

**CYTOLOGIC FINDINGS OF THE  
PROSTATE GLAND AT AUTOPSY BY  
TOUCH IMPRINT CYTOLOGY**

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REGISTRATION NUMBER: H568/71018/09

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UNIVERSITY OF NAIROBI

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**A Dissertation Submitted in Partial Fulfillment for the Award of a  
Master of Science in Clinical Cytology at the University of Nairobi**

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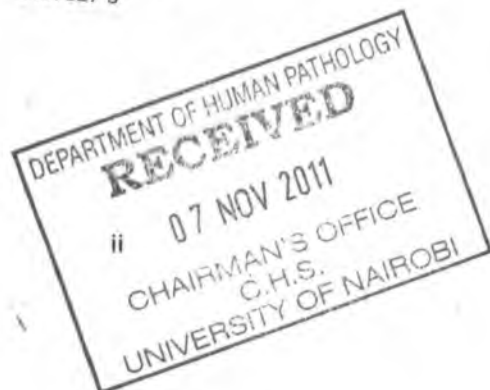
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## DECLARATION

I, Gladys Esendi Change, hereby declare that this is my original work and that it has not been submitted in any other institution previously and is being presented for the first time for the award of Master of Science in Clinical Cytology at the University of Nairobi.

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## **DEDICATION**

*To my late father, H.M Makwana,  
who prior to his demise,  
was diagnosed of prostatic carcinoma*

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## LIST OF ABBREVIATIONS

ASAP.....	Atypical Small Acinar Proliferations
AAH.....	Atypical Adenomatous Hyperplasia
CZ.....	Central Zone
CK.....	Cytokeratin
DRE.....	Digital Rectal Examination
DPX.....	Di-N-Butyle Phthalate in Xylene
EAMJ.....	East African Medical Journal
ERC.....	Ethics and Research Committee
FNAC.....	Fine Needle Aspiration Cytology
FPA.....	Focal Prostate Atypia
GS.....	Gleason Score (Gleason Sum)
H&E.....	Haematoxylin and Eosin
HGPIN.....	High Grade Prostatic Intraepithelial Neoplasia
IARC.....	International Agency for Research on Cancer
KNH.....	Kenyatta National Hospital
LGPIN.....	Low Grade Prostatic Intraepithelial Neoplasia
UoN.....	University of Nairobi
PAP.....	Prostatic Acid Phosphatase
PC.....	Prostatic carcinoma
PI.....	Principal Investigator
PIA.....	Proliferative Inflammatory Atrophy
PIN.....	Prostatic Intraepithelial Neoplasia
PSA.....	Prostate Specific Antigen
PZ.....	Peripheral Zone
SOP.....	Standard Operating Procedure
SPSS.....	Statistical Package for Social Scientists
TIC.....	Touch Imprint Cytology
TNM.....	Tumour, lymph Node, Metastasis
TRUS.....	Trans Rectal Ultra Sonography
TZ.....	Transition Zone

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## ABSTRACT

**Introduction:** Pathological conditions of the prostate include inflammation, prostate intraepithelial lesions and many types of carcinomas, 95% of which are adenocarcinomas. The gold standard in diagnosing prostatic carcinoma is trans-rectal ultra sound directed-histological biopsy. Nevertheless, touch imprint cytology in autopsy diagnosis has been investigated and found to have good agreement with histology results.

**Objective:** To describe the cytologic findings of the prostate gland at autopsy by touch imprint preparations.

**Rationale:** To demonstrate the use of imprint cytology in the rapid laboratory diagnosis of neoplasms in autopsy and intra-operative surgical procedures.

**Study design:** Descriptive cross-sectional study conducted from June to October, 2010.

**Setting:** University of Nairobi cytology laboratory, Kenyatta National Hospital and City mortuaries.

**Sample size:** Sixty one prostates excised at autopsy from men aged above 30 years.

**Methodology:** The glands were weighed and step-sectioned at regular intervals to increase sample homogeneity. Touch imprints were made from cut surface onto glass slides and fixed immediately in 95% ethanol and sent to the laboratory for processing using Haematoxylin and Eosin technique and examined by use of light microscopy. The imprints were screened and signed out by the two supervisors. Evaluation of cytomorphologic criteria was based on the microscopic appearance of cellular architecture, cytoplasmic and nuclear features, staining characteristics and the background milieu. Interpretations were made depending on the pattern and the degree of cellular atypia. The imprints were then validated by an independent pathologist blinded to the initial findings for quality control

**Outcome measures:** Age, weight of gland, benign prostate hyperplasia, prostatitis, prostate intraepithelial neoplasia and prostatic carcinoma.

**Statistical considerations:** Statistical comparisons of age groups, weight of glands and the resultant diagnostic parameters were determined using the Statistical Package for Social Sciences, version 17. Continuous data (age and weight) was summarized using mean, median, minimum and maximum. Categorical data (diagnostic outcomes) were presented in frequencies and percentages. Analysis of Variance was used to compare the mean differences on weight of the prostate gland for the various age groups. The t-test was used to make pairwise comparisons of the two means, to ascertain the groups which are actually different. The associations of various conditions of the prostate were assessed against age, weight and statistical conclusions were drawn as to the significance.

**Results:** The average weight of the 61 prostate glands was 29.6g and the mean age of the decedents was 50.9 years. Prostatic carcinoma was found in 6.2%, suspicious for malignancy in 1.6%, Prostatic intraepithelial neoplasia in 8.2%, benign prostate hyperplasia in 8.2%, prostatitis in 8.2% and corpora amylacea in 13.2% of the study population. Benign prostate hyperplasia, prostatic intraepithelial neoplasia and prostatic carcinoma were detected at the age of 30, 38 and 46 years respectively.

**Conclusion:** Touch imprint cytology detected malignancy, prostatitis, benign prostate hyperplasia and other findings that compared well with histological studies. The procedure will be of future use both in autopsy and in intra-operative procedures as an adjunct to histology, for rapid detection of tumours in Kenyan district hospitals that lack histology facilities

## CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

### 1.0 Introduction

Prostatic carcinoma (PC) is one of the most common internal malignancies and the leading cause of cancer deaths in men.<sup>1</sup> More than 75% of men diagnosed with prostate cancer are above 50 years and the tumour is rarely found in children and adolescents. The International Agency for Research on Cancer (IARC), estimates that Kenya has 1007 new cases, 850 new deaths and a prevalence of 790 per year.<sup>2</sup>

Carcinogenesis of PC is not clearly established but the adenocarcinoma begins when normal semen-secreting prostate gland cells mutate into neoplastic cells through several grades of prostatic intraepithelial neoplasia to carcinoma in situ.<sup>3</sup> Over time these cells invade the surrounding prostate stroma forming a tumour which metastasizes to other organs. The greatest risk factors for prostate neoplasms are age and race. The disease is common in black men who are over 50 years and it tends to worsen with advancing age.<sup>4</sup>

Prostatic carcinoma affects the prostate gland which is physiologically and pathologically divided into four distinct regional zones: peripheral, central, transition, and the anterior fibromuscular. Pathological conditions affecting the prostate can be summed up as prostatitis, hyperplasia, pre-neoplastic lesions and carcinoma.<sup>5,6</sup>

Prostatic carcinoma is further classified into incidental, clinical, latent and occult types.<sup>7</sup> The most powerful triad in diagnosis of PC comprises digital rectal examination (DRE), transrectal ultra-sonography (TRUS) and estimation of prostate specific antigen (PSA) in serum. Diagnosis of cancer is made either after prostatectomy and subsequent histology of the specimen, or after serum PSA screening followed by needle biopsy.<sup>8</sup>

In experienced hands, fine needle aspiration cytology (FNAC) is very effective in detecting prostate neoplasms with high accuracy.<sup>9</sup> However, the TRUS-directed histological biopsy is the gold standard for confirmation of prostatic carcinoma.<sup>8,10</sup>

Various publications have addressed the application of touch imprint cytology (TIC) in the intra-operative diagnosis of different types of surgical specimens and in autopsy diagnosis.<sup>11</sup>

The potential value of TIC is the reduced cost relative to routine histologic sections, its reliability and high concordance with histology. Despite its proven reliability, imprint cytology is not a common practice.<sup>12</sup>

Treatment and management of conditions of the prostate remain controversial and pose challenges to both researchers and clinicians. Development of novel therapeutic approaches is, currently, an active area of clinical research.<sup>13,14</sup>

Staging describes the presence of regional and distant extension of the tumour while grading assesses the microscopic degree of differentiation and growth pattern of the tumour. The Gleason scoring system is currently the *de facto* grading standard for prostatic carcinoma.<sup>5</sup>

The grading and the staging correlate and bear prognostic significance.<sup>15</sup>

This study used the TIC to demonstrate its utility in autopsy diagnosis of prostatic neoplasms by evaluating cytologic features of conditions that affect the prostate gland. The resultant proportions were compared to previous studies and conclusions drawn with possible extrapolation of the value of the technique in intra-operative surgical procedures for rapid diagnosis and improved laboratory diagnosis of tumours.

## **1.1 Epidemiology of Prostatic Neoplasms**

### **1.1.1 Occurrence and Distribution**

Prostatic carcinoma is one of the most common internal malignancy and leading cause of cancer deaths in men second only to lung cancer. Over 75% of men diagnosed with PC are over 50 years old and the tumour is rarely found in adolescents and even children. Frequency of PC at autopsy varies between 15-75% and is related to patient age and thoroughness of sampling. The most recent study of autopsy cases ( $n = 139$ ; 18–95 years) with no history of urological disease were examined for PC and high grade prostatic intraepithelial neoplasia (HGPIN). In the age group 81–95 years, 86.6% and 60% of men had PC and HGPIN, respectively.<sup>1</sup> The GLOBOCAN 2002 database (compiled by Ferlay *et al* for the International Agency for Research on Cancer) estimates that in Kenya, there are an estimated 1,007 of new cases, 850 new deaths and a prevalence of 790 per year.<sup>2</sup> A Kenyan study conducted on transperineal trucut needle biopsies of the prostate revealed that 25.42% of patients had undifferentiated prostate cancers. Within one year of being followed, there was 100% mortality.<sup>16</sup> A retrospective review of the cancer registry in Kenya by Tenge *et al* revealed that prostatic carcinoma is the second most common non-hematological malignancy among men in Kenya.<sup>17</sup>

### **1.1.2 Pathogenesis of Prostate Neoplasia**

Carcinogenesis of PC is not clearly established at this time but the condition begins when normal semen-secreting prostate gland cells mutate into neoplastic cells through several grades of prostatic intraepithelial neoplasia (PIN) which then progresses to carcinoma in situ. Over time these cells begin to multiply and spread to the surrounding prostate stroma forming a tumour.<sup>3</sup>



Eventually, the tumour may grow large enough to invade nearby organs via the lymphatic channels or by hematogenous spread.<sup>3</sup> The most common sites of metastatic spread of prostatic carcinoma are lymph nodes, rectum, urinary bladder and bones. Other metastatic sites include the liver, adrenal gland, central nervous system (including dura), eye, skin, lung, breast and unusual locations such as penis and salivary gland.<sup>3,18,19</sup>

### **1.1.3 Classic Risk Factors for Prostatic carcinoma**

The major risk factor for prostatic carcinoma is age.<sup>4</sup> The disease is more common in men who are above 50 years and it worsens with advancing age. Smoking, ethnicity (race), diet, obesity, environmental exposure, genetics (family history), and lifestyle have also been linked to higher incidence and more aggressive forms. Exposure to venereal diseases has been studied but the link to prostatic carcinoma is yet to be established.<sup>4,20</sup> Every man is at risk for prostate neoplasia as the precursor lesion PIN, has been detected in men as young as 30 years old. In 2007, Wasike and Magoha reported on 65 patients diagnosed with prostate cancer at the Kenyatta National Hospital in Nairobi. The crude hospital incidence was 76.5/100,000 patients, and the crude hospital death rate was 5.8/100,000. About 87.5% of these patients presented late in the course of disease, with clinical stage III or IV prostate cancer.<sup>20,21</sup>

