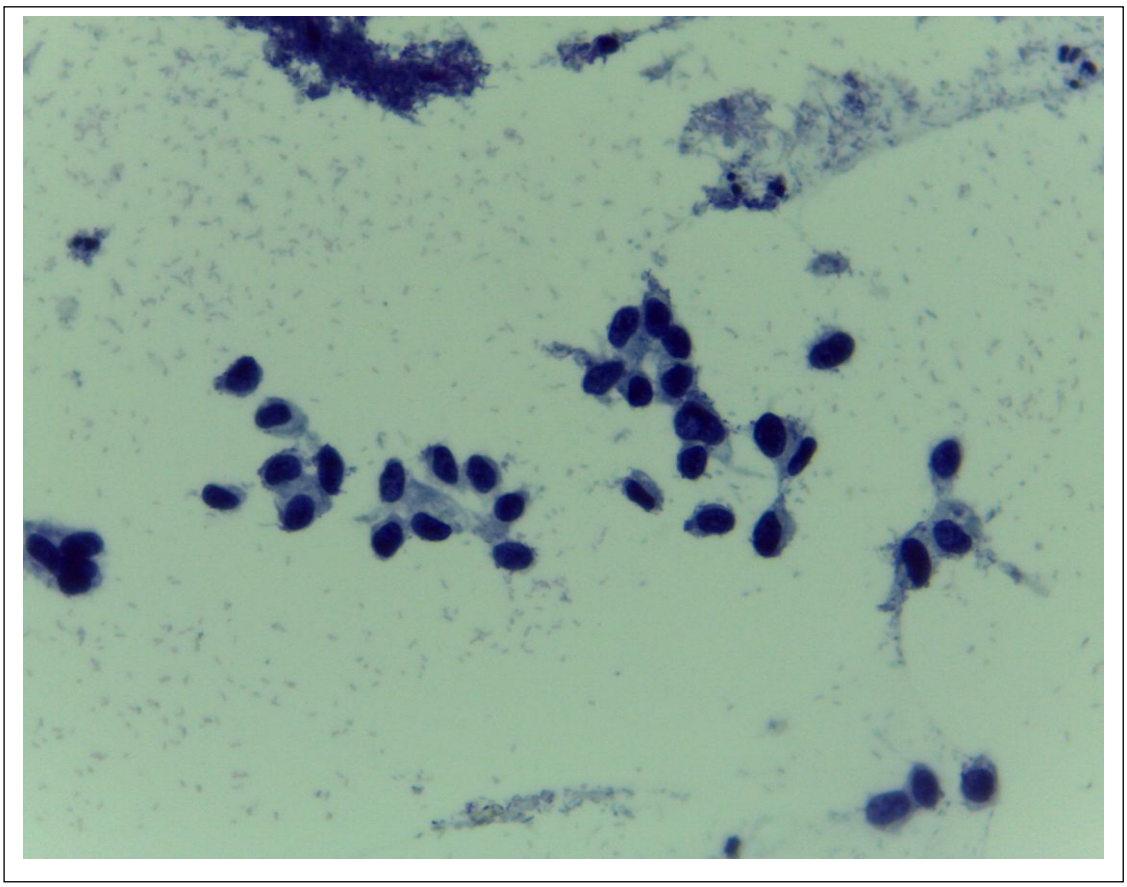


Figure 8a: Smear displaying urothelial epithelial cell with marked atypia (Papanicolaou's Stain x40).

