

**PATHOLOGICAL CHARACTERISTICS OF
PROSTATE CARCINOMA IN ARCHIVAL TISSUES
AT THE AFRICA INLAND CHURCH KIJABE HOSPITAL,
KIAMBU COUNTY - KENYA**

BY

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DECLARATION

I declare that this thesis is my original work. It has not been presented for a degree award in any other University or for any other award.

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DEDICATION

I dedicate this thesis to my wife, Jackie, my daughters Tina and Talia and to my son Tian for their unwavering and consistent support and understanding during the study period and Thesis preparation.

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OPERATIONAL DEFINITIONS OF KEY CONCEPTS AND TERMS

Archival tissue blocks: Previously fixed and processed tissue specimen within a block that have been stored for future use.

Paraffin embedded tissue blocks: Tissue blocks impregnated and embedded in paraffin wax.

Formalin fixed tissue: Tissue that has been preserved using 10% neutral buffered formalin as a fixative.

Prostate carcinoma: Malignant neoplasm arising from the epithelial cells of the prostate gland.

Histopathology: Examination of diseased tissue under a microscope after staining using histochemical stains.

Immunohistochemistry: A specialized test whereby known antibodies are used to detect for corresponding antigens within tissues.

Lymphovascular invasion: The spread of a cancer cells into the blood vessels or lymphatic channels

LIST OF ABBREVIATIONS AND ACRONYMS

34BE12	High molecular weight keratin relatively specific for prostate basal cells
AIC	Africa Inland Church
AJCC	American Joint Committee on Cancer
AMACAR	Alpha-methylacyl-CoA racemase
AR	Androgen receptor
CDKN1B	Cyclin dependent Kinase 1B
CHEK2	Cell-cycle checkpoint kinase2
cPSA	Complexed prostate specific antigen
CYP17	Cytochrome P17
DRE	Digital rectal examination
fPSA	Free prostate specific antigen
GSTP1	Glutathione S-transferase protein 1
H & E	Hematoxylin and Eosin stain
HER ₂	Human epidermal receptor 2
HPCs	Histopathological characteristics
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
ISUP	International society of urological pathology
LVI	Lymphovascular invasion
MSR1	Macrophage scavenger receptor 1 gene
NACOSTI	National commission for science, technology and innovation
NPH	Nodular prostatic hyperplasia
NSB1	Nijmegen breakage syndrome
p504s	Protein involved in the beta-oxidation of branched chain fatty acids
p53	Protein 53
p63	Protein 63
PCa	Prostate carcinoma
PNI	Perineural invasion
PSA	Prostate specific antigen
RNASEL	Ribonuclease L
SRD5A2	Steroid 5- α reductase type II
TLR4	Toll like receptor 4
TNM	Tumor, Node, and Metastases
tPSA	Total prostatic specific antigen
WHO	World Health Organization

ABSTRACT

Prostate carcinoma (PCa) is the leading cancer among middle and elderly men worldwide. It accounts for 15% of all cancer cases and 6.6% of all cancer associated-deaths in men. PCa is a leading cause of cancer burden among men in Africa with Kenya reporting an incidence of 16.6/100,000. The exceedingly high burden of PCa is largely due to lack of population-based screening and delayed diagnosis in the background of genetic and environmental predisposing factors. Histopathological features of PCa offer prognostic indicators while immunohistochemical (IHC) staining provide insights into the molecular characteristics of PCa which have both prognostic and predictive value in the management of PCa. The androgen receptor (AR), Human epidermal growth receptor (HER₂/neu), and protein p53 genes are among the most important genes regulating PCa pathogenesis. IHC staining patterns of these gene products have been associated with different therapeutic and prognostic outcomes. This cross-sectional study determined the histopathological and AR, HER2 and p53 IHC expression on archival simple prostatectomy specimens (n=210) at AIC Kijabe Hospital, a tertiary faith-based health facility in Kiambu County, Kenya. Patient demographic and gross characteristics of the prostatectomy specimens were obtained from the patient files. The tissue blocks (n=210) were retrieved, sectioned and stained using standard H&E and immunohistochemical staining protocols. The slides were then examined using light microscopy and histopathological and immunohistochemical features recorded. Data analyses showed that the median age of the subjects was 74.0 years (44.0 – 99.0). Ethnic groups were represented as follows; Bantu (68.0%), Nilotes (31.0%) and Cushites (1%). Histopathologically, 97.6% of the PCa were of acinar adenocarcinoma type with the rest being of ductal adenocarcinoma type. Perineural invasion (PNI) was present in 57.6% of the PCa while 25.2% had lymphovascular invasion (LVI). Low grade PCa (Gleason score <8) constituted 56.6 % of the cases while high grade PCa (Gleason score ≥8) constituted 43.4% of the cases. The frequencies of the different ISUP grade groups were as follows; ISUP grade 1 (23.3%), grade 2 (16.2%), grade 3 (17.1%) grade 4 (10.5%) and grade 5 (32.9%). This study showed that 34.8% of the PCa were AR negative, 4.5% had HER₂ over-expression while 19.7% of the cases showed p53 protein over-expression. Binary logistic regression analyses revealed that Gleason score ≥8 was associated with higher odds for PNI (OR, 40.407; 95% CI, 11.036-147.949; *P*<0.0001) and gland involvement of ≥50% (OR, 30.447; 95% CI, 7.220-128.397; *P*<0.0001), but lower odds for AR expression (OR, 0.109; 95% CI, 0.019-0.621; *P*=0.013). In addition, PCa pathological grade III was associated with PNI (OR, 37.710; 95% CI, 8.762-162.288; *P*<0.0001), LVI (OR, 33.531; 95% CI, 13.757-81.729; *P*<0.0001), and gland involvement of ≥50% (OR, 2.291; 95% CI, 1.040-50.500; *P*=0.040). Likewise, p53 expression was associated with a lack of PNI (OR, 0.157; 95% CI, 0.032-0.778; *P*=0.023). Altogether, this is the first PCa study outlining the pathological characteristics of PCa in Kenya. This is the first African study that has used ISUP grade groups for PCa. These findings will influence the diagnosis, treatment and prognosis of PCa and might become the standard of care in the country. Further studies to compare patient characteristics, biochemical test results and pathological findings of PCa are recommended.