## **1.2 An Overview of Prostate Pathology**

### **1.2.1 Anatomy of the Prostate Gland**

The average weight of the prostate gland in a normal adult male is 20g. The gland is divided pathologically into four distinct regional zones: peripheral, central, transitional, and anterior fibro-muscular (stroma). Anatomically, the gland is divided into five lobes: anterior, middle, posterior left-lateral and right lateral.<sup>5</sup>

The peripheral zone (PZ) is the sub-capsular portion of the posterior aspect of the gland that surrounds the proximal urethra. From this portion, more than 64% of prostatic carcinomas originate.<sup>6</sup> The central zone (CZ) surrounds the ejaculatory ducts and accounts for roughly 2.5% of prostate cancers. Transition zone (TZ), is the origin prostate cancer in roughly 34% of patients. It surrounds the proximal urethra and is responsible for benign prostatic enlargement. The anterior fibro-muscular zone (stroma) is usually devoid of glandular components, and composed of smooth muscle and fibrous tissue.<sup>5,22</sup> In the lobe classification, the anterior lobe (or isthmus) roughly corresponds to part of transitional zone, the posterior lobe spans all zones, the lateral lobes roughly corresponds to peripheral zone and the median lobe (or middle lobe) roughly corresponds to part of central zone.<sup>5</sup>

### 1.2.2 Benign Conditions of the Prostate

**Inflammation** of the prostate gland (prostatitis) presents in three main forms: Acute prostatitis is caused by bacterial organisms similar to those causing urinary tract infections, e.g. *E.coli* and *Pseudomonas* species. A related complication of prostatic abscess is uncommon. Chronic prostatitis may be bacterial or abacterial in origin. Granulomatous prostatitis may be due to tuberculosis that spreads from the lungs. In idiopathic granulomatous prostatitis, the prostate becomes firm and irregular and can be mistaken for PC on palpation as prostatic surface antigen (PSA) levels become elevated.<sup>5,6</sup>

**Benign prostatic hyperplasia (BPH)** also termed nodular prostatic hyperplasia is a common condition as men age. However, in only about 10% will this hyperplasia be symptomatic and severe enough to require surgical or medical therapy. The mechanism for hyperplasia may be related to accumulation of dihydrotestosterone in the prostate, which then binds to nuclear hormone receptors which then trigger

### 1.2.3 Pre-neoplastic Lesions of the Prostate

*Atypical adenomatous hyperplasia (AAH)* is a small glandular proliferation that has histological similarities with Gleason grade 1 and 2 but may be mistaken for prostatic adenocarcinoma. The incidence is reported as 1.6-36.9%. They are seen most commonly in the transitional zone.<sup>23,24</sup>

*Prostatic Intraepithelial Neoplasia (PIN)* is a concept borrowed from the well established and universally accepted term cervical intraepithelial neoplasia. Prostatic intraepithelial neoplasia is characterized as a neoplastic transformation of the secretory epithelium lining prostatic ducts and acini. A two-tier classification has been recommended: low-grade PIN (LGPIN) and high-grade PIN (HGPN).<sup>15</sup> High grade PIN is the most likely precursor of prostatic adenocarcinoma according to available evidence. This lesion is characterized by cellular proliferation within pre-existing ducts and acini, with nuclear and nucleolar enlargements similar to those seen in prostatic carcinoma except, unlike cancer, HGPN retain a basal-cell layer. PIN coexists with cancer in more than 85% of cases. The incidence and extent of PIN seem to increase with age.<sup>11</sup> Low grade PIN is considered clinically insignificant because progression to higher grade has not been reported. Due to the loss of prostatic secretions, cytology shows piling up of cells and a pale cytoplasm. Currently, LGPIN is not documented in pathology reports due to a fairly low risk of cancer following repeat biopsy.<sup>6,23-26</sup>

*Focal Prostate Atrophy* lesions are associated with chronic inflammation, or less commonly with acute inflammation. Pathologists have long recognized focal areas of epithelial atrophy which occur most commonly in the peripheral zone of the prostate. The term Proliferative Inflammatory Atrophy (PIA) has been proposed.<sup>22,25</sup>

#### 1.2.4 Malignant tumours of the prostate

Almost 95% of prostatic tumours are adenocarcinomas with the characteristic malignant features such as hyperchromasia, nuclear molding, and prominent nucleoli. Conventional prostatic adenocarcinomas sometimes exhibit neuroendocrine differentiation.<sup>7</sup>

**Small cell carcinoma** of the prostate is a high grade malignant neoplasm of neuroendocrine differentiation. The characteristic morphologic features are nuclear hyperchromasia, nuclear molding, extensive tumour necrosis and minimal cytoplasm.<sup>27</sup>

**Transitional cell carcinoma** may be found as a primary arising in the transitional epithelium of the prostatic urethra or as a metastasis of urinary bladder tumour into the prostate. Cytology shows loose clusters and single malignant cells with dense cytoplasm and distinct cell borders.<sup>7,27</sup>

**Papillary duct carcinoma** shows papillary groups of atypical glandular cells with subtle malignant features and characteristic grooved nuclear membranes.<sup>7</sup>

**Endometrioid carcinoma** is so-named because of its histologic resemblance to endometrial carcinoma. It is a rare tumour that is thought to be of prostatic ductal origin.<sup>7</sup>

**Squamous cell carcinoma** and adenosquamous carcinoma are rare tumors although **squamous metaplasia** is common in the prostate. However, hormonal manipulation of ordinary adenocarcinoma of the prostate can induce squamous changes in the cancer. Squamous cells which sometimes arise from squamous metaplasia of the prostatic ducts may appear singly or in sheets. Polygonal intermediate cells have small round nuclei, while superficial cells have pyknotic nuclei.<sup>27</sup> The other rare tumours of the prostate include mucinous adenocarcinoma, signet ring carcinoma, sarcomatoid carcinomas and sarcomas (rhabdomyosarcoma, osteosarcoma, leiomyosarcoma and fibrosarcoma).<sup>7</sup>

### 1.2.5 Types of Carcinoma of the Prostate

Carcinomas of the prostate are further divided into four types based on the method of detection:

**Latent PC** discovered by the pathologist during autopsy of a decedent who had no signs or symptoms referable to prostate. These tumours may occur anywhere in the prostate, but are usually in the central and peripheral zones, and are well differentiated. The frequency of latent carcinoma ranges from 26-73%.<sup>7,28</sup>

**Incidental PC** is discovered incidentally in prostatic tissues removed, during life, in transurethral resection of the prostate (TURP) for benign nodular hyperplasia or benign enlargement of the prostate or during cystoprostatectomy for bladder cancer. Frequency increases with age and incidence at autopsy is 15–70% and 6-20% in TURP.<sup>28</sup>

**Clinical PC** is detected by which digital rectal examination and other investigations. It is confirmed by histopathologic evaluation of the prostatic biopsy.<sup>7,29</sup>

**Occult PC** is the type of carcinoma is found in the biopsy of bone or lymph node in a patient without symptoms of prostatic disease. The prostatic origin of these metastatic lesions is confirmed by elevated serum levels of PAP and/or PSA and by a prostatic biopsy.<sup>7,30</sup>

### 1.2.6 Cytomorphological Features of Cells in the Prostatic Ducts

Epithelial cells derived from prostatic ducts appear in large cohesive sheets with well defined cell borders, forming a honeycomb pattern when viewed *en face*. Uniformly sized round-oval nuclei with smooth membranes are regularly spaced within the sheets. The chromatin is evenly distributed and finely granular while the nucleoli are indistinct. Basal cells of the prostate are primarily localized in the proximal region of the duct. The nuclei appear elongated to oval, and sometimes out of plane of focus of the sheets of cells.

The cytoplasm of the basal cells is decreased in the atrophic epithelium but increased in the hyperplastic epithelium.<sup>27</sup> Squamous cells which sometimes arise from squamous metaplasia of the prostatic ducts may appear singly or in sheets. Polygonal intermediate cells have small round nuclei, while superficial cells have pyknotic nuclei. Parabasal cells contain large vesicular nuclei with moderate amount of cytoplasm. Transitional cells may arise from the transitional epithelium of the prostatic ducts. They are found in clusters, sheets or as single cells. They are characterized by round to oval nuclei with fine evenly distributed chromatin. The transitional epithelium of the prostatic ducts does not show the superficial umbrella cells seen in the urethra. The epithelial cells that line the lumen of the ducts are surrounded by stromal cells. The cells in the lumen close to the urethra are cuboidal while those in the distal regions are tall columnar.<sup>27</sup>

### **1.3 Methods in Diagnosis of Prostatic Carcinoma**

Early detection of cancer is an important issue in the field of oncology. The prostate gland is no exception to this rule. The most powerful diagnostic triad for PC is DRE, TRUS and determination of serum PSA levels. Routine screening of the vulnerable elderly male population using the three-pronged approach has led to marked increase in the frequency of prostatic biopsies. Direct rectal examination can detect the size, location, volume and extension of tumour especially those in the posterior and lateral aspects of the gland.<sup>3,31</sup> Accuracy depends on experience of the examiner. Since TRUS is not highly sensitive in detecting PC, it is mainly used to guide prostate biopsy for histology and to give an indication of the size of the prostate in order to plan radiation therapy. Serum PSA estimation allows early detection of PC but its use as a screening tool remains controversial as changes in the epidemiology of PC have strongly limited its prognostic role.<sup>5,31</sup>

Thompson *et al* showed that there is a significant number of men with PSA values  $<4.0$  ng  $\text{mL}^{-1}$  who actually have PC. Not all patients with a relatively high-grade prostate cancer have elevated PSA levels, nor do elevated PSA levels always signify disease progression. Diagnosis of cancer is made either after prostatectomy and subsequent histology of the specimen or PSA detection followed by needle biopsy.<sup>5,31-32</sup>

### **1.3.1 Blood Profile and Culture**

Hematological workup of full blood count and a blood chemistry profile, including serum creatinine, liver function tests, serum PSA, and acid and alkaline phosphatase are useful aids in differential diagnosis. Abnormal urinalysis (i.e. haematuria, increase in protein levels, presence of inflammatory cells and atypical cells) from symptomatic patients, should be followed by a urine culture to rule out bacterial infections.<sup>7,33</sup>

### **1.3.2 Molecular Markers**

The role of p53 marker in predicting the outcome of prostatic adenocarcinoma is not clear. Certain molecular markers, such as E-cadherin, p53 and p21, DNA ploidy analysis, human kallikrein 2, and microvessel density (histologic marker of tumour angiogenesis) are also being evaluated to characterize disease progression.<sup>34</sup>

### **1.3.3 Immuno-histochemistry and Immuno-cytochemistry**

Immuno-staining for high molecular weight keratin is very useful in biopsies suspicious of adenocarcinoma. It specifically stains basal cells that are present in normal glands but not seen in carcinoma. In 56% of cases of HGPIN, the basal layer is only disrupted but not lacking. Immuno-staining for PSA and PAP is helpful to confirm the prostatic origin of a metastatic adenocarcinoma.<sup>34</sup> Cells that are acquired as touch imprints can provide information on membrane receptors.<sup>35</sup>

### **1.3.4 Histology**

Histology biopsy is the gold standard in diagnosis of PC in many settings. Many types of needles and punches (core, sextant, saturation, etc) have been devised for obtaining prostatic tissue biopsies. There is general agreement that the accuracy of detecting carcinoma is up to 98% especially when done under ultrasound guidance. Radical prostatectomy specimens can also be examined as whole mount sections. Pathologic diagnosis of PC is based on nuclear anaplasia, nuclear pleomorphism, invasion, and architectural disturbances. Mitotic figures and giant cells are rare, except in high-grade tumours. These changes are easily discernible in low-power microscopic view.<sup>10,36</sup>

### **1.3.5 Frozen Sections**

Frozen sections have specific limitations that allow error to occur. These include the initial selection and sampling of tissue by the pathologist, technical expertise required to prepare slides and errors of interpretation and delivery of results. Frozen sections have a high accuracy rate but the 15% incidence of false negative results and frequent loss of tissue during processing are major disadvantages.<sup>5,22</sup>

### **1.3.6 Fine Needle Aspiration**

Fine needle aspiration cytology (FNAC) is generally highly sensitive for the presence of disease and highly specific for the absence of disease, with very rare false positive results in experienced hands. Epstein correlated findings of FNAC and histology needle biopsy and the accuracy was 86.6%. Despite these facts, FNAC has fallen in disuse and has been replaced by automated spring-loaded 18-gauge needle core biopsy which may obtain sufficient tissue material for frozen sections and touch preparations.<sup>9,15</sup>



### **1.3.7 Prostate Secretions**

Cytologic diagnosis of prostatic secretions, obtained by prostate massage, has proved a futile exercise because of the high number of false negative results and frequent problems of specimen inadequacy. This is because in adenomatous glands the ducts are often blocked by stromal hyperplasia making obtaining prostate fluid for cytology in living individuals a painful, tedious procedure that often results in inadequate material and distortion of cells making PC studies difficult.<sup>9,22</sup>

### **1.3.8 Touch Imprint Cytology**

Touch Imprint cytology (TIC) was first described, in 1927, by Dudgeon and Patrick who used imprint smears of fresh tissues in the rapid microscopic diagnosis of tumours. The cells are obtained by touching a wet tissue with a glass slide.<sup>11</sup> Cells adhere to the glass in roughly the same orientation as they exist on the surface of the lesion touched. Such touch preparations or imprints enable the examination of the whole cell apart from the tissue aspect. Touch imprint cytology in autopsy diagnosis has consistently demonstrated excellent diagnostic accuracy. The application of the technique in autopsy examinations has received far less attention, although the few publications on the subject have noted its potential value including low cost preparation relative to routine histologic sections. Shirley *et al* recorded a cyto-histologic concordance of 92.2% and later reported accuracy rates 95.9% by Liu *et al*.<sup>6,11,12,37</sup>

Various publications have addressed the application of TIC in the diagnosis of surgical specimens including intra-operative diagnosis (Table 1).<sup>38</sup> Although many useful studies have been done locally on PC, none has employed TIC (Table 2).