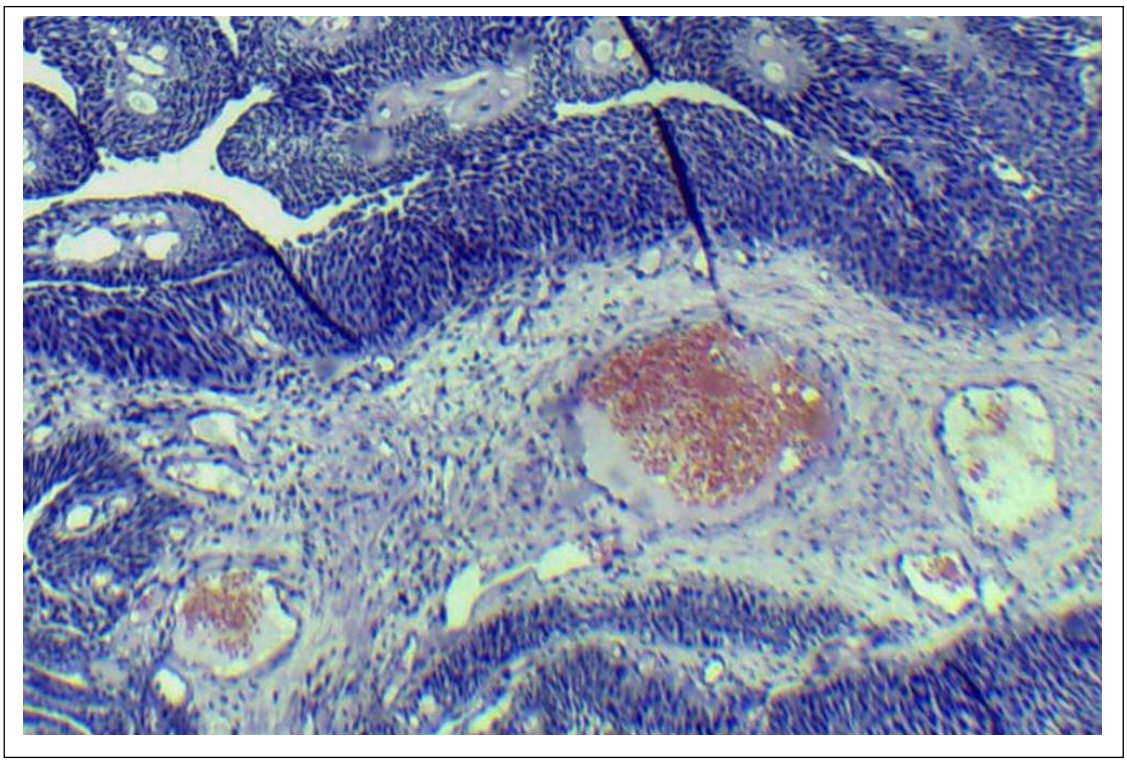


Figure 8b: Section from 74 years Female. Papillary urothelial carcinoma with area of squamous metaplasia. (H&E X10).

4.0 Discussion

4.1 Study demographics

In this study, the mean age at diagnosis regardless of the histological type was 52.3 years and a median of 52 years. In Mwanza, Tanzania the mean age at diagnosis was found to be 54.3 years (26), and similar findings were obtained in a study done by Kane et al., in Senegal (28). Also, in this study we found out that majority (61.4%) of patients were below the age of 60 years with the trend of diagnosis of bladder cancer going towards younger patients. Similar findings were also obtained in another study done in Senegal (28). Similar to what has been shown before in the literature (26,29–31), male patients were found to be the most affected (62.5%) by urinary bladder tumors compared to females. Other two studies done in the Tanzania (19, 24, 25) reported that squamous cell carcinomas was more common than urothelial carcinoma, which supports the current study findings. These findings are however, contrary to frequency of the types of urinary bladder cancer seen in developed countries were urothelial carcinomas are the commonest (29). This could be explained by environmental differences and thus different etiological factors and pathogenesis for urinary bladder neoplasms in developing and developed countries (29).

Majority (45.7%) of the study population were diagnosed to have high-grade urothelial carcinoma (HGUC) lesions by urine cytology. Using urine cytology fewer (7%) cases of squamous cell carcinoma (SCC) were identified. Urinary bladder cystoscopy only managed to describe urinary bladder tumors by their gross appearance as well as other lesions, which were confirmed to be benign by histological diagnosis. This was an expected finding due to the already known abilities and limitations of bladder cystoscopy in diagnosing urinary bladder tumors (1).