CHAPTER ONE: INTRODUCTION

1.1 Background information.

Globally, prostatic cancer (PCa) is the second most often diagnosed malignancy amongst males and the sixth ranked cause of cancer mortality in men (Jemal et al., 2011). PCa incidence portrays notable and quite baffling geographical and racial differences worldwide (Ekman, 1999). White Americans show a PCa incidence of 50 - 60 males per 100,000 people. The incidence of PCa amongst African Americans is higher than for the White Americans pushing the United States of America national age - adjusted PCa incidence to 69 males per 100,000 people. Among the Japanese, PCa age - adjusted incidence is 3-4 males per 100,000 people while in Hong Kong the age - adjusted PCa incidence is just 1 male per 100,000 people. African population-based data on PCa are scanty. The International Agency for Research on Cancer (IARC <http://www-dep.iarc.fr/>) reveals that rates amongst Africans are higher in the Eastern countries (10.7–38.1 per 100,000 man-years, age-adjusted world standard) than in the Western countries (4.7–19.8) (Lisa et al., 2011). According to the Nairobi Cancer Registry (Mutuma and Anne, 2006), PCa is the commonest cancer in Kenyan males with an age specific rate of 17.3%. Recently released data indicates that 2,864 new PCa cases were diagnosed in Kenya in 2018 constituting 14.9% of all new cancers in Kenyan males in 2018 (Globocan, 2018).

Prostate cancer is a heterogeneous disease. On one extreme, some PCa patients are asymptomatic while on the other extreme some PCa patients have a rapidly fatal systemic malignancy (Shah et al., 2004; Hughes, Murphy, Martin, Sheils, & O’Leary, 2005). Early stages of PCa symptoms are indistinguishable from those due to nodular prostatic hyperplasia (NPH). Therefore, PCa should be excluded before concluding these

symptoms to be due to NPH. In patients with PCa, per rectal digital examination can demonstrate firm to hard nodule(s), loss of normal rubbery consistency of the gland with loss of median sulci, periprostatic spread or uniformly hard gland. In high stage PCa cases, a palpable urinary bladder, iliac and supraclavicular lymph nodes can be detected. In metastatic PCa, bony pain can be picked (William, Deborah, Tim, & Alison, 2006; James, 2014).

A patient whose serum PSA value is elevated above the normal range and in whom PCa is suspected, the diagnosis is verified by taking a biopsy for histopathology before treatment can be instituted. The prostate sample types include; prostatic needle core or trucut biopsies, transurethral resection of the prostate (TURP), simple prostatectomy specimen or radical prostatectomy specimen (John et al., 2012). Histopathological characteristics including cancer type, tumour bulk, perineural invasion, lymphovascular invasion, grading and tumor stage are crucial characteristics to elicit to guide in deciding on the management options and prognostication purposes. Molecular diagnostics like immunohistochemistry for different molecules can also be performed to aid in determining the treatment of choice and for prognostication of the disease. PCa treatment modalities include; expectant management or watchful waiting for the asymptomatic cases or surgery, radiotherapy, hormonal manipulation and chemotherapy for the symptomatic cases (Chodak et al., 2017).

1.2 Statement of the problem

Among Kenyan men, prostate cancer is the commonest malignancy with an age specific rate of 17.3% according to the Nairobi Cancer Registry (Mutuma & Anne, 2006).

Recently released data indicates that 2,864 new PCa cases were diagnosed in Kenya in 2018 constituting 14.9% of all new cancers in Kenyan males in 2018 (Globocan, 2018).

PCa is not always lethal but rather it is a diverse disease spanning a full spectrum of being asymptomatic to a swiftly lethal systemic cancer (Shah et al., 2004; Hughes et al., 2005). However, there is minimal published data on the pathological characteristics of PCa in Kenyan setting. PCa characteristics including histological type, Gleason score/ISUP/WHO grade group, angiolymphatic /lymphovascular invasion (LVI), tumor bulk, perineural invasion (PNI), and tumor stage are crucial in determining the management and prognosis of PCa.

PCa can advance from being an androgen hormone-sensitive, hormone-dependent stage to a hormone-independent androgen-refractory stage neoplasm. This has a bearing on the treatment options since an androgen-refractory, hormone-independent tumor can't respond to androgen therapy or surgical castration. The proportion of the androgen hormone sensitive to the androgen insensitive cancer in the Kenyan settings has not been established. The human epidermal growth receptor-2 (HER₂/neu) and the p53 protein have been shown to be of prognostic importance in PCa (Day et al., 2017; Mozhgan, 2015). Understanding the histopathological and immunohistopathological characteristics of PCa in our locality will elucidate its biology and help us infer prognosis and predict response to treatment. It is this gap in knowledge of the pathological characteristics of PCa in our locality that prompted this study to be undertaken.

1.3 Justification of the study

There is paucity of data on prostate cancer pathology in Kenya. There is no Kenyan data available on the pathological characteristics of prostate cancer. In Africa, PCa population-based data is scarce. The International Agency for Research on Cancer (IARC <http://www-dep.iarc.fr/>) shows that rates among Africans are higher in the Eastern African countries (10.7–38.1 per 100,000 man-years, age-adjusted world standard) than in the Western African countries (4.7–19.8) (Lisa et al., 2011).

The pathogenesis of PCa is complex involving multiple genes and environmental factors. Therefore, it is important to determine the pathological characteristics of PCa in Kenya. This information is important for screening, diagnostic, therapeutic and prognostic purposes for PCa. The study was performed on retrieved 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks routinely archived at AIC Kijabe pathology department. AIC Kijabe pathology department receives prostate samples from hospitals across Kenya for histopathological analysis and reporting. These archived PCa tissue blocks had been labeled with unique accession numbers as per archiving protocol and are reasonably resistant to adverse conditions thus allowing archival and retrieval for research purposes. The archival and retrieval process is often done locally by surgical pathology laboratories (Ashokkumar et al., 2007).

1.4 Research questions

- a) What are the demographics of PCa subjects and the gross characteristics of prostate gland samples seen at the AIC Kijabe Hospital?

- b) What are the histopathological characteristics of PCa in archival tissue blocks at the AIC Kijabe Hospital?
- c) What is the IHC expression of the AR, HER₂/neu and p53 proteins in archival PCa tissue blocks at the AIC Kijabe Hospital?
- d) What are the associations between different histopathological and IHC staining characteristics on archival prostate cancer tissue at the AIC Kijabe Hospital?