**Table 1: Literature Data on Accuracy of TIC in Intra-operative Procedures**

References	Body site	% accuracy
Scopa <i>et al. Diagn Cytopathol</i> 1990;6:5-8.	All	94.3
De Rosa <i>et al. Diagn Cytopathol</i> 1993;9:623-631.	Breast	95.3
Blandamura <i>et al. Acta Cytol</i> 1993,39:23-29.	Pancreas	100
Ghandur-Mnayneh <i>et al. Cancer</i> 1985;56:339-344.	Lymph nodes	99
Eltoum <i>et al. Diagn Cytopathol</i> 1998;18:62-66.	All	60.00
Liu <i>et al Diagn Cytopathol.</i> 2002;26:329 -333.	All	95.90

**Table 2: Summary of PC-Related Studies in Kenya**

Reference	Comment and Findings
Magoha & Wasike. <sup>21</sup> <i>East African Medical Journal (EAMJ)</i> 2007;84(9):S31-35	Incidence study, n=65, 87.5% late stage (II & IV)
Magoha <sup>16</sup> <i>EAMJ.</i> 2000;77(5); 260-263	Prospective study on survival of PC patients. n=59, GS>7;25.42%, GS<6; 25.42%,GS<4;49.2%
Ngugi & Byakika. <sup>35</sup> <i>EAMJ.</i> 2007;84(8) 363-366	Histology & Needle aspirates. Morphology. n=108 PC:26 %,BPH: 76%.
Mbuuko <i>et al.</i> <sup>38</sup> <i>Master of Medicine Thesis. UoN.</i> 2007	Prevalence Histology n=103. HGPIN;15.5%. PC:1% AAH;10.7%.
<b>n: sample size</b>	

## **1.4 Treatment and Management Conditions of the prostate**

Given the high prevalence of the disease and the aging of the population, the clinical dilemma today in the management of PC is to distinguish men who need definitive treatment from men who have indolent disease. Treatment consists of: radical prostatectomy, radiation therapy, androgen deprivation or watchful waiting. Controversies regarding treatment for PC continue to challenge researchers and clinicians. The discovery of novel therapeutic approaches (kinase inhibitors, antisense oligonucleotides, and angiogenesis inhibitors) is an active area of clinical research and currently in various stages of clinical trials. Watchful waiting, radical prostatectomy and chemotherapy are the common modes of management.<sup>13,14</sup>

## **1.5 Staging and Grading of Prostatic Neoplasms**

### **1.5.1 Staging of Prostate Neoplasms**

Staging describes the anatomical extent of the tumour. Is it *in situ* i.e. confined to the primary site or has it metastasized to adjacent and distant sites? Staging is important for identifying appropriate treatment options for a particular cancer in an individual.<sup>40-41</sup>

The tumor, lymph node, and metastasis (TNM) staging system is commonly used for individual tumours and identifies three factors: tumour size, lymph node involvement, and identification of distant metastases. An overall stage I, II, III, IV (W.H.O international histological classification of tumours designed by Mostofi et al) or A, B, C, D (American Urologic Society) is assigned for many cancers based on the TNM values. An extra stratification 'D' is for hormone-insensitive prostate cancer (Appendix 1).<sup>6,22,40,41</sup>

### 1.5.2 Gleason Grading System

Grading assesses the microscopic appearance of the tumour. More than 30 grading systems have been proposed over the years for grading prostate tumours, but the Gleason scoring system is currently the *de facto* grading standard in many settings (Appendix 2). The system is based on the glandular differentiation and pattern of the tumour and ignores nuclear cytology. Microscopically, prostatic adenocarcinoma exhibits four major cyto-architectural patterns: diffuse, medium-sized glands, small glands and cribriform. In order to determine the Gleason score, the predominant pattern is assigned a grade of 1 to 5 and the same is done with the secondary pattern. The sum of these patterns gives a Gleason score (GS) which in theory can range from 2 to 10. If a tumour contains a single pattern of growth then primary and secondary scores are the same.<sup>5,15</sup>

Poorly differentiated cancers have a GS of 8-10, moderately differentiated cancers have a GS of 5-7 and well differentiated cancers have GS of 2-4. Cancers with a Gleason score of less than 6 are generally low grade and not aggressive. Magoha published data on 59 patients diagnosed with locally advanced or metastatic prostate cancer in three hospitals in Nairobi. Fifteen patients had Gleason score of 8-10; another 15 patients had Gleason score of 6; and 29 patients had Gleason score of 2-4. Survival of the 15 patients with GS greater than 8 was very poor, with all patients dying within 12 months of diagnosis, despite treatment. Only 22 of the 59 patients were still alive 48 months after diagnosis, regardless of treatment.<sup>17</sup> The system can also be used in cytology based on structural and morphologic features. This was demonstrated by Rupp *et al* who used the Gleason's score to grade PC in urine. The grade and the stage correlate well with each other and with the prognosis. The prognosis of prostatic adenocarcinoma varies widely with tumour stage and grade.<sup>22</sup>

Advanced PC typically cause urinary obstruction; metastasize to regional (pelvic) lymph nodes and to the bones, causing blastic metastases in most cases. Metastases to the lungs and liver are seen in a minority of cases.<sup>6,42,43</sup>

### **1.6 Rationale**

To demonstrate the use of touch imprint cytology as a rapid method of laboratory diagnosis of neoplasms in autopsy and intra-operative surgical procedures.

### **1.7 Justification**

The incidence of prostate cancer continues to rise with changing lifestyles. According to GLOBOCAN 2002 data source<sup>16</sup>, the mortality rate of the PC in East Africa is high and it almost equals that of incidence since most patients present with late stage disease die despite treatment.<sup>17</sup> The method of obtaining prostate fluid for cytology by prostatic massage, in living individuals, is a painful, tedious procedure that often results in inadequate material which is unsuitable for evaluation. Histology is accurate but it is expensive and takes a long time to process while frozen sections are frequently associated microbial contamination, tissue loss and high false negative rates.<sup>8</sup> The rising costs of autopsy services and the high cost of setting histology laboratories makes histology facilities out of reach for majority of Kenyans. Conversely, TIC at autopsy allows thoroughness of sampling thus increasing the detection rate of tumours. It enables the examination of the entire cellular aspect of the gland. The cells adhere to the glass in roughly the same orientation just as they exist on the surface of the lesion touched. The method can be applied in intra-operative surgical procedure for rapid cytologic diagnosis of tumours from most body sites. Studies have demonstrated that TIC is rapid, reliable and affordable. It correlates well with histology without problems of specimen adequacy.<sup>8</sup>

## **1.8 Research Question**

What are the cytologic findings of the prostate gland at autopsy when using touch imprint preparations?

## **1.9 Broad Objective**

To describe cytologic findings of the prostate gland in men aged over 30 years at autopsy by touch imprint cytology

### **1.9.1 Specific Objectives**

1. To describe the cytomorphology of the prostate gland at autopsy
2. To analyze the distribution cytologic findings of the prostate gland in relation to age and weight of gland.

## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Study Design and Population

#### 2.1.1 Study Site

The study area was the University of Nairobi cytology laboratory located within the Kenyatta National Hospital, a national referral and teaching hospital located south of the central business district, in the Kenya capital, Nairobi. The hospital offers a wide range of specialised diagnostic services such as diagnostic imaging, TURP, TRUS and cytopathology. The KNH mausoleum is located next to the School of Medicine's library. Autopsies are performed by qualified consultant pathologists and registrars. On average, six autopsies done each week are male. The city mortuary is located on Mbagathi–Ngong road junction, 300m west of KNH mortuary. A minimum of 50 autopsies are performed weekly, between Tuesday and Friday.

#### 2.1.2 Study Design

Descriptive cross-sectional study, conducted between June and October 2010

#### 2.1.3 Study Population

Decedents of all decedents preserved in KNH and City mortuaries, Nairobi.

#### 2.1.4 Eligibility Criteria

##### *Inclusion criteria:*

- Decedents aged above 30 years of age
- Decedents with prostate gland *in situ*
- Decedents that were well-preserved but not fixed in formalin
- Decedents who had been dead for less than seven days prior to the date of autopsy

***Exclusion criteria:***

- Evidence of putrefaction and autolysis
- Pre-demise history of PC, hormonal therapy and/or radiotherapy for PC
- History of prostatectomy
- Embalmed decedents

**2.1.5 Sample Size Determination**

The Fisher's formula was used to calculate sample size.

$$n = \frac{Z^2(p)(1-p)}{D^2}$$

- $p$  = Estimated percentage histologic correlation (95.9%)<sup>33</sup>
- $Z$  = Standard value(1.96) assuming normal distribution at 95% confidence level
- $D$  = Allowable type I Error (alpha, 0.05)
- $n$  = Sample size

$$n = \frac{(1.96)^2 (0.959) (0.0419)}{(0.05)^2}$$
$$= 61$$

**2.1.6 Sampling Methodology and Procedure**

Sampling methodology was by convenient sampling of 61 prostates from men who met the inclusion criteria. The PI liaised with the registrar or pathologist on duty for autopsy service at both mortuaries. At the autopsy site, the fulfillment of the inclusion criteria was ascertained and a study number assigned. Decedents' data, obtained from the funerals superintendent's office, were recorded in a data collection sheet (appendix 3). Microscope glass slides were labeled accordingly. After autopsy procedure, the gland was excised.



The PI weighed it on the AVer<sup>TM</sup> balance and recorded the weight in grams. One step-section was made for every 10g of the total weight. An imprint was made for each section by pressing a labeled slide, gently but firmly, on the exposed surface of the tissue and fixed, within 5-10 seconds, in 95% ethanol (appendix 4). The specimens were sent to the laboratory for processing by the H&E technique (appendix 5) and mounted in Di-N-Butyle Phthalate in Xylene (DPX). The preparations were screened by the PI, counterchecked by the two supervisors and signed out for dispatch on a pre-designed reporting format (appendix 6). Interpretations were classified as: negative for malignancy (i.e. normal prostate), BPH, PIN, suspicious for malignancy, PC and prostatitis. These were based on the cytodagnostic criteria described by De May and Herzeberg *et al*<sup>7,27</sup> (appendix 7). The results were entered in a well structured data sheet (Appendix 3). An independent pathologist, blinded to the study, validated 10% of the imprints for quality control.