4.2 Sensitivity, specificity, positive and negative predictive values

In this study, sensitivity for urine cytology was found to be 90.5%, similar to a study done by Koss LG et al., (33) with a sensitivity of 94.2 %. The finding however, was slightly higher in comparison to a study by Planz et al, (4) were the sensitivity for high grade tumor was found to be 78.3%. The differences are explained by the fact that only cases diagnosed as high-grade urothelial carcinoma (HGUC) were used in calculating specificity and sensitivity (3). Generally urine cytology has a very good sensitivity for high grade bladder tumors, making it easier to identify the lesions early through a method which

is not invasive but cost effective (34). Urine cytology therefore proves to be an ideal technique for surveillance and assessment of tumor recurrences in previously diagnosed individuals or those who have already received treatment for urinary bladder tumors (2).

The acceptance of screening and early detection methods depend on the sensitivity and specificity (4). Using urinary bladder tissue biopsy as the gold standard, urine cytology was put to test, with the specificity of 92% similar to a study done by B. Planz et al., (4) and that of Raitanen et al., (16). This means there is a good chance in ruling out urinary bladder tumors by using this convenient and reliable technique especially in resource limited setups.

The positive and negative predictive values were also found to be 96.6% and 79.3% respectively supporting earlier findings by Planz et al., (4) and Bastacky et al., (27) again advocating the usefulness of urine cytology as a diagnostic option as well as a screening tool.

4.3 Effectiveness of urine cytology in diagnosis of urinary bladder tumors.

The level of agreement between diagnosis by urine cytology and histological diagnosis on tissue biopsy was measured using the Kappa (κ) coefficient with the value of 0.79 proved to be statistically significant with the p value of < 0.05. This means there is a substantial agreement between urine cytology diagnosis and that of histological findings, similar to studies by Raitanen et al., (26) and Ramakumar S. et al., (28) with the coefficient of 0.70 for all the primary bladder tumors.

4.4 Study Limitations

Time was a limiting factor in this study, and due to this reason, the estimated sample size could not be reached but approximately 70% of the expected samples which can affect the results of the study. If more patients were recruited the statistical interpretation of the results would have had more meaning and better compared with larger studies done elsewhere.

In some patients, tissue biopsies were not collected during cystoscopy procedure. These cases lacking tissue biopsies were not included in the calculation of specificity, sensitivity, positive and negative predictive values. As a result, less number of patients were eligible to fit in comparison groups for the calculation of specificity and sensitivity which further reduced the sample size.

Lack of recent literature in urine cytology was observed in various stages of this

project. Most studies are more than ten years old with the few recent studies focusing on ancillary test done on urine cytology samples an aspect which was out of the scope of the study. This led to limited number of studies to compare with the currently obtained results.

5.0 Conclusion

Results obtained from this study show that urine cytology had a good sensitivity and a high specificity allowing it to be used routinely in screening and diagnosis of urinary bladder tumors. Being effective and convenient despite its known limitation's urine cytology was effective in detecting high-grade tumors, with a sensitivity of 90.5% and a specificity of 92%.

However, bladder cystoscopy and biopsy was still a superior diagnostic test as it managed to detect bladder tumors accurately, and the current urine bound techniques cannot replace cystoscopy but it will offer practical solutions especially in resource limited settings where cystoscopy techniques are not available but also expensive for those who can reach them.

With a substantial [Kappa coefficient (κ) 0.78] measure of agreement between urine cytology and bladder tissue histological results close to the perfect (0.81-1.0), meaning that 78% of bladder cytology results are concordant with bladder histological results hence from this study, cytology can well be employed as routine screening and diagnostic technique in remote resource limited areas, especially in high risk patients.