1.5 Null hypotheses

- a) There is no difference in the histopathological and immunohistopathological characteristics of PCa on 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded retrieved tissue blocks at the AIC Kijabe Hospital and those from western population.
- b) There is no association between different histopathological characteristics and immunohistochemical staining characteristics on 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded retrieved tissue blocks at the AIC Kijabe Hospital

1.6 Research objectives

1.6.1 Main objective

To assess the histopathological characteristics (HPCs), immunohistochemical (IHC) expression of AR, HER₂/neu and p53 protein and the associations between HPCs and IHC on retrieved PCa 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks at the AIC Kijabe Hospital pathology laboratory.

1.6.2 Specific objectives

- a) To establish the demographics of PCa subjects and prior gross prostate weight for PCa 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks retrieved at the AIC Kijabe Hospital.
- b) To determine the histopathological characteristics of PCa on retrieved 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks at the AIC Kijabe Hospital.
- c) To evaluate the AR, HER₂/neu and p53 protein expression on retrieved prostate cancer 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks at the AIC Kijabe Hospital.
- d) To determine the associations between different histopathological characteristics and IHC staining characteristics on retrieved prostate cancer 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks at the AIC Kijabe Hospital.

1.7 Significance of the study

The findings from this thesis will add to the existing academic knowledge on the characteristics of PCa at the AIC Kijabe Hospital, Kiambu County, Kenya and Sub-Saharan Africa. The findings of this study will provide much needed local baseline data on the pathological characteristic of PCa. This will shed more light on the biology of PCa seen locally. The results of this study will contribute in determining which PCa patients might benefit from androgen ablation by castration and anti androgen therapy. The findings of this study might become the standard of care.

Using immunohistochemistry, it may be possible to distinguish androgen-sensitive and androgen-insensitive prostate cancers by staining for AR and HER₂/neu receptor over expression (Guo et al., 2009). It is hypothesized that in future determination of androgen sensitivity in patients with prostate cancer will be determined before instituting anti-androgen therapy or androgen ablation through castration (Watson, Arora, & Sawyers, 2015).

The study will also lead to the establishment of a functional surgical pathology and oncology unit at Kenyatta University to offer cancer diagnosis at the Kenyatta University Teaching, Research and Referral Hospital.

1.8 Scope and limitations of the study

This was a cross-sectional study that utilised 210 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded prostate cancer retrieved blocks. Limited demographic data of interest was collected from the patients' files. Histopathological characteristics were deduced from the archival tissue. This was a unicenter cross sectional study. Further studies are highly recommended for more findings.

1.9 Conceptual framework

The independent variables for this study were age, ethnicity and prostate gland weight. The dependent variables that were being tested included; histopathological and immunohistopathological characteristics including; tumour type, tumour bulk, Gleason score/ISUP grade group, perineural invasion, lymphovascular invasion, AR, HER₂/neu and p53 expression.

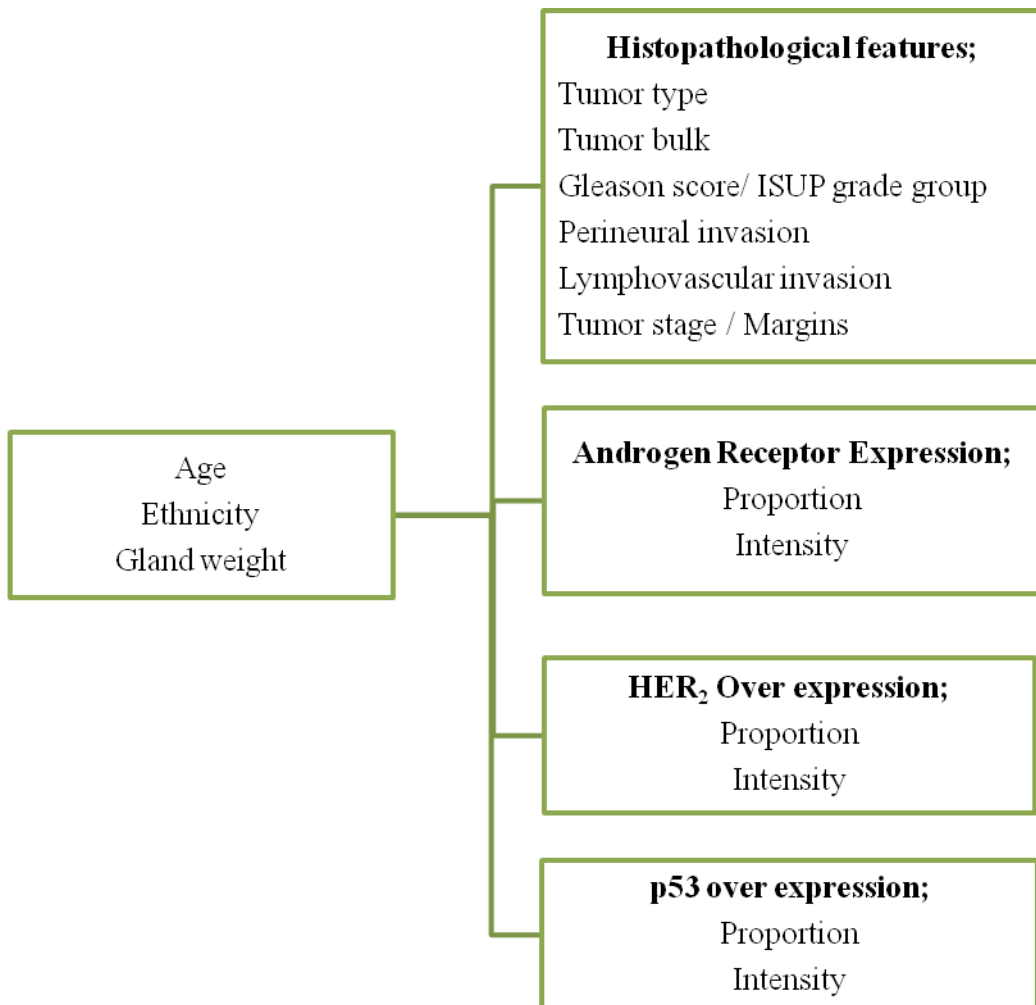
Independent Variables**Dependent Variables**

Figure 1.1: Conceptual framework; Relationship between variables.

Source: Adopted and modified from Researcher (2017).

CHAPTER TWO: LITERATURE REVIEW

2.1 The gross anatomy and histomorphology of the prostatic gland

The prostatic gland is an anatomic component of the male genital system. The gland is found behind the pelvic peritoneum around the neck of the urinary bladder and proximal urethra. The average weight of the prostatic gland in an adult male is 20 grams. It lies within the anatomic true pelvis with its base dorsal to the urinary bladder neck and the apex caudal to the urogenital diaphragm/levator ani muscle (James, 2014).