## **2.2 Data Management**

### **2.2.1 Data Entry and Storage**

Raw data was first entered in well-structured data sheet (Appendix 3), then it was cleaned and entered into excel spread sheets that were password-protected before analysis by SPSS version 17. Hard copies and back-ups of software copies were made and locked in a cabinet to avoid data loss. Prepared slide imprints and data entry sheets were stored under tight security to avoid unauthorized access.

### **2.2.2 Statistical Analyses**

The samples were divided in six distinguished age groups each corresponding to a decade of life. Statistical comparisons of age groups, weight of glands and the resultant diagnostic parameters were determined using SPSS version 17.

Continuous data (age and weight) was summarized using mean, median, minimum and maximum. Categorical data (diagnostic outcomes: prostatitis, BPH, PIN, and PC) were presented in frequency and percentage. Analysis of variance (ANOVA) was used to compare the mean differences of weight of prostate glands for various age groups. The t-test was used to make pair-wise comparisons of the two means. The association of conditions of the prostate was done against age, weight and statistical conclusions drawn as to their significance.

### **2.3 Variables**

Independent variables were age of decedent and weight of prostate gland. Dependent variables were the cyto-diagnostic outcomes (e.g. prostatitis, BPH, PIN and malignancy).

### **2.4 Quality Assurance Procedures**

Laboratory standard operating procedures (SOPs) in specimen collection and methods were followed. Reagents were prepared according to manufacturers guidelines (Appendix 4, 5 and 8). Good laboratory practices such as use of personal protective equipment, and disinfection of surfaces were practiced. Laboratory items were obtained from reputable suppliers used by the KNH and UoN. To minimize interpretation bias and as a quality control measure, an independent pathologist who was blinded to the study, provided an independent report on 10% of the specimens.

### **2.5 Ethical Considerations**

Permission to carry out this study and a waiver of consent administration were granted by the Ethical Review Committee of KNH/UoN (appendix 9). Clearance to conduct the study at the city mortuary was obtained from the Nairobi City Council (appendix 10). Strict confidentiality of decedents' record was maintained throughout the study. Decedents were treated with and tissues were disposed off appropriately according to standard protocol

## CHAPTER 3: RESULTS

### 3.1 Baseline Characteristics of Decedents

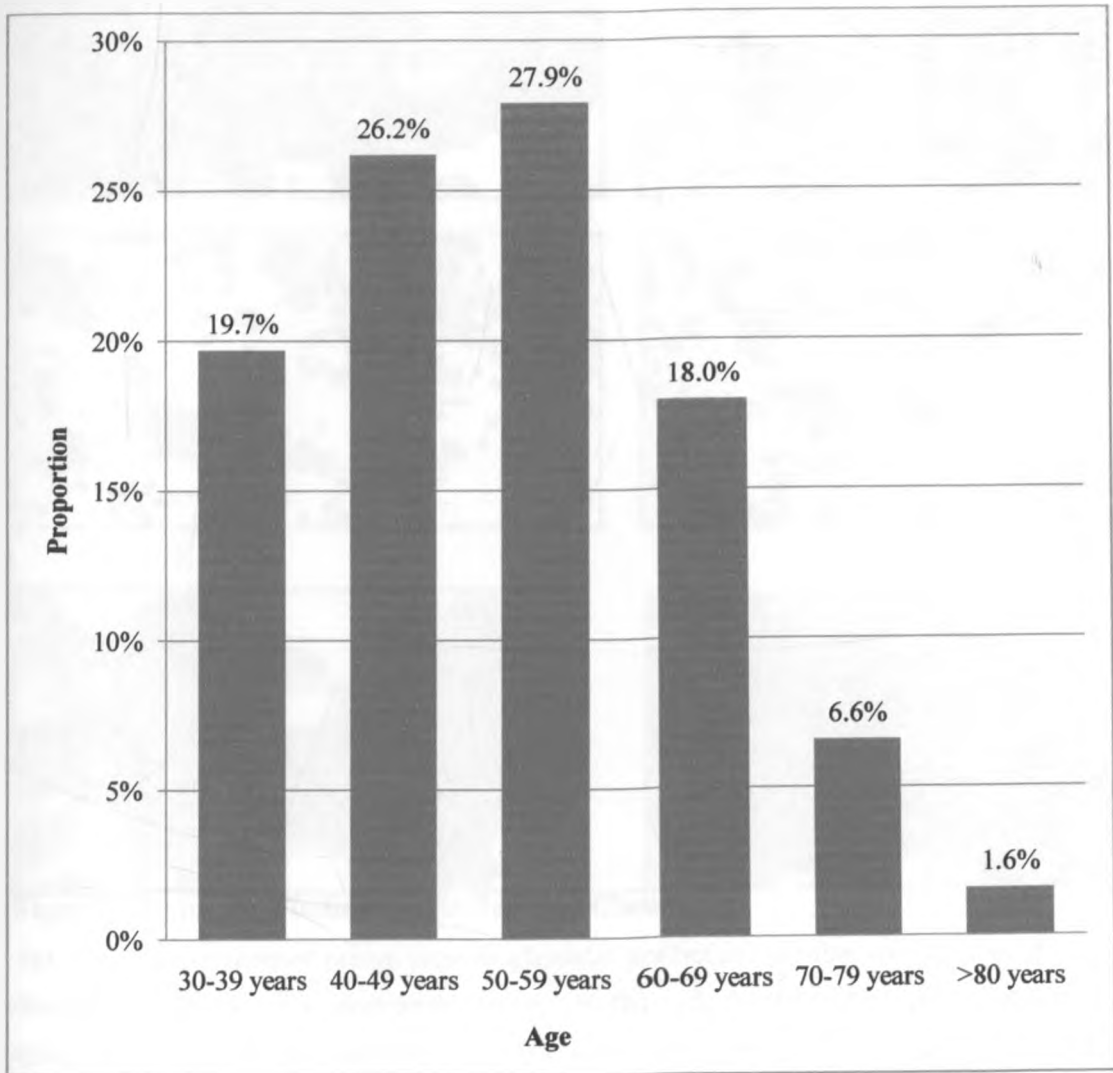
A total of 61 male participants were recruited. All men were of African origin except for one Asian. All imprints were satisfactory for evaluation in terms of specimen adequacy, cellularity, and staining characteristics. The mean age of the subjects was 50.9 years with a median and mode of 50 years, (range 30-80 years). The mean weight of the prostate glands was 29.6g ranging from 17-83g, with a mode of 27g. Only 16.4% of the study participants died of natural causes while Motor Vehicle Accident (MVA) constituted 54.1% of the remaining unnatural deaths (table 3).

**Table 3: Baseline Characteristics**

<b>Characteristic</b>	<b>Statistic</b>	<b>Findings</b>
<b>Age in years</b>	Mean	50.9
	Median/Mode	50.0
<b>Weight of gland in (g)</b>	Mean	29.6 (13.2)
	Range	17 – 83
	Median	26
	Mode	27
<b>Manner of death {n, (Frequency %)}</b>	MVA	33 (54.1)
	Poison	4 ( 6.6)
	Natural	10 (16.4)
	Assault	11 (18.0)
	Murder	2 ( 3.3)
	Burns	1 ( 1.6)

### 3.1.1 Distribution of Age of Decedents

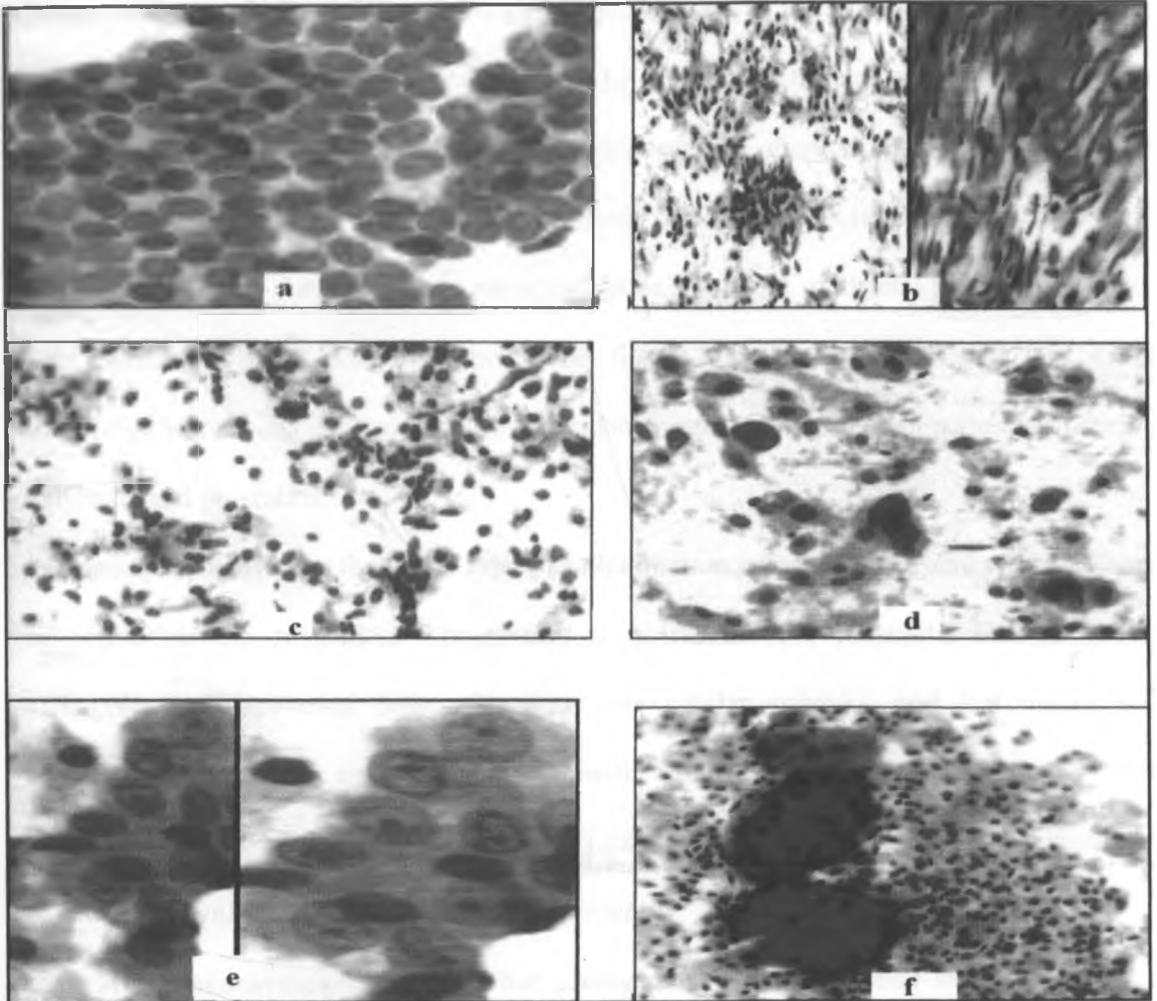
More than half (54.1%) of the study subjects were obtained from age group 40-59 years, many of whom died after MVA. There were difficulties in obtaining men who were above 70 years due to restrictions imposed at the city mortuary. Only 5/61 (8.2%) in this age group were recruited (**figure 1**).



**Figure 1: Age Group Distribution**

### 3.2 Cytomorphology of Conditions of the Prostate at Autopsy

A variety of conditions of the prostate detected on cytology as illustrated in figure 2. These included normal glandular prostate epithelium, prostatic hyperplasia, PIN, and prostatic adenocarcinoma. Other non-specific findings were corpora amylacea, spermatozoa, and proteinaceous material.



**Figure 2: Cytologic Findings of the Prostate Gland.**

(a) A monolayer sheet of benign prostate glandular epithelium showing cohesive ductal cells with distinct cell borders, in a honeycomb pattern, x40 (b) *Left*; Prostatic stromal cells with elongated cytoplasm. *Right*; stromal cells in a prostatic duct (x10). (c) Chronic Prostatitis with abundant lymphocytes. (d) Prostatic carcinoma tumor cells that are markedly hyperchromatic and pleomorphic. (e) PIN showing mildly atypical cells with prominent nucleoli (*Right*; x40, *Left*; x10). (f) Concentrically laminated spherules of corpora amylacea in a case of chronic prostatitis

**Normal prostate** imprints were cellular smears in monolayer sheets of cohesive, ductal cells with distinct cell borders, in honeycomb patterns. The nuclei were uniform in size, round to oval in shape, with smooth nuclear margins and finely granular chromatin. The nucleoli were indistinct with a clean background.