6.0 Recommendations

This study provides a platform for introduction of urine cytology techniques in screening and diagnosing bladder at Muhimbili National Hospital and all health system levels in the country. Pathology laboratories in the country can start using this convenient and reliable diagnostic tool particularly in areas were bladder cystoscopy services are not available.

With the available technology and laboratory facilities at MNH, this study has proved that it is possible to introduce this service without much interfering with the hospital logistics but yet provide an appropriate service to patients whenever necessary.

7.0 Dissemination of results:

The findings from this study will be presented at the Department of Pathology meetings at Muhimbili University of Health and Allied Sciences and Muhimbili National Hospital. The dissertation will be available for the public through the University Library and on-line through the Directorate of Research and Publications. The results of this study will be published as article(s) in peer-reviewed journal(s).

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9.0 APPENDICES

Appendix I: Data Collection Sheet

Part A (Patient demographic data)

1. A) Patient MNH ID number …….
   B) Study ID number …….
   C) Laboratory ID number …….

2. A) Telephone number of the patient ……
   B) Next of kin relationship …….
   C) Next of kin telephone number …….

3. Current residence
   A) District …….
   B) Region …….

4. Gender (tick)
   A) Male ……
   B) Female ……

5. Age at diagnosis ……..yrs.

6. Occupation……

Part B (Urinary Bladder Cancer)

7. Presenting clinical history

……

……

…….....

8. Associated Signs and Symptoms …….....

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…….....

9. Urine cytology results……

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10. Bladder cystoscopy results …….

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11. Bladder biopsy results …….....

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Appendix II: Papanicolaou staining procedure

1. Rinse smear in descending grade of alcohol (80%, 70% ethyl alcohol)
2. Rinse in tap water.
3. Stain in Harris haematoxylin for 5-10 minutes depending on the strength of the haematoxylin.
4. Rinse in running tap water.
5. Differentiate in 1% acid in 70% ethyl alcohol: 1 dips.
6. Rinse in running tap water.
7. Blue in running tap water for 10 minutes.
8. Rinse smear in ascending grades of alcohol (70%,80%, ethyl alcohol) 10 dip each.
9. Rinse in two changes of 95% ethyl alcohol: 10 dips each.
10. Stain in orange G-6 for 3 minutes.
11. Rinse in two changes of 95% ethyl alcohol: 10 dips.
12. Stain in EA-36 solution for 3 minutes.
13. Rinse in 95% alcohol2 changes 10 dips each.
14. Complete dehydration in 2 changes of absolute ethyl alcohol: 10 dips each.
15. Clear alcohol in xylene two changes four dips each.
16. Mount the slide with DPX and cover slip.

Appendix III: Haematoxylin and eosin staining procedure

1. Retrieve blocks and make tissue sections using microtome [3-5 microns]
2. Pick the section on a slide from water floatation and place a hot plate 60^0C for 50 minutes.
3. Deparaffinized sections, hydrate through graded alcohol to water
4. Remove fixation pigments if necessary
5. Stain in alum hematoxylin for a suitable time.
6. Wash well in running tap water until section ‘blue’ for 5 minutes
7. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5-10 seconds.
8. Wash thoroughly in tap water until sections are again ‘blue' (10-15 minutes)
9. Stain in 1% eosin Y for 10 minutes.
10. Wash in running water for 1-5 minutes
11. Dehydrate through alcohols (70, 95, 100%), clear and mount

Appendix IVa: Informed Consent English Version

Informed Consent to volunteer in a study Titled: The role of urine cytology in the diagnosis of urinary bladder tumors at MNH.

Introduction

My name is Dr. Gilbert Z. Nkya a Resident pursuing a Master's degree of Medicine in Anatomical Pathology at the Muhimbili University of Health and Allied Science. My Dissertation project is aimed at determining the role of urine cytology in the diagnosis of urinary bladder tumors at MNH.

Purpose of the study

The study aims at determining the effectiveness of urine cytology in diagnosing urinary bladder tumors at MNH.