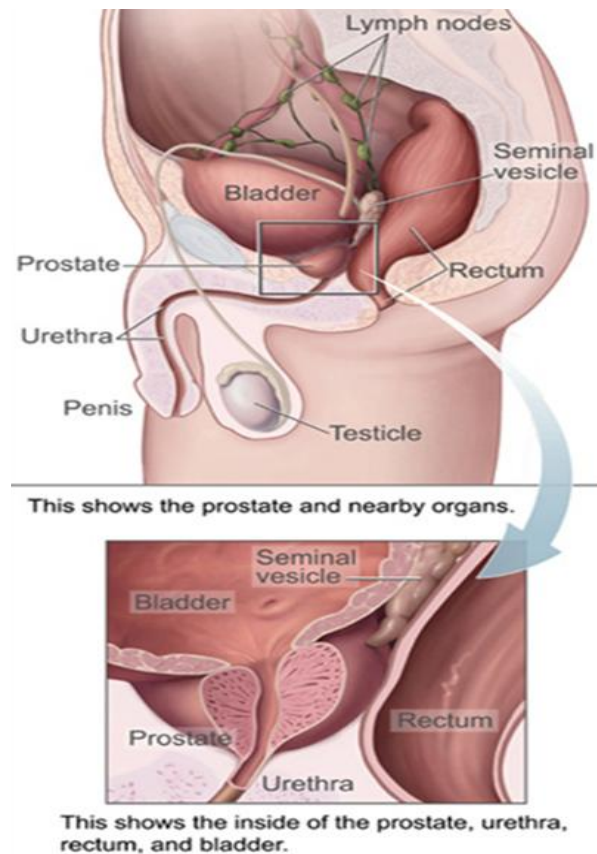


Figure 2.1: The anatomic location of the prostate gland in relation to surrounding organs. (<https://en.wikipedia.org/wiki/Prostate>, 2018, May 10).

McNeal modeled the anatomy of the prostate gland into 4 main anatomical zones namely; central, transitional, peripheral and anterior fibromuscular zones (McNeal, 1981). This zonal mapping is crucial in understanding the signs and symptoms of different prostate gland pathologies. Nodular prostatic hyperplasia usually arises from the transitional zone and hence causes early urinary tract obstructive symptoms. 70 – 80% of all PCas arise from the peripheral zone and hence urinary tract obstructive signs and symptoms appear later and are milder (McNeal, 1981).

Regarding histomorphology, the prostate is a compound tubuloalveolar gland composed of fibromuscular stroma and glands. The glands are lined by a bilayered epithelium composed of a low cuboidal basal myoepithelium and an overlying secretory columnar luminal layer. The epithelium sometimes forms pseudopapillary infoldings into the lumen. Intraluminal secretions sometimes crystallize to form corpora amylacea. The glands have a distinct basement membrane and testicular androgens control prostatic growth (McNeal, 1988).

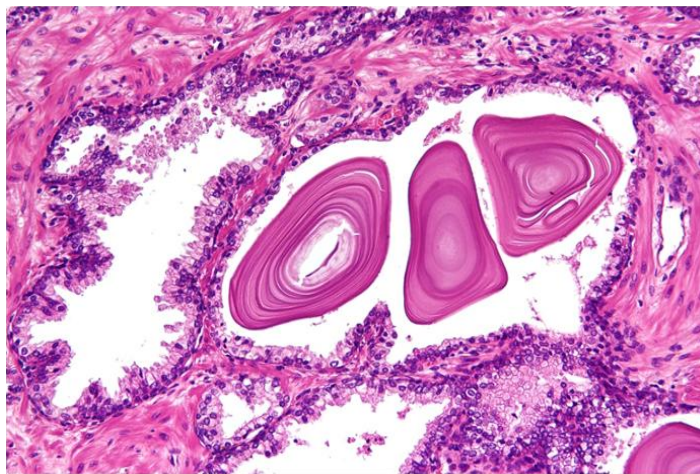


Figure 2.2: Micrograph showing the normal histology of the normal prostate gland.

Image from Webpathology (www.webpathology.com, 2018, May 10).

2.2 Physiology of the prostate gland

The prostate gland secretes a milky or whitish mildly alkaline fluid. Its function is to nourish the spermatozoa and enable mobility for fertilization. In human beings, prostatic fluid makes up to 30% of the overall volume of semen. The rest of the semen is made up of spermatozoa and seminal vesicle fluid. Due to its alkalinity, it helps neutralize the acidity in the vaginal vault thus prolonging the survival of spermatozoa. The prostatic fluid with most of the spermatozoa forms the first ejaculate fraction while seminal vesicular fluids constitute the later ejaculate fractions. Comparatively, spermatozoa ejaculated in prostatic fluid are more motile, survive longer and exhibit superior protection of the genetic material than those ejaculated with mainly seminal vesicular fluid (Owen & Katz, 2013). The prostate gland stroma contains some smooth muscles that contract to expel semen during ejaculation (Knobil, E., Knobil, J. & Neill, 2006).

2.3 Epidemiology, burden and challenge of prostate carcinoma

PCa is a malignant epithelial neoplasm of the prostate gland. It is typically a disease of men over 50 years. The Nairobi Cancer Registry ranks PCa as the commonest malignancy in Kenyan males with an age-specific rate of 17.3% (Mutuma & Anne, 2006). There are evident unexplained differences in the incidence of PCa. Population-based data on PCa in Africa are quite limited. Available data from the IARC indicate that rates among Africans are higher in the Eastern countries (10.7–38.1 per 100,000 man-years, age-adjusted world standard) than in the Western countries (4.7–19.8), (Lisa et al., 2011; IARC <http://www-dep.iarc.fr/>).

The incidence of PCa in the USA is 69 men per 100,000 after age adjustment, Japan 3-4 men per 100,000 and 1 man per 100,000 in Hong Kong (Zhang, Bangma, & Roobol, 2017). According to Zhang et al., (2017), the disease is more prevalent among black than whites in the USA. The differences in the incidences are attributed to both inherited genetic factors and environmentally induced mutations (Lisa et al., 2011). PCa is the second most often diagnosed malignancy amongst males and the sixth ranked cause of cancer mortality in men globally (Jemal et al., 2011). In 2012, estimates showed that there were 1,100,000 PCa cases with 307,000 deaths (Humphrey, 2014). The prevalence of PCa is high enough to an extent that it can be taken to be as a normal ageing occurrence (Hughes et al., 2005). PCa isn't always fatal but it has a disease spectrum varying from an asymptomatic incidental finding to a swiftly lethal systemic cancer (Shah et al., 2004; Hughes et al., 2005).