**Prostatic hyperplasia** (BPH and stromal hyperplasia) was characterized by hypercellular smears composed of sheets of benign epithelial cells with normal stromal and glandular elements. Some smears showed coarse intra-cytoplasmic granules but most of them had finely granular chromatin with indistinct nucleoli. The basal cells (akin to the myoepithelial cells seen in breast cytology) were often visible on the edges of sheets. Stromal cells had a pale pink cytoplasm, elongated nuclei and finely granular chromatin that was evenly distributed were seen. Stromal cells were frequently encountered in conjunction with corpora amylacea and prostatitis.

**Chronic prostatitis** was the major type of inflammation encountered. There were two cases of active chronic prostatitis. The smears in active chronic prostatitis had an orderly arrangement of epithelial cells with good intercellular cohesion and features of mild epithelial atypia such as prominent nucleoli. There were many inflammatory cells, lymphocytes, polymorphs and macrophages. Lymphocytic prostatitis was the dominant form of prostatitis with no organisms or bacteria present.

**Prostatic adenocarcinoma** imprints had tumour cells that were either singly dispersed or crowded in small dispersed clusters. There was marked pleomorphism, loss of cohesion and irregular nuclear margins. The nuclei were hyperchromatic with coarse granular or clumped chromatin and very prominent nucleoli (macro nucleoli). Large, single, bizarre nuclei were occasionally seen with very high nuclear:cytoplasmic ratio.

The background had abundant necrotic debris from remnants of inflammation, as well as cytoplasmic and nuclear fragments. The basal cells, which are normally present in the normal prostate were absent in adenocarcinoma.

**Prostatic Intraepithelial Neoplasia** was characterized by cellular smears with some cells in clusters, exhibiting mild to moderate pleomorphism and prominent nucleoli. It was not possible to grade the various grades of PIN on cytology alone.

**Corpora amylacea** were frequently encountered in many touch preparations. They were seen as small hyaline-like masses in various shapes and sizes. Their color ranged from pink to orange in H&E. The presence of concentric laminations was variable but they were visible even at low power magnification.

**Suspicious for malignancy lesions** were those that lacked sufficient cytologic criteria or atypia to establish a definitive malignant diagnosis. The cells were minimally atypical and PIN could not be ruled out. It was difficult to differentiate atrophy from reactive atypia as the lesions were associated with inflammation. Cytologic features such as nuclear enlargement, hyperchromasia, prominent nucleoli, and the absence of basal cells were noted.

There were no cases of the other types of prostatic cancers or squamous metaplasia. Other cellular elements that were seen but were of no diagnostic value were spermatids and erythrocytes.

### 3.3 Distribution of Cytologic Findings of Prostate Gland

In general, the frequency of BPH, prostatitis and PIN in the study population were the same (8.2%). The occurrence of lesions that were classified as suspicious for malignancy and PC constituted 1.6% and 6.6% respectively (Figure 3).

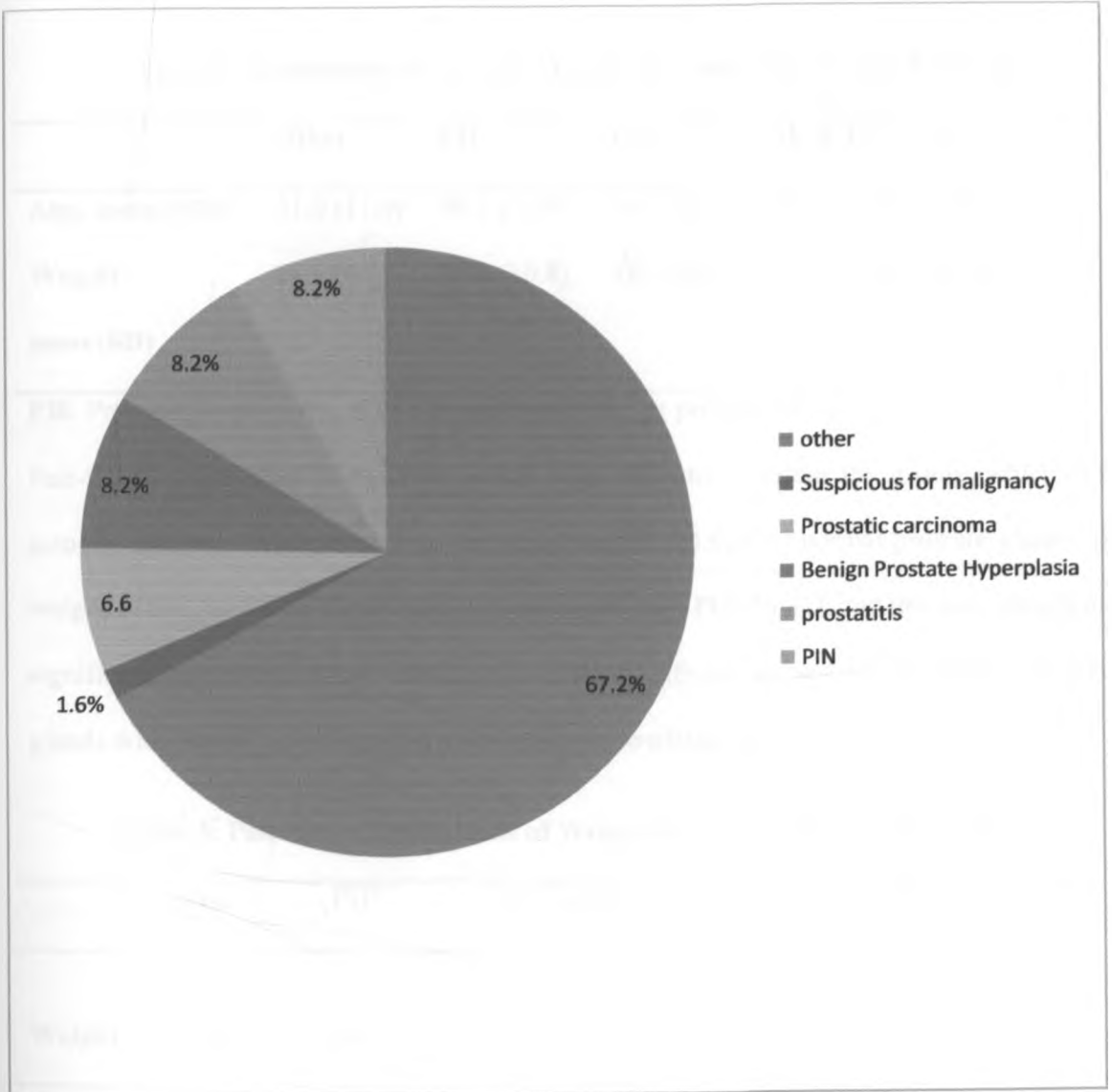


Figure 3: Distribution of Cytologic Findings



### 3.3.1 Association of Age and Weight of Gland with Benign Findings

Age was found not to be significantly associated with all the categories of cases of benign findings ( $p=0.817$ ). However, the association between benign findings and the weight of the prostate gland was statistically significant ( $p=0.001$ ). Prostates with prostatitis and hyperplasia weighed heavier than the normal prostates (table 4).

**Table 4: Association of Age and Weight of Gland with Benign Findings**

	Other	PH	CP	AP & CP	p
Age, mean (SD)	51.0 (11.0)	50.1 (12.9)	56.7 (15.6)	47.5 (17.7)	0.817
Weight mean(SD)	24.9 (6.2)	36.0 (16.8)	28.3 (4.9)	52.5 (31.8)	0.001

PH: Prostatic Hyperplasia, CP: Chronic Prostatitis, p: probability

Pair-wise comparisons of the two means were done to ascertain the groups which were actually different. As compared to the mean weight (24.9g), of normal prostate glands, the weight of the gland was higher among those found with PH (36.0g), and this was statistically significant, ( $p=0.007$ ). There was a statistically significant association, ( $p=0.011$ ) between glands with prostatitis and those with prostatic hyperplasia (table 5).

**Table 5: Pair-wise Comparisons of Weight of Gland with Benign Findings**

	Other <sup>1</sup>	PH <sup>2</sup>	$p^{1 \& 2}$ (t test)	AP & CP <sup>3</sup>	$p^{2 \& 3}$ (t test)
Weight	24.9 (6.2)	36.0 (16.8)	0.007	52.5 (31.8)	0.011

### 3.3.2 Non-Specific Findings

Non-specific findings encountered in this study included corpora amylacea, stromal components, spermatozoa, erythrocytes, amorphous material and proteinaceous secretions. Corpora amylacea and stromal hyperplasia constituted 13.1% and 9.8% of non-specific findings. Statistically, these findings were found to be neither associated with age of the decedent nor weight of the prostate glands ( $p>0.05$ ) were both statistically insignificant.

### 3.3.3 Prostatic Intraepithelial Neoplasia

The only precursor lesion detected microscopically in this study was PIN. There were no cases of focal prostate atypia and atypical adenomatous hyperplasia. It could not be cytologically determined whether the lesions were high grade or low grade. PIN was found in 8.2% of the samples. PIN was found in 8.2 % of the study population but it occurred with a higher frequency in glands that weighed more than 20g which is cut-off weight of a normal prostate gland (table 6). When subjected to a statistical test, ages of decedents and weight of the prostate glands were not significantly associated with cases of PIN;  $p>0.05$  in both cases

**Table 6: Frequency of occurrence of PIN Relative to Weight of Gland**

Weight (g)	Nil	PIN	Total
≤25	30 (96.77%)	1(3.23%)	31(100%)
>25	26(86.67%)	4(13.33%)	30(100%)
<b>Total</b>	56(91.80%)	5(8.20%)	61(100%)

### 3.3.4 Summary of Cytologic Findings by Age Group

A final cytologic diagnosis of negative for malignancy, suspicious for malignancy and prostatic carcinoma was made. Out of the 61 study subjects, 4/61(6.6%) had obviously malignant lesions while 1.6% had lesions that were suspicious but not diagnostic for malignancy. Age and weight of the prostate gland was not statistically associated with final diagnosis, i.e. negative for malignancy, suspicious for malignancy or prostatic carcinoma;  $p=0.444$  and  $p=0.554$

All the findings were analysed together against different age groups and the distribution of all the findings were illustrated. This study found that prostatic carcinoma and PIN occur in men at an early age (46 and 38 years respectively). All the afore-mentioned findings (PC, PIN, hyperplasia, corpora amylacea, and normal) were detected in 40-59 years age group. The normal prostates were found in highest proportion (64%) among the 60-69 year old men but no normal prostates were found in the age group 80-89 year as illustrated in figure 4 below.

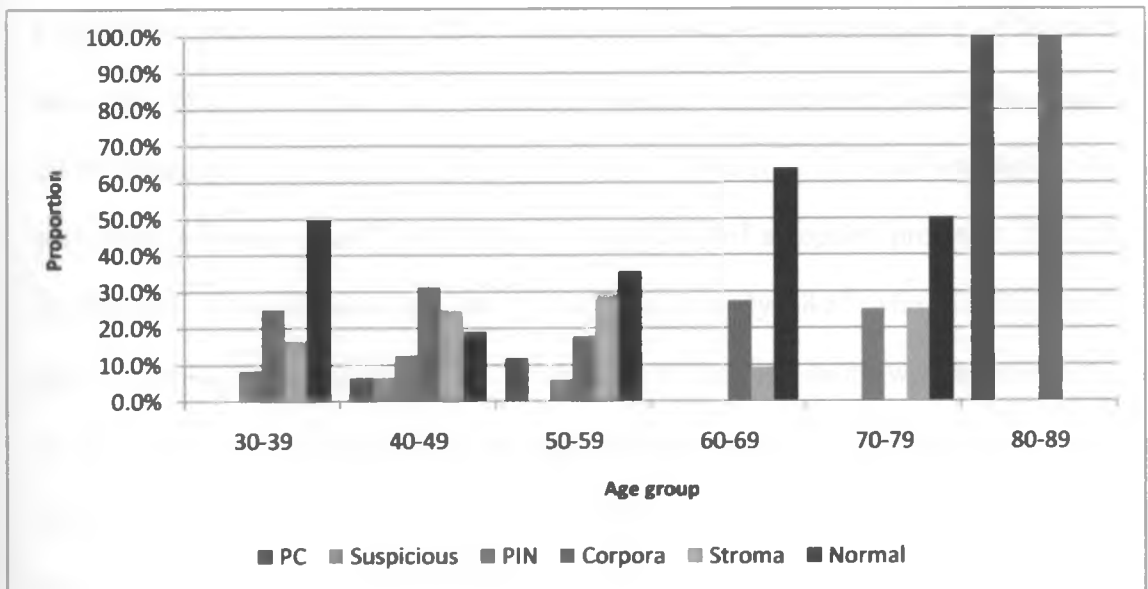


Figure 4: Overall Proportions of Cytological Findings per Age Group

## CHAPTER 4: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

### 4.1. Discussion

The incidence and mortality of prostatic carcinoma are increasing at alarming rates globally. Several autopsy studies have been used to evaluate the prevalence and incidence of prostatic carcinoma among men of various ages, races, and in distinct geographic areas.<sup>44-47</sup> The use of cytologic preparations in autopsy diagnosis is simple and has the potential for major cost savings because the basic tools required are additional glass slides and relevant routine stains only. In contrast, expensive processing equipment required for histological sample processing.<sup>11,38</sup> This study investigated prostate cytology, by touch imprint method, of 61 decedents aged between 30-80 years and analysed them against baseline cytomorphologic characteristics.