This study will introduce and create a platform for urine cytology practice as a diagnostic tool for bladder tumors. This method is cheap and less invasive but also allows easy screening and patient follow-up.

Procedure

In case you agree to participate in the study, the investigator will collect your urine and biopsy for cytological and histological examination respectively. The investigator will as well have access to your urine cystoscopy results.

Confidentiality

All the samples and information obtained from this study will be entered on the database with a study identification number, and the electronic data will be password protected.

Right to withdraw

Participation in this study is voluntary, therefore withdraw at any point does not involve any penalty.

Risk involved

Loss of confidentiality and privacy of patient’s information during processing or analysis. Otherwise, there is almost no risk in participating in this study

Benefits

The results obtained from this study will help to improve urinary bladder cancer diagnosis and treatment regarding early detection and follow-up for recurrences in managed patients.

Contacts

In case you have any additional question regarding this study. Contact Dr. Gilbert Z. Nkya Mobile +255767-724900, Email: gznkya@gmail.com

Signature:

I, ……., have read and understood the content of this consent form and I agreed to participate in this study.

Signature of the participant: …… Date: ……

Signature of the investigator: …….

Appendix IVb: Informed Consent Swahili Version

Fomu ya ridhaa kujitolea sampuli kwa ajili ya utafiti uhusio kutambua manufaa ya kipimo cha mkojo katika kugundua chembechembe za viashiaria vya saratani ya kibofu cha mkojo katika Hospitali ya Taifa Muhimbili.

Utangulizi

Mimi naitwa Gilbert Z Nkya Mwanafunzi wa udhamili katika chuo kikuu cha Sayansi na tiba Muhimbili. Nafanya utafiti juu ya uhakika wa kupima chembechembe za viashiria vya saratani ya kibofu cha mkojo kwa kutumia sampuli ya mkojo katika Hospitali ya Taifa Muhimbili.

Lengo la utafiti

Lengo la utafiti huu ni kuangalia uwezekano wa kutumia mkojo kupima chembechembe za viashiria vya saratani ya mkojo katika Hospitali ya Muhimbuli.

Mtindo wa utoaji wa sampuli ikiwa utaridhia kushiriki kwenye utafiti huu, mtafiti atachukua sampuli ya mkojo kutoka kwa wagonjwa watakao fanyiwa kipimo cha kuangalia kibofu kwa kamera maalum, pamoja na samlupi ya nyama endapo itahitajika. Vilevile mchunguzi ataweza kuona majibu ya kipimo cha kuangalia kibofu cha mkojo kwa kamera maalum.

Usiri

Sampuli zote na habari zitokanazo na utafiti huu zitaingizwa kwenye mfumo wa elektroniki na zitalifungiliwa na mtafiti pekee kutumia kwa numbari ya siri.

Uhuru wa kujitoa kujitoa kwenye utafiti

Kushiriki utafiti huu ni kwa hiari na kujitoa hakutaambatana na adhabu.

Madhara yatokanayo na ushiriki

Hakuna madhara yatokanayo na kushiriki utafiti huu.

Faida za ushiriki

Matokeo ya utafiti huu yatasaidia kuwapunguzia gharama za uchunguzi kwa wagonjwa. Pia kipimo hiki kitawezesha madaktari kugundua mapema zaidi chembechembe za viashiria vya saratani ya kibofu cha mkojo na kuwafuatilia wagonjwa waliokwisha kupata matibabu.

Mawasiliano

Endapo una swali la ziada juu ya utafiti huu usisite kuwasiliana na Dr Gilbert Z Nkya simu; +255767-645477, **Barua pepe: gznkya@gmail.com**

Saini ya ridhaa

Mimi..…….nimesoma na kuelewa maelezo katika fomu hii ya ridhaa na nimekubali kushiriki utafiti huu.

Saini ya mshiriki……

Saini ya mtafiti…….. Tarehe……..