2.4 Pathogenesis of prostate carcinoma

The exact etiology of PCa is not well known. Some risk factors like advancing age, racial extraction, positive ancestry and androgen hormonal status. Environmental risks including excessive consumption of fat and processed foods have all been implicated (Boyle, Severi & Giles, 2003; Gronberg, 2003). However, some dietary products have been shown to prevent or slow down the progression of PCa. These include antioxidants like (a) lycopenes found in tomatoes (Yang, Lu, Chen & Hu, 2012), (b) vitamin D (Gregory et al., 2010), (c) vitamin E (Antwi et al., 2015), (d) selenium (Xiang, Zhao & Zhong, 2009), (e) soy products (Hamilton-Reeves, Rebello, Thomas, Kurzer, & Slaton, 2008) and (f) pomegranate (Hong, Seeram & Heber, 2008; Lansky et al., 2005; Koyama et al., 2010).

Molecular pathogenesis of PCa is multifaceted. Several genes acting as the bullets are involved in the pathogenetic pathway. Environmental risks such as foods and inflammatory reactions act as the triggers that release the bullets. Research into PCa has demonstrated a multifaceted interaction of several genes and environmental risks, some of which could be more crucial in the biology of some PCa cases than others (Hughes et al., 2005). Epidemiologically, PCa can be grouped into inherited and random types (Carter et al., 1993). Suspected inherited PCa susceptibility genes have been identified including RNASEL gene, MSR1 gene, NSB1 gene, and CHEK2 gene in some affected lineages (Cybulski et al., 2004; Dong et al., 2003; Zheng et al., 2004).

However, most PCa are random (Hughes et al., 2005). Some gene polymorphisms have been linked with increased PCa risk. They include the following genes; TLR4 gene (Kibel et al., 2003), CDKN1B-p27 gene, (Gionannuci et al., 1997), the androgen receptor (AR) gene (Stanford et al., 1997; Freedman et al., 2005; Chang et al., 2001), CYP17 gene (Noonan et al., 2002; Makridakis et al., 1999), SRD5A2 gene (Nam et al., 2001; Skowronski, Peehl & Feldman, 1993), Vitamin D receptor gene (Morrison, Yeoman, Kelly & Eisman, 1992; Ingles et al., 1997; Knudson, 1984), Glutathione S-transferase (GSTP1) gene (Millar et al., 1999).

The AR gene is positioned at the Xq locus. The growth of prostatic cells is controlled by androgens. Androgens influence cell proliferation, survival and differentiation. The AR is now targeted in the treatment of PCa (Alia, Shadan & Fazlul, 2014). The AR contains polymorphic polyglutamine (CAG) trinucleotide base repeats. Shortening of the base repeats has been linked with greater PCa risk. A shorter CAG length is associated with

higher grade, higher stage, metastatic, and poor prognosis PCa. It is hypothesised that short CAG repeat causes an increase in ignition of androgen dependent genes (Stanford et al., 1997; Freedman et al., 2005; Chang et al., 2001).

The p53 gene is a frequently mutated cancer suppressor gene in several human malignancies. Nonetheless, research has indicated that p53 mutations are uncommon in PCa but when present are associated with advanced disease. Usually, elevated expression is linked to point mutations in one p53 gene allele with loss of the other allele. P53 over expression in a tiny PCa population has been linked to poor prognosis related to disease progression and overall survival (Grignon et al., 1997; Thomas et al., 1993). Accumulated p53 protein product can be demonstrated on biopsies using immunohistochemical techniques (Yaman et al., 1997).

2.5 Clinical presentations

PCa patients with clinically non advanced disease usually don't present with urinary symptoms. This is because most PCa arise in the peripheral zones away from the urethra. In these patients, the PCa is usually suspected during DRE when a suspicious nodule is palpated or by elevated PSA levels.

2.6 Progression of PCa

Patients with advanced PCa can exhibit urinary tract symptoms including difficult in initiating or terminating micturition, painful urination or blood in urine, urinary retention and back pain due to vertebral metastases. Osteoblastic metastases in vertebral bones in men are virtually diagnostic of PCa. Urinary symptoms like frequency, urgency, terminal

urine dribbling and weak urine flow usually signify NPH rather than PCa (Sarma & Wei, 2012).

Advanced stage PCa develops when there is spread of the cancer through lymphatic route, hematogenous route, or local spread of the disease to adjacent structures. Bone symptoms and signs are a frequent presentation since PCa has a penchant for spreading to bone. Clinical presentations of advanced stage PCa may comprise: Cancer cachexia, early satiety, anaemia, and bony tenderness (Grossmann et al., 2011). Pathological fractures, spinal cord compression with neurological deficits, lower limb pain and edema due to lymphovascular obstruction by metastatic tumor cells into lymph nodes. Uremia can be caused by obstruction of ureters by local prostate gland enlargement or by retroperitoneal metastatic lymphadenopathy (Ramos et al., 2013).

The clinical biology of PCa strides from a 'benign' like histopathologic, low grade neoplasm that is clinically silent to a very potent grade that ultimately metastasizes leading to advanced disease and death. PCa progresses from an androgen-sensitive, androgen-reliant stage to an androgen-insensitive, androgen-noreliant malignancy. The AR pathway these androgen-insensitive neoplasms are resistant to anti-androgen treatment (Michael, Haojie & Tindall, 2001).

2.7 Diagnosis of prostate carcinoma

In patients clinically suspected to have PCa, a digital rectal examination is performed. If the patient has PCa, on DRE palpation can reveal (a) firm to hard nodule(s), (b) non-rubbery prostate gland and obliteration of the median sulcus, (c) periprostatic spread or

uniformly hard gland. In advanced PCa, a palpable urinary bladder, engorged iliac and supraclavicular lymph nodes and foci of bone tenderness may be detected due to metastases (Lowrance et al., 2012).