#### 4.1.1 Benign Conditions of the Prostate

Cytological BPH was the most common benign lesion of the prostate gland, constituting 8.2% of the study population. Other researchers have found a frequency of BPH ranging from 5%-76%.<sup>25,36,45</sup> The 8.2% frequency found in this study was within the range of 5-20.5% frequency of prostatic hyperplasia found by Bharat *et al*<sup>25</sup> but was lower than that with Delongchamps *et al*.<sup>46</sup> who found BPH in 29% of autopsied prostates. This autopsy finding was far much lower than that found Ngugi and Byakika<sup>36</sup> who found a histologic BPH frequency of 76% in patients of a hospital in Kenya. Prostatitis was detected in 8.2% of all the cases, 3.3% of which was chronic prostatitis and the remaining 4.9% constituted chronic active prostatitis. This frequency is in the range described by Delongchamps *et al*<sup>46</sup> reported a 4-53% occurrence of prostatitis.

Several population-based cross-sectional surveys and series of clinical data have shown that the prevalence of prostatitis in a given population may be up to 25% whereas the prevalence based only on questionnaires and physicians' diagnoses is reported to be between 4% and 11%.<sup>48-51</sup> There was no significant association ( $p=0.827$ ) between age and all the categories of benign findings (prostatic hyperplasia and prostatitis). However, pair-wise statistical comparisons of the weight of normal prostate and benign findings revealed a significant association ( $p=0.007$  and  $p=0.011$  respectively) between the weight of normal prostates (24.9g), BPH (36.0g) or prostatitis (52.5g). Delongchamps *et al*<sup>55</sup> also observed a similar association between hyperplasia and prostatitis.

The prostates with hyperplasia and prostatitis were heavier than those with normal findings because hyperplasia causes both glandular and stromal enlargement of the gland resulting in an increase in weight of the gland beyond the 20g limit regarded as normal in a healthy adult male individual.<sup>22</sup>

#### **4.1.2 Prostatic Intraepithelial Neoplasia**

Prostatic intraepithelial neoplasia was detected in the study population towards the end of the 4th decade, at 38 years of age. PIN was more frequent in the prostates of men aged between 70 and 79 years, 1/4 (25%) than in the prostates of 50-59 year old men 1/17(5.88%). PIN was also found to be more frequent in glands weighing more than 20g, (probably due to hypertrophy) than those that weighed less than 20g (3.2%). This trend is similar to that found by Stomatiou *et al*<sup>52</sup> in an age specific autopsy study ( $n=212$ , age range 50-79 years). PIN was found to be less frequent, 8/38(21%) in 50-59 years than in 70-79 year age group 17/36 (47.2%). The overall frequency of PIN was 8.2% which was just below the upper limit of that found by Bostwick *et al*<sup>53</sup> who described PIN in 9%-13.5% in autopsy prostates.

The finding was, however, slightly higher than the frequency of 5.2% (n=330) described by Mettilin *et al*<sup>54</sup>. Age and weight of the prostate gland were not significantly associated ( $p>0.05$ ) with cytological detection of PIN ( $p=0.735$  and  $p=0.442$  respectively). These findings contrast earlier views by Cotran *et al*<sup>55</sup> who wrote that prostatic carcinoma is only 1% in the United States of America before age 50 years. This study has shown that lesions of PIN manifested at the early age of 38 years. These would probably have developed in high grade lesions of the prostate in later life or even in late 40s if the subjects had survived. Therefore, prostatic carcinoma should no longer be viewed as a disease of the aged population because it has been established by Harvei *et al*<sup>56</sup> that PIN is a precursor of carcinoma.

#### **4.1.3 Malignancy**

Malignancy (prostatic adenocarcinoma) was detected in 6.6% of the autopsy prostates. Statistical observations in this study showed no association between age and weight of gland with the detection of malignancy ( $p=0.444$  and  $p=0.554$ ). Statistically significant associations were observed by Haas *et al*<sup>44</sup> ( $p<0.01$ ) between tumour detection and age in a needle biopsy autopsy (n=164) of decedents with no history of PC.

This finding was 6.1 times higher than what Mbuuko *et al*,<sup>39</sup> (2007) found in a histological study at the city mortuary, Nairobi. It should not be misconstrued to indicate that TIC is six times more sensitive than histology but instead the age factor should be considered. A possible explanation is that Mbuuko sample included men who were 20 years old. The vast majority of his study population was < 40 years (61.2%).

The overall occurrence of latent prostatic carcinoma in the current study was lower than the 26% found by Mostoffi and Davis, 2002<sup>41</sup> in an autopsy study.

Two other studies<sup>32,36</sup> both with n=194 found higher occurrences of prostatic carcinoma. Thompson *et al*<sup>31</sup> found 11% occurrence of PC. A study by Ngugi and Byakika<sup>36</sup> in Kenya, found a histology-FNA prevalence of 26%. Several international studies have shown higher age specific prevalence rates than those observed in this study, especially among men aged 50–70 years.<sup>41,45-49, 53</sup>

This discrepancy may be attributed to the magnitude of some of these studies, such as one with n=1.3m, as accuracy increases when sample size is increased.<sup>53,54</sup> In addition, the thickness of the step sections during histological processing varies from 2-5mm in autopsy studies. Such thin step sections increase the possibility of detecting of small tumours.<sup>44</sup> The relatively thick sections which ranged 5-25mm, (1 section for every 10g of gland weight), used in this study may have negatively influenced detection of very small tumours.

The early occurrence of PIN in the Kenyan male population may explain the corresponding early detection of PC, at 46 years of age. Decedents aged above 70 years (8%) were not readily available during the study period because at the city mortuary, there was a provision for relatives request for a waiver of autopsy.

This provision was taken advantage of by relatives of the elderly deceased. This provision resulted in a shortage of elderly men which in turn may have influenced detection rates of PC because studies have shown that detection of PC increases with advancing age among the elderly men.<sup>1,57-59</sup> A study done in Pakistan by Ahmed and Muzaffer<sup>58</sup> (n=2708, mean age 70 years), found an incidence 14.2% of the population.

#### 4.1.4 Non-specific Findings

Corpora amylacea were present in prostates of approximately 13.1% of the study population. This is within the range of the 5-13% incidence found by Christian *et al*<sup>60</sup> involving transurethral resection specimens.

#### 4.1.5 Suspicious for Malignancy

Prostates found with lesions in the 'suspicious for malignancy' category were 1.6% of the study population, a finding that falls in the range 1.5%-2.5% observed by Bostwick and Meiers.<sup>59</sup>

Although lesions that are suspicious for malignancy are sometimes difficult to categorize by other cytologic preparations such as FNAC, a fraction of these lesions were detected in this study. This was possible because the TIC method of preparing smears allows the cells to be viewed in roughly the same orientation as they appear in the body.<sup>11,12,37</sup> Since there was no smear or crash artifact that usually disrupt cellular arrangement as in FNAC, subtle changes that were more than PIN but equivocal for malignancy could therefore be classified as "suspicious for malignancy".

Urologists prefer the term atypical small acinar proliferations (ASAP) in describing lesions suspicious for malignancy in prostate pathology because they are an important predictor of carcinoma.<sup>61</sup> Bostwick and Meiers<sup>59</sup> confirmed, by histology, the presence of prostatic carcinoma, in 60% of ASAP while Deiter *et al*<sup>62</sup> found that these lesions had a 92% sensitivity in prediction of carcinoma. Such studies indicate that lesions that are suspicious for malignancy are important predictor of prostatic carcinoma.



#### 4.1.6 Ancillary Testing

There is need to gain maximum diagnostic information from lesions described in this study by use of special stains and immunocytochemistry techniques with appropriate antibodies.<sup>63</sup>

This is because in neoplastic conditions the complex secretory mechanism of the prostate is severely altered and this can be evidenced through morphologic, histochemical and immunohistochemical techniques. Benign conditions of the prostate may stimulate the basal cell layer but in a discontinuous fashion and in ASAP, it may be absent or distorted.

Immuno-histochemical stains can also be important in differential diagnosis of other lesions. For instance, the glandular component of the prostate is composed of acini and ducts which contains secretory, neuroendocrine and basal cells. The luminal side of the gland is the site of secretory cells which produce PAP and PSA. These products are of great diagnostic utility due to being organ-specific, and can be readily identified immunohistochemically.<sup>22</sup>

Basal cells contain keratins: 34 $\beta$  E12, cytokeratin (CK) 8,12, 312C8 and stain strongly for antikeratin antibody 903. Under normal circumstances, basal cells lack immune-reactivity for S-100 protein or smooth muscle actin.

Other prostate markers are: prostate membrane specific antigen, low molecular weight cytokeratins e.g. CK7 and CK20, carcinoembryonic antigen, epithelial membrane antigen, androgen and progesterone receptors which are all positive for PC. The normal prostate secretes neutral mucins; therefore, prostatic carcinoma is negative for mucicarmine. Molecular markers used in confirming diagnosis of prostate neoplasms include E-catherin, p53, and kallikren-2. The luminal content secretes acid mucins which produce a blue tinge in alcian blue or colloidal iron.<sup>22,34</sup>

## **4.2. Conclusion**

This study has shown that TIC is a simple procedure in autopsy that can be valuable in autopsy studies. The method demonstrated that malignant lesions, PIN and prostatitis were evenly distributed in a section of the Kenyan male population irrespective of individual age. Touch imprint cytology may, therefore, be applied to a variety of tissue types for detection of tumours. As the cost of autopsy services continues to rise drastically, TIC method is likely to be of particular value in Kenya's district level hospital settings which lack histology facilities due to the high cost of operating and setting up such facilities. Nonetheless, the continued role for histological sampling in autopsy diagnosis remains crucial as cases which are inconclusive need to be resolved by histology. However, assessment by touch imprint cytology may be utilized to facilitate preliminary diagnosis of tumours during autopsy and intra-operative surgical procedures.

## **4.3 Study Limitations**

- City mortuary does not routinely perform autopsy examinations on warm decedents and the refrigeration conditions were sub-optimal. A degree of microscopic autolysis may have affected cellular morphology.
- Data on imprint cytology autopsy studies were scarce locally resulting in unfair and subjective comparisons with data from very different settings in international studies some of which had large sample size.
- Assumptions were made as to pre-demise exposure to risk factors as a detailed clinical history was not available.
- The category of "suspicious for malignancy" remains a gray area in the cytology report and there is need for immuno-staining to confirm lesions detected in this study.

#### 4.4 Recommendations

- District level hospital facilities need to consider adopting imprint cytology as valuable and inexpensive tool in autopsy diagnosis of prostate tumours for epidemiological purposes with a view of putting control measures in place.
- Touch imprint cytology is recommended for use in intra-operative surgical procedures for preliminary detection of tumours in a variety of organs due to its reliability and simplicity.
- Due to scarcity of data on prostate cancer epidemiology in Kenya, a larger country-wide study, with complete cyto-histological correlation, immuno-cytochemistry and detailed clinical data, taking into account previous exposure to pre-disposing factors is recommended, to provide more information on the epidemiology of this disease in Kenya.

## REFERENCES

1. **Yin, M., Bastacky, S., Chnadran, S., Becich, M.J. and Dhir, R.** Prevalence of incidental prostatic carcinoma in the general population: A study of healthy organ donors. *Journal of Urology*. 2008;179:892-895
2. **Ferlay, J., Bray, F., Sankila, R. and Parkin, D.M.** Cancer incidence, mortality and prevalence worldwide. *International Agency for Research on Cancer Press*. 2004. No. 5 Version 2 (online-accessed 05/04/2010)
3. **Fernandes, H., Naik, C., Marla, N.T. and Arora, D.** Supraclavicular lymphadenopathy - Initial manifestation of prostatic carcinoma. *Journal of Cytology* 2007;24(3):157-158
4. **Huxley, R.** Asia Pacific Cohort Studies Collaboration. The impact of modifiable risk factors on mortality from prostate cancer in populations of the Asia-Pacific region. *Asian Pacific Journal of Cancer Prevalence*. 2007;8(2):199-205.
5. **Damjanov, I., Linder, J. and Anderson, W.A.D.** Anderson's Pathology. 10<sup>th</sup> Edition Vol II. Mosby Elsevier, St Louis Missouri. 2009. Chapter 67 pp2197-2230
6. <http://library.med.utah.edu/WebPath>. Pathology tutorial of prostate tutorial (Accessed on 08/03/2010)
7. **De May, R.** The art and science of Cytopathology. Vol II. American Society of Clinical Pathologist press, Chicago. 1996;27 pp1127-1146
8. **Kim, H.L and Beldegrum, A.** Prostate. In; Schwartz's Principals of Surgery. 8<sup>th</sup> Edition. McGraw-Hill. New York. 2005 pp1528-1535
9. **Muchiri, L.W., Penner, D.W., Adwok, J. and Rana, F.S.** Role of Fine Needle Aspiration in the Diagnosis of Neoplasms. *East African Medical Journal*. 2001;78(5):225-226

10. **Eichler, K., Hempel, S., Wilby, J., Myers, L., Bachmann, L.M and Kleijnen, J.** Diagnostic value of systematic biopsy methods in the investigation of prostate cancer: a systematic review. *Journal of Urology* 2006;175:1605–12
11. **Shirley, S.E. and Escoffery, C.T.** Usefulness of Touch Preparation Cytology in Autopsy Diagnosis: A Study from the University Hospital of the West Indies. *The Internet Journal of Pathology*. 2005; vol 3 No. 2. Available online at <http://www.ispub.com/internet> Accessed 13th March, 2010.
12. **Liu, Y., Silverman, J.F., Sturgis, C.D., Brown, H.G., Dabbs, D.J and Raab, S.S.** Utility of Intra-operative Consultation Touch Preparations. *Diagnostic Cytopathology*. 2002;26:329-333
13. **Albertsen, P.C.** Controversies in the treatment of prostate cancer. *New England Journal of Medicine* 2009;360(3):311-312
14. **Rubin, M.A.** Targeted Therapy of Cancer: New Roles for Pathologists- Prostate Cancer. *Modern Pathology*. 2008;21(2):S44-55
15. **Epstein, J.I., Allsbrook, W.C. Jr. and Amin, M.B.** The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. *American Journal of Surgical Pathology*. 2005; 29(9):1228-1242.
16. **Magoha, G.A.O.** Management and survival in advanced prostate cancer in Nairobi. *East African Medical Journal*. 2000;77(5):260-263
17. **Tenge, C.N., Kuremu, R.T., Buziba, N.G., Patel, K. and Were, P.A.** The burden & pattern of cancer in western Kenya. *East African Medical Journal*. 2009;86(1):1-9
18. **Tremont-Lukats, I.W., Bobustuc, G., Lagos, G.K, Lolas, K., Kyritsis, A.P. and Pudevalli, V.K.** Brain metastasis from prostatic carcinoma. *Cancer* 2003;98:363–368.

19. **Tu, S.M., Reyes, A., Maa, A., Bhowmick, D., Pisters, L.L. and Pettaway, C.A., et al.** Prostatic carcinoma with testicular or penile metastases: clinical, pathologic, and immune-histochemical features. *Cancer*. 2002;94:2610–2617
20. **Delongchamps, N.B., Singh, A. and Haas, G.P.** The role of prevalence in the diagnosis of prostate cancer. *Cancer Control*. 2006;13(3):158-68
21. **Wasike, R.W. and Magoha, G.A.** Descriptive case series of patients presenting with cancer of the prostate and their management at KNH, Nairobi. *East African Medical Journal*. 2007;84 (9):S31-5
22. **Rosai, J.** Rosai and Ackerman's Surgical Pathology. Vol II. 9<sup>th</sup> Edition. Mosby Elsevier, St Louis Missouri. 2009. Chapter 18 pp 1361-1411
23. **Nelson, W.G., De Marzo, A. and Isaacs, W.B.** Prostate Cancer. *New England Journal of Medicine* 2004;349(4):366-381
24. **Midi, A., Tecimer, T., Bozkurt, S. and Ozkan, N.** Differences in the structural features of atypical adenomatous hyperplasia and low-grade prostatic adenocarcinoma. *Indian Journal of Urology* 2008;24:169-77
25. **Rekhi, B., Jaswal, T.S. and Arora, B.** Premalignant lesions of prostate and their association with nodular hyperplasia and carcinoma prostate. *Indian Journal of Cancer* 2004;41:60-5
26. **Montironi, R., Mazzucchelli, R., Lopez-Beltran, A., Cheng, L. and Scarpelli, M.** High-Grade Prostatic Intraepithelial Neoplasia and Other Proposed Pre-neoplastic Lesions in the Prostate. *National Clinical Practice in Urology*. 2007;4(6):321-332
27. **Herzeberg, A.J., Raso, D.S. and Silverman, J.F.** Color Atlas of Normal Cytology. 1<sup>st</sup> Edition. Churchill Livingstone, Michigan. 1999. Chapter 11 pp 377-380.

28. **Gittes, R.F.** Carcinoma of the prostate. *East African Medical Journal*. 1994;324:236-245
29. **Mazzucchelli, R., Barbisan, F., Scarpelli, M., Lopez-Beltran, A., van der Kwast, T.H, Cheng, L. and Montironi, R.** Is Incidentally Detected Prostate Cancer in Patients Undergoing Radical Cystoprostatectomy Clinically Significant? *American Journal of Clinical Pathology*. 2009;131(2):279-283
30. **Iguchi, T., Ching Y.W, Delongchamps, N.B., Sunheimer, R., Nakatani, T., de la Roza, G. and Haas, G.P.** Occult Prostate Cancer Affects the Results of Case Control Studies Due to Verification Bias. *Anticancer Research* 2008;28:3007-3010
31. **Yamamoto, T., Ito, K., Ohi, M. and Kubota, Y.** Diagnostic significance of digital rectal examination and transrectal ultrasonography in men with prostate-specific antigen levels of 4 NG/ML or less. *Urology*. 2001; 58(6): 994-8.
32. **Thompson, I.M., Goodman, P.J., Tangen, C.M., Lucia, M.S., Miller, G.J, Ford, L.G. et al.** The influence of finasteride on the development of prostate cancer. *New England Journal of Medicine*. 2003; 349: 215-224.
33. **Terris, M.K., Rhee, A. and Qureshi, S.M.** Prostate Cancer - Metastatic and Advanced Disease. Differential diagnoses and work up. <http://emedicine.medscape.com>. (Accessed on 17<sup>th</sup> March, 2010)
34. **Chitale, A. and Khubchandani, S.** Interpretation of prostatic biopsies: A review (for practicing urologists). *Indian Journal of Urology*. 2004;20:3-10
35. **Javois, L.C** Immuno-cytochemical methods and protocols. Vol 34. Humana press, New York.2005 pp 75-77. Online ; Accessed, 03/02/2010
36. **Ngugi, P.M. and Byakika, B.** Histology of specimens taken by prostatectomy and needle biopsy. *East African Medical Journal*. 2007;84(8):363-366

45. **Delongchamps, N.B., De la Roza, G., Chandan, V., Jones, R., Sunheimer, R., Gregory, T., Jumbelic, M. and Haas, G.P.** Evaluation of prostatitis in autopsy specimens; Is chronic inflammation more associated with benign prostate hyperplasia or carcinoma? *Journal of Urology* 2008;179(5):1736-1740
46. **Delongchamps, N.B., De la Roza, G., Jones, R., Jumbelic, M. and Haas, G.P.** Saturation biopsies on autopsied prostates for detecting and characterizing prostatic carcinoma. *International Brazilian Journal of Urology* 2009;103(1): 49-54
47. **Barros, M.S., Silva, V.R/S., Santos, G.B., Hughes, A. and Silveira, M.A.** Prevalence of prostate adenocarcinoma according to race in a university hospital. *International Brazilian Journal of urology*. 2003;29(4):306-312.
48. **Moon, T.D.** Questionnaire survey of urologists and care physician's diagnostic and treatment practices for prostatitis. *Urology*. 1997; 50: 543–547.
49. **Roberts, R.O., Lieber, M.M., Rhodes, T., Girman, C.J., Bostwick, D.G. and Jacobsen, S.J.** Prevalence of a physician-assigned diagnosis of prostatitis: the Olmsted County study of urinary symptoms and health status among men. *Urology*. 1998; 51: 578–584.
50. **Pavone, C., Calderera, E., Liberti, P., Miceli, V., DiTrapani, D., Serretta, V., Porcu, M. and Pavone-Macaluso, M.** Correlation between chronic prostatitis syndrome and pelvic venous disease. *European Urology*. 2000; 37: 400–403.
51. **Nickel, J.C., Downey, J., Hunter, D and Clark, J.** Prevalence of prostatitis-like symptoms in population based study using the National Institutes of Health chronic prostatitis symptom index. *Journal of Urology*.2001;165: 842–845



52. **Stamatiou, K., Alevizos, E., Agapitos, F and Sofras, F.** Incidence of impalpable carcinoma of the prostate and of non-malignant and pre-carcinomatous lesions in Greek male population: An autopsy study. *The Prostate*. 2006. 66;(12): 10319–1328.
53. **Bostwick, D.G.,** HGPIN-The most likely precursor of prostatic carcinoma. *Cancer*.1995;75:1823-1836
54. **Mettillin C.** American cancer detection project and national patterns of prostate cancer detection and treatment. *Cancer Journal for Clinicians*. 1997; 47;265-272
55. **Cotran, R.S., Kumar, V. and Collins, T.** Robins pathologic Basis of disease. 6th Edition. 2006. Saunders, Philadelphia. Chapter 23:1011-34
56. **Harvei, S., Skjorten, F.G., Robsahm, T.E., Berner, A. and Treti, S.** Is PIN in the transition zone, a true precursor of prostatic carcinoma? A long term retrospective study in Norway. *British Journal of cancer*. 1998;78(1):46-49.
57. **Aydin, O., Cosar, E.F. and Varinli, S., et al.** Prostatic Intraepithelial Neoplasia in prostate specimens-a retrospective study of 121 cases. *International Journal of Urology and Nephrology*.1999;31:687-697
58. **Ahmed, Z. and Muzaffer, S.** Prostatic carcinoma with an emphasis on Gleason's grading; an institution-based experience. *Journal of Pakistan Medical Association (JPMA)*. 2002;52(2):54-56
59. **Bostwick, D.G. and Meiers, I.** Atypical Small Acinar Proliferations; Clinical Significance. *Archives of Pathology and Laboratory Medicine*. 2006;130(7):952-957.
60. **Christian, J.D., Lamm, T.C., Morrow, J.F. and Bostwick D.G.** Corpora amylacea in the prostate: incidence and histology within needle core biopsies. *Modern Pathology*. 2005; 18: 36–39

61. **Montoroni, R., Scattoni, V., Mazucheli, R., Lopez-Betran, A., Bostwick, D.G. and Montosi, F.** Atypical foci, suspicious but not diagnostic for malignancy in prostate needle biopsies. *Urology*.2006;50(4):666-674.
62. **Deiter, S.W., Constantinople, N.L., Bresette J.F., Spence, I.J. and Reicert, C.M.** Cytologic diagnosis of prostatic carcinoma by FNA biopsy; a community hospital experience. *Urology*. 1998;3(3):255-259
63. **Iczkowzi, K.A., Bassier, T.J., Schwob, V.S., Bassier, I.C., Kunnei, B.S., Orozco, R.E. and Bostwick, D.G.** Diagnosis for 'suspicious for malignancy' in prostatic biopsies; predictive value for cancer. *Urology*. 1998;51(5):748-758.