Prostatic specific antigen (PSA) levels in serum and a DRE are performed to screen men for PCa. PSA is a prostate organ specific glycoprotein released nearly solely by the prostatic epithelial cells. In the blood, PSA is either bound to alpha 1-antichymotrypsin (cPSA) serum proteins or it can exist freely as fPSA which is the minor fraction. The cPSA together with the fPSA make up total PSA (tPSA). The tPSA is less than 4 ng/mL in a healthy prostate gland. However, it is not PCa specific as some other prostate pathologies can also lead to elevated tPSA levels ((Ezenwa et al., 2012). These include; NPH, prostatitis, prostatic infarction, prostate instrumentation and ejaculation. Also, 20 – 40% of patients with prostate organ confined PCa have tPSA measurements of 4.0 ng/mL or even less (Jung et al., 2000). Due to this, several advancements in inference and elucidation of PSA levels have been suggested. These include; PSA density that measures the ratio between the serum PSA and the transrectal ultrasound determined volume of the prostate gland. The upper normal value for this ratio is 0.15 (Stavros & Petros, 2012). Another novel advancement is the determination of PSA velocity done by calculating change in PSA over a certain time period. The PSA velocity that identifies males with PCa is 0.75ng/ml annually. Upto three PSA tests within 1.5 to 2 years are recommended (Marc & Stacy, 2013).

Another advancement is the use of age-specific PSA reference cut offs. The suggested age-specific serum PSA reference cut off is 2.5 ng/ml for men 40 – 49 years of age, 3.5

ng/ml for men 50 – 59 years old, 4.5 ng/ml for men 60 -69 years old and 6.5 ng/ml for men 70 -79 years old (Hans-Joachim, Joachim & Herbert, 2007). Measurement of the percentage of fPSA ($fPSA/tPSA \times 100$) is recommended when the tPSA is in the gray zone (4 – 10 ng/ml) (Ezenwa et al., 2012). When the percentage of the fPSA is more than 25% of the tPSA, it signifies a lower PCa risk whereas a fPSA percentage of less than 10 % signifies a higher PCa risk (Shariat, Scardino, & Lilja, 2008). These refinements are still being worked on and therefore serum PSA alone is not sufficient for the detection of PCa. However, when measurement of serum PSA is combined with DRE and transrectal prostate ultrasound, it is a crucial test in the screening for early stage PCa (Mehraj et al., 2007).

When an elevated serum tPSA value is measured and PCa is supposed, definitive diagnosis is done by taking a biopsy for histopathology before treatment can be started. The biopsy sample can either be needle core biopsies, trucut biopsies, transurethral resection of the prostate gland (TURP), a simple prostatectomy or a radical prostatectomy specimen. Histopathological features like carcinoma typing, Gleason scoring / ISUP/WHO grade grouping, PNI, LVI, tumor bulk and evaluation of the cancer stage should be determined before the management and prognostication. Immunohistochemistry can also be used to confirm diagnosis and prognosis of the prostate carcinoma.

2.8 Histopathology of PCa

The histopathological criteria for diagnosis of PCa use architectural features and cellular features. Architectural features include; invasive growth pattern, perineural invasion, and

irregular glandular ducts. Cellular features include; enlarged hyperchromatic nuclei with conspicuous nucleoli. Suspicious tiny cancer foci need immunohistochemical techniques for conclusive determination. Invasive prostatic adenocarcinoma has no basal cells which are present in benign glandular ducts and hence basal cell markers like 34BE12 and p63 are negative (Gleason, 1977). There is upregulation of the alpha-methylacyl-CoA racemase (AMACR/p504s) marker in prostate carcinoma and this can be demonstrated using immunohistochemical staining techniques (Humphrey, 2017).

PCa is an assortment of malignant prostate gland neoplasms as per WHO classification (Mathers, Sadana, Salomon, Murray, & Lopez, 2000). Ninety-five percent of these malignancies are adenocarcinomas arising from the glandular ducts of the prostate gland. The malignancy grows multifocally without producing grossly visible nodules. As Mathers et al. (2000) noted, approximately 75% of the neoplasms originate in the periphery of the gland. This leads to a change in the consistency of the gland that can be palpated during DRE. Majority of the prostate malignancies are of the acinar adenocarcinoma type and hence these are commonly called prostate carcinomas. Approximately, 1% of prostate malignancies are made up of other subtypes which have worse prognosis than the acinar variant. These include; ductal adenocarcinoma, signet ring carcinoma, mucinous carcinoma and small cell prostatic carcinoma. Five percent of prostatic malignancies arise either from the urethral transitional epithelial cells, sarcomas arising from the supporting mesenchymal tissue, or lymphomas arising from the lymphoid tissue. Metastatic secondary malignancies to the prostate gland can also occur. However, it is more likely for neoplasms from bordering organs like the urinary bladder, anorectum, or testicular tissue to invade the prostate gland (Humphrey, 2017).

Important histopathological features in PCa include; perineural invasion (PNI) which is the penetration of malignant cells into the perineurium, epineurium or endoneurium of nerve bundles. It is a less acknowledged pathway of metastatic cancer spread for a number of malignancies including prostatic carcinoma, pancreatic, colorectal, head and neck neoplasms, gastric malignancies and biliary tract malignancies. In these malignant neoplasms, PNI has been shown to be a poor prognostic marker with increased mortality (Liebig, Ayala, Wilks, Berger & Albo, 2009). D'Amico and his fellow researchers (D'Amico et al., 2001) demonstrated PNI to be a non-dependent prognostic signature for PCa reappearance following radical prostatectomy procedure.

However, Reeves et al., (2015) in their study claimed that PNI on radical prostatectomy histopathology is not a non-dependent forecaster for biochemical cancer reemergence and hence they don't advocate for routine reporting of PNI. A study by DeLancey et al., (2013) demonstrated that PNI is autonomously linked to unfavourable pathological characteristics and poorer survival results following radical prostatectomy procedures. Therefore, PNI in prostatic biopsy specimens ought to be factored in PCa management evaluation and clinical care decisions. The histomorphology-based prognostication characteristics committee during the 2004 WHO facilitated International conference on handling and reporting of prostate cancer protocol (Epstein et al., 2005) recommends that PNI status be included in all PCa reports. Future research should be targeted at the biology of PNI to increase the understanding of its role in PCa progression.

LVI is identified morphologically in the peritumoural area. LVI is a critical stage in the infiltration-metastasis flow for malignancies and is taken as a pointer of metastatic

probability and is sturdily linked to worse outcome in several solid malignancies (Yong et al., 2016). Malignant cells have the potential to intermingle with endothelium, permeate the lymphovascular wall and endure the intralymphovascular stresses through different molecular mechanisms (Epstein et al., 2005). LVI is the commonest route of metastases for malignancies. The other routes of spread are direct spread and transcoelomic spread. When the cancer cells invade the lymphatics or blood vessels, the chance of this malignancy to spread to regional and distant parts of the body is very high. LVI has been demonstrated as a very crucial prognostic feature in PCa since it is significantly linked to elevated presurgery serum PSA, higher pathological stage, superior Gleason score, positive surgical excision margins, extra prostatic spread, infiltration of seminal vesicle, nodal spread and PNI (Mitsuzuka et al., 2015; Matthias, Olaf, Fränze, Volker, & Michael, 2006). This is also backed by the histomorphology-based extrapolative features committee of the 2004 WHO facilitated International Consultation on handling and reporting of prostate cancer protocol (Epstein et al., 2005).

The Gleason grading and scoring system is among the greatest predictive characteristics ever invented for Prostate adenocarcinoma (Gleason, 1977). Combined with other factors it is also used to stage prostate cancer. It was developed in 1966 by Dr. Donald Gleason who was a pathologist based at the Veterans Affairs Hospital, Minneapolis. Together with his team, Gleason devised grades ranging from grade 1 to grade 5, using glandular duct structure at microscopic low power to medium power magnification. These Gleason grades were demonstrated to forecast behaviour of PCa.

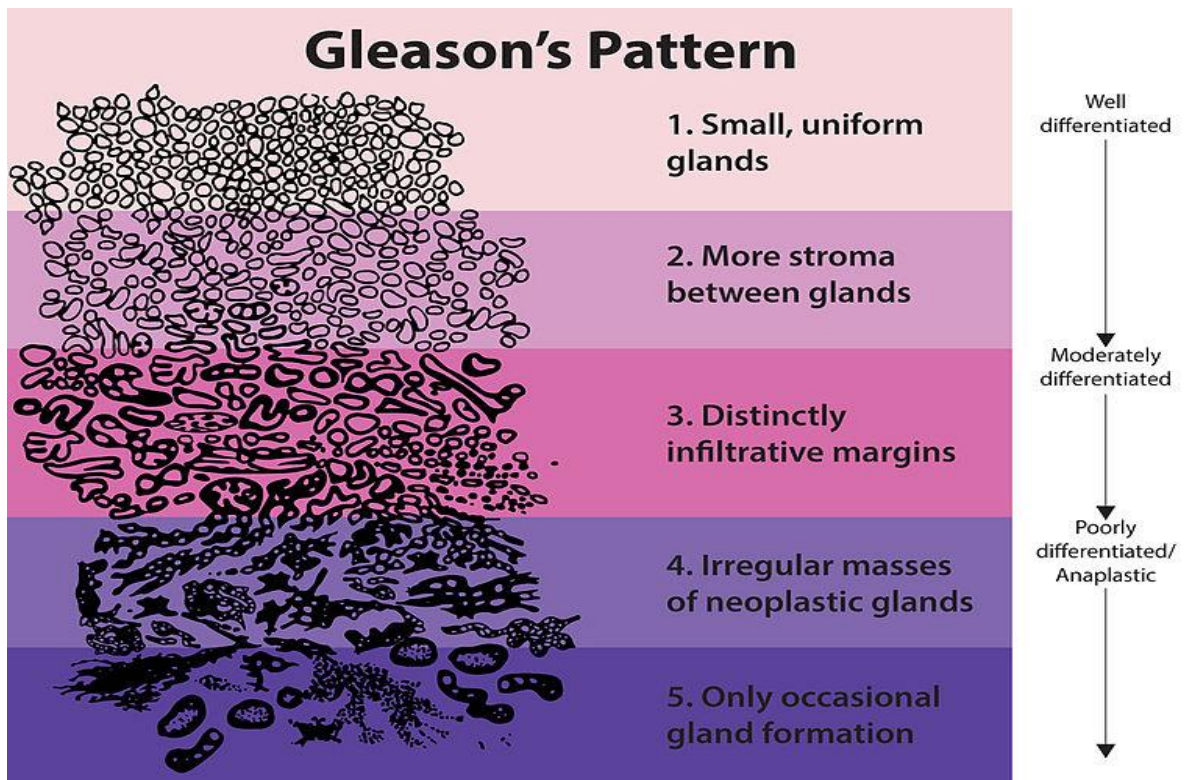


Figure 2.3: Gleason grading system. Artistic demonstration of Gleason patterns.

The Gleason score is a product of adding the two most common Gleason grades named the primary and the secondary patterns. If only one pattern is present it is multiplied by two to get a Gleason score. The Gleason score potentially ranges from a score of 2 to a score of 10. Though the Gleason grading system has been modified over the years, it still has some challenges. Theoretically the Gleason grading system can have up to 25 different grades. Several improvements on the Gleason grading system have been instituted. In 2005, the Gleason grading and scoring system was improved to become the 'Modified Gleason score' by the ISUP (Gleason, 1977). The scoring criteria were polished and the ascription of some morphological patterns altered (Epstein et al., 2005). Gleason grades 1 and 2 were dropped because prostate cancer with these patterns has a similar outcome to Gleason grade 3 PCa. Additionally, pure Gleason grade 3 cancers very rarely

metastasize and should be managed by close watch and follow up. This has ignited a hot debate on whether or not Gleason grade 3 should even be regarded as prostatic cancer (Lepor & Donin, 2015). However, the jury is still out there on whether Gleason score of 6 has a potential to progress to higher-grade cancer and therefore Gleason grade 3 was retained in the modified Gleason grading system.

The International Society for Urological Pathologists (ISUP) and the WHO in 2014, embraced an easy patient-oriented PCa grading system composed of 5 prognosis based grade groups (Epstein et al., 2016) which had been suggested in 2013 using Johns Hopkins' Hospital data (Pierorazio, Walsh, Partin & Epstein, 2013).

Table 2.1: The 2014 WHO / ISUP prostate adenocarcinoma grading system.