## APPENDIX 1: TNM STAGING FOR PROSTATE TUMOURS

Stage T1-T2c - Organ-confined disease

Stage T3a – Extra-capsular extension of the tumour

Stage T3b - Invasion of the seminal vesicle(s)

Stage T4 - Tumour fixed or tumour invading adjacent structures other than seminal vesicles (e.g., bladder neck, external sphincter, rectum, levator muscles, and/or pelvic floor)

Stage NX - Regional lymph nodes cannot be assessed

Stage N0 - No regional lymph node metastasis

Stage N1 - Regional lymph node(s) metastasis

Stage MX - Distant metastasis cannot be assessed.

Stage M0 - No distant metastasis

Stage M1 - Distant metastasis

Stage M1a - Distant metastasis other than regional lymph nodes

Stage M1b - Metastasis to bone(s)

Stage M1c - Other site(s)

Stage pM1c - Metastasis to more than 1 site





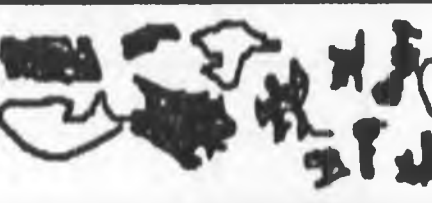

The stage D Includes subset of hormone-insensitive PC patients:

Stage D1 - Involvement of pelvic lymph nodes

Stage D1.5 - Rising PSA level after failure of local therapy (i.e., biochemical failure)

Stage D2 - Metastatic disease to bone and other organs

APPENDIX 2: GLEASON GRADING SYSTEM

Gleason Scale		Well differentiated
	1 Small, uniform glands	
	2 More space between glands	
	3 Infiltration of cells from glands at margins	
	4 Irregular masses of cells with few glands	
	5 Lack of glands, sheets of cells	

Gleason Score (GS) equals the 1<sup>st</sup> most common pattern plus the 2<sup>nd</sup> most common pattern.  
GS 2-5; Well differentiated, GS 6-7; Moderately differentiated and GS 8-10; Poorly differentiated

**APPENDIX 3: DATA COLLECTION SHEET**

S/N	Age	Weight	M.O.D	Cytologic Findings			
				Benign Findings	Precursor Lesions	Non-specific Findings	Final Diagnosis
			MVA..1				
			Murder..2				
			Natural..3	Normal—0	Nil—0		NM—0
			Burns ..4	PH—1	PIN—1	Nil—0	SM—1
				AP—2	AAH--2	SC—1	PC—2
				CP —3	FPA--3	CA—2	
				GP—4		PS—3	
					SqM—4		

## APPENDIX 4: TOUCH IMPRINT TECHNIQUE

### METHOD OF OBTAINING TOUCH IMPRINT FROM PROSTATE GLAND

1. Weigh intact prostate gland and record weight
2. Divide the total weight by 10 to determine the number of sections to be made
3. Section the prostate perpendicular to the long axis of the urethra
4. Gently scrap off excess secretions using a clean slide.
5. Press a labeled slide on the exposed tissue surface, avoiding sliding or gliding motion, and lift up the slide
6. Fix immediately ethanol
7. Repeat 4 and 5 until all exposed surfaces for each gland are exhausted
8. Submit to the cytology laboratory for processing

## APPENDIX 5: HARRIS H&E STAINING TECHNIQUE

### **Principal of the stain**

The mordant forms a lake on the tissue. It is on the lake that the stain gets attached thus colouring the cell nuclei. The nuclei having an affinity for the basic radical in the dye retains the colour even after treatment with 1% acid alcohol. Eosin stains the cytoplasm as a counter stain

### **Staining technique**

1. Bring section to water
2. Stain in Harris haematoxylin for 5 minutes
3. Rinse in tap water
4. Differentiate in 1% acid alcohol, 3 dips
5. Rinse in tap water
6. Blue in Scotts tap water for 30 seconds or in running tap water for 10 minutes
7. Counter stain in Eosin for 5 minutes
8. Rinse in tap water to remove excess eosin followed by 70% ethanol to obtain the desired shades of red and pink.
9. Dehydrate in the 3 changes of absolute alcohol
10. Clear in 3 changes of Xylene
11. Mount with D.P.X

**APPENDIX 6: CYTOLOGY REPORT FORM**

**Touch imprint Preparation**

S/No..... Age.....

**Autopsy prostatectomy**

Prostate measuring.....

Weight.....

**Microscopy**

.....  
.....  
.....  
.....  
.....  
.....  
.....  
.....

**Diagnosis**

.....  
.....  
.....

**Comment**

.....  
.....  
.....  
.....

Signature.....

Date.....



## APPENDIX 7: CYTODIAGNOSTIC CRITERIA

### **Normal Prostatic Epithelium**

Small groups of cells and monolayer sheets

Columnar and basal nuclei

Round to oval uniformly sized cells

Pale, finely vacuolated granular cytoplasm

Smooth nuclear membranes

Evenly distributed chromatin

Inconspicuous small nucleoli

Acinar structures

### **Benign prostatic hyperplasia**

Monolayered sheets

Cohesive honeycombed

Bland nuclei

Small round fine chromatin

Absent or inconspicuous nucleoli

Monolayered sheets

Cohesive honeycombed

Bland nuclei

Small round fine chromatin

Absent or inconspicuous nucleoli

### **Prostatic Carcinoma**

Cellular smear

Nuclear enlargement

Prominent nucleoli

Microacinar complexes

Dyshesion and single cells

### **Prostatic Intra-epithelial Neoplasia**

Cohesive crowded cells

Increased nuclear size

Slightly hyperchromatic

Conspicuous nucleoli

### **Prostatitis**

Decreased dissociation of epithelial sheets

Many inflammatory cells- polymorphs, lymphocytes, and macrophages

Mild epithelial atypia

Mild irregularity of cell membranes

Slightly enlarged nuclei , variable sizes

No cytoplasmic granules

Degenerative changes such as vacuolations

### **Suspicious for Malignancy ( i.e ASAP)**

Nuclear Enlargement

Prominent nucleoli

Hyperchromasia

Cytoplasmic amphophilia

Pink dense secretions

Basophilic mucinous secretions

Crystalloids

Inflammation

Adjacent PIN

## APPENDIX 8: PREPARATION OF REAGENTS

### To prepare 2000mls Harris Alum Hematoxylin

1.	Hematoxylin	10gm
2.	Absolute alcohol	200ml
3.	Ammonium alum	200gm
4.	Distilled water	2000ml
5.	Mercuric oxide	6gm
6.	Glacial acetic acid	80ml

### Methodology

1. Dissolve Hematoxylin in absolute alcohol (Solution 1).
2. Dissolve Ammonium alum in water (Solution 2).
3. Mix solution 1 and solution 2 and heat to boil.
4. Add mercuric oxide and cool rapidly.
5. Add glacial acetic acid.
6. Solution is ready for use as soon as it cools.
7. Filter before use.

### To prepare 4 litres of E.A.36

1.	Light green	2gm
2.	Bismark brown	2gm
3.	Eosin yellow	10gm
4.	Phosphotungstic acid	8gm
5.	Distilled water	1200ml
6.	Absolute ethanol	2800ml
7.	Glacial acetic acid	40ml

### **Method of Preparation of EA 36**

1. Dissolve light green then Bismarck brown then eosin yellow into water.
2. Add phosphotungstic acid slowly in stages while agitating
3. Add alcohol slowly while agitating
4. Add glacial acetic acid then agitate (pH should be 4.5-5 for maximum results).

### **To prepare 1 litre of 0.05% acid alcohol**

- |    |                 |         |
|----|-----------------|---------|
| 1. | Distilled water | 999.5ml |
| 2. | Conc.HCL        | 0.5ml   |

### **To prepare 1000ml Scotts tap water**

- |    |                    |          |
|----|--------------------|----------|
| 1. | Sodium bicarbonate | 3.5gm    |
| 2. | Magnesium sulphate | 20gm     |
| 3. | Distilled water    | 1000ml   |
| 4. | Thymol             | 1 tablet |

### **Quality Check**

1. Stains were stored in dark coloured, stoppered bottles.
2. Fresh amounts of filtered Haematoxylin were added to replace stain lost to evaporation.
3. Water rinses were done under running tap water.
4. Alcohol was replaced on a rotating basis
5. Xylene was changed as soon as it becomes tinted with any of the cytoplasmic stains or becomes slightly milky due to presence of water.
6. Agitation of the slides was done to remove excess dye.
7. Dipping was done gently to avoid cell loss and the slide carrier will not hit the bottom of the staining dish.

## APPENDIX 9: APPROVAL BY ERC UoN/KNH



### KENYATTA NATIONAL HOSPITAL

Hospital Rd along Ngong Rd  
P O Box 20723 Nairobi  
Tel 726300-9  
Fax 725272  
Telegrams MEDSUP Nairobi  
Email [KNHplan@KenHealthnet.org](mailto:KNHplan@KenHealthnet.org)  
16<sup>th</sup> June 2010

Ref KNH-ERC/ A/509

Gladys Esendi Change  
Dept of Human Pathology  
School of Medicine  
University of Nairobi

Dear Ms Change

#### RESEARCH PROPOSAL: "CYTOLOGIC FINDINGS OF THE PROSTATE GLAND AT AUTOPSY USING TOUCH IMPRINT CYTOLOGY" (P153/5/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above cited research proposal for the period 16<sup>th</sup> June 2010 to 15<sup>th</sup> June 2011

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

**PROF A N GUANTAI**  
**SECRETARY, KNH/UON-ERC**

c c Prof K M Bhatt, Chairperson, KNH/UON-ERC  
The Deputy Director CS, KNH  
The Dean, School of Medicine, UON  
The Chairman, Dept of Human Pathology, UON  
The HOD, Records, KNH  
Supervisors Dr. Lucy Muchiri, Dept of Human Pathology, UON  
Dr. A. Gachii, Dept of Lab Medicine, KNH

APPENDIX 10: CLEARANCE BY CITY COUNCIL OF NAIROBI

CITY COUNCIL OF NAIROBI

TOWN CLERK  
TELEGRAM: "MUNICIPALITY" NAIROBI  
TELEPHONE: 224282  
EXT 2387



CITY HALL  
P. O. BOX 300  
NAIROBI  
KENYA

DEPARTMENT OF HUMAN RESOURCES MANAGEMENT

Ref: CCN/HRM/22/VOL II/2017/10

Date: 28/06/2016

CLAUDE ESCOFFIER CHURCH  
UNIVERSITY OF NAIROBI  
P. O. BOX 20123  
NAIROBI

**RE: RESEARCH**

Reference is hereby made to your research letter dated 15-06-2016 on the above subject.

The City Council of Nairobi has approved your request subject to the following:-

1. The period of research will be three (3) months w.e.f. July, September to end October 2016
2. You will be attached to the Public Health Department
3. You are expected to adhere to the rules and regulations pertaining to your research.
4. That during your study there will be no cost devolving on the Council.
5. That you undertake to indemnify the Council against any claim that may arise from your research.
6. You are expected to be decently dressed at all times;
7. You are required to submit a copy of the final research document within \_\_\_\_\_ weeks/month after completion.

8. You are expected to pay research fees as advised by Medical officer of health

By a copy of this letter M.O.H is/are requested to accord you/or you the necessary assistance.

Please report to the Chief Administrative Officer Public Health Department for your research.

**R. M. MUEMA**  
**For: DIRECTOR - HUMAN RESOURCES MANAGEMENT**

S.S.  
CAC - Public Health Department  
H.R.O. - Training