ISUP grade group	Gleason score	Characteristics
Grade group 1	3 + 3 =6	Histomorphologically made up of distinct well delimited glandular ducts.
Grade group 2	3 + 4 =7	Histomorphologically principally made up of distinct glands with minor constituent of defectively shaped, merged or cribriform glandular ducts.
Grade group 3	4 + 3 =7	Histomorphologically principally made up of defectively shaped, merged or cribriform glandular ducts with minor constituent of distinct glands.
Grade group 4	4 + 4 or 3 + 5 or 5 + 3 = 8	Histomorphologically principally made up of defectively shaped, merged or cribriform glandular ducts or principally a mix distinct glands and no glandular formation.
Grade group 5	9-10	Histomorphologically tumor lacks glandular formation (or necrotic glands) with or devoid of defectively shaped, merged or cribriform glandular ducts

The 2014 WHO/ISUP grading system for PCa was consequently evaluated using biochemical reappearance risk ratios on patients from 5 big scholarly sites (Epstein et al.,

2016). The current PCa grade grouping system gives a better precise grading stratification than the Gleason scoring system. It has cut down grading to five grades groups with the lowest grade group being 1 as opposed to a score of 6 in the Gleason system. This will potentially reduce overtreatment of PCa. This novel grade grouping system and the vocabularly grade groups 1 - 5 were received and adopted by the WHO in the 2016 publication titled 'Pathology and Genetics: Tumors of the urinary system and male genital organs'.

Cancer staging systems are a yardstick of describing how far a malignancy has spread. The commonly adopted cancer staging system for PCa is the American Joint Committee on Cancer (AJCC) system that evaluates Tumor characteristics, Nodal spread, and distant Metastases (TNM) system (Buyyounouski et al., 2017). Prostate cancer patients undergo two types of staging; the clinical cancer stage is the clinician's best approximation of how far the prostate cancer disease has spread, informed by digital rectal examination findings, laboratory tests, prostatic biopsy and imaging diagnostic studies performed. After surgery, the pathologists establish the PCa pathological stage informed by the PCa clinical stage together with the histopathological results of the submitted prostate specimen. The pathological stage is more precise than the clinical stage. Determination of the cancer stage offers a methodical approach to express the bulk and how far the cancer has spread at a particular time (Buyyounouski et al., 2017).

2.8 Hormone receptors in PCa

The AR and the HER₂/neu receptor have generated a lot of interest in PCa research. The AR gene is located at Xq locus. The growth of prostatic epithelial cells is reliant on androgen hormones. Androgens influence cell proliferation, survival and differentiation.

The AR is presently a pharmaceutical target in the management of PCa. Also, surgical castration in advanced PCa targets androgen production.

The AR has polymorphic polyglutamine (CAG) trinucleotide base repeats (Stanford et al., 1997). Reduction of these base repeats has been associated with amplified PCa hazard. Shortened CAG span has also been linked to poor grade, advanced stage, metastatic, and lethal PCa. A theory that has been advanced for the power of the short CAG repeat on PCa pathogenesis is that due to its role in AR function, it leads to an upsurge in inauguration of androgen reliant genes (Freedman et al., 2005; Chang et al., 2001).

Studies conducted by Noah, Yuriy, Michael & Sawyers (1999) and Shi et al. (2001) have shown that androgen-nonreliant PCa portrayed elevated levels of the HER₂/neu tyrosine kinase receptor compared to their androgen-reliant counterparts. HER₂/neu is a proto-oncogene that encodes a transmembrane receptor that belongs to the epidermal growth factor receptors family. Available research evidence shows that HER₂/neu overexpression may have a role in androgen hormone resistance in prostate cancer. Therefore, HER₂/neu receptor over expression can be used as a surrogate marker for Androgen independent or insensitive PCa. HER₂ is over expressed in some breast, ovarian, colon and PCa. The gene programming the HER₂/neu protein is overexpressed in 20% to 25% of breast cancer patients. An anti-HER₂/neu antibody called trastuzumab has been confirmed to be tremendously useful in their treatment (David & Neal, 2007). Therefore, it can be hypothesized that trastuzumab or its newer generations can be useful

in treating the PCa that over express HER₂/neu receptor. The AR and HER₂/neu can be detected using immunohistochemistry.

2.9 Tumour suppressor gene mutation

The p53 gene has been shown to be one of the most commonly mutated cancer suppressor genes in human cancers (Vogelstein, Sur & Prives, 2010). Research has indicated that p53 mutations are rare in PCa but when present they have been associated with advanced disease (Eastham et al., 1995). An increased p53 expression is linked to point mutations in one p53 gene allele and loss of the other allele. Over expression of p53 in a tiny group of PCa has been associated with a worse outcome (Grignon et al., 1997; Thomas et al., 1993). Buildup of the p53 protein product can be demonstrated using immunohistochemistry (Yaman et al., 1997).

2.10 Management of prostate carcinoma

The treatment modalities for PCa include; (a) expectant management or watchful follow up for the asymptomatic cases and for the symptomatic cases, surgery, radiotherapy, hormonal therapy and chemotherapy are the available options, (b) bilateral orchidectomy for the symptomatic cases to reduce the growth of PCa by androgen ablation (both the anti-androgen hormonal therapy and the androgen ablation surgery are only effective in androgen sensitive prostate cancers and (c) palliative care for advanced cases (Chodak et al., 2017).

CHAPTER THREE: MATERIALS AND METHODS

3.1 The study design

A hospital-based cross-sectional descriptive and analytical study design on archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded histopathology tissue blocks was utilised to examine the histopathological and immunohistochemical characteristics of PCa.

3.2 The study variables

3.2.1 The independent variables

The following were the independent variables;

- 1) Age of the patients when the simple prostatectomy samples were collected.
- 2) Patients' ethnicity according to ethnic grouping as either Bantu, Nilotic or Cushitic ethnic group.
- 3) Prostate gland weight at the time of grossing the prostatectomy biopsy in the laboratory.

3.2.2 Dependent variables

The dependent variables included:

- 1) Histopathological characteristics: Tumor type, tumor bulk, Gleason grade, WHO/ISUP grades, PNI, LVI and tumor stage
- 2) AR expression: Quantity of tumor cells expressing nuclear staining and their staining strength.

- 3) HER₂/neu receptor over-expression: Quantity of tumor cells expressing membrane staining and their staining strength.
- 4) p53 protein over expression: Quantity of tumor cells expressing nuclear staining and their staining strength.

3.3 Study area

The study was conducted at the AIC Kijabe hospital pathology department. The AIC Kijabe hospital is located in Lari Subcounty of Kiambu County. The County adjoins the northern edge of Nairobi County, the capital city of the Kenyan Republic. It has a population of 1,623,282 as per the 2009 census. Farming is the main economic activity in the county. AIC Kijabe Hospital is a faith based hospital run by the AIC Church. The Hospital caters for surgical patients from across Kenya and neighbouring East African and Horn of Africa countries. The pathology department receives biopsy specimen from the hospital theatres and also from more than 10 other faith based hospitals in the East African and Horn of Africa region (Muchendu, 2017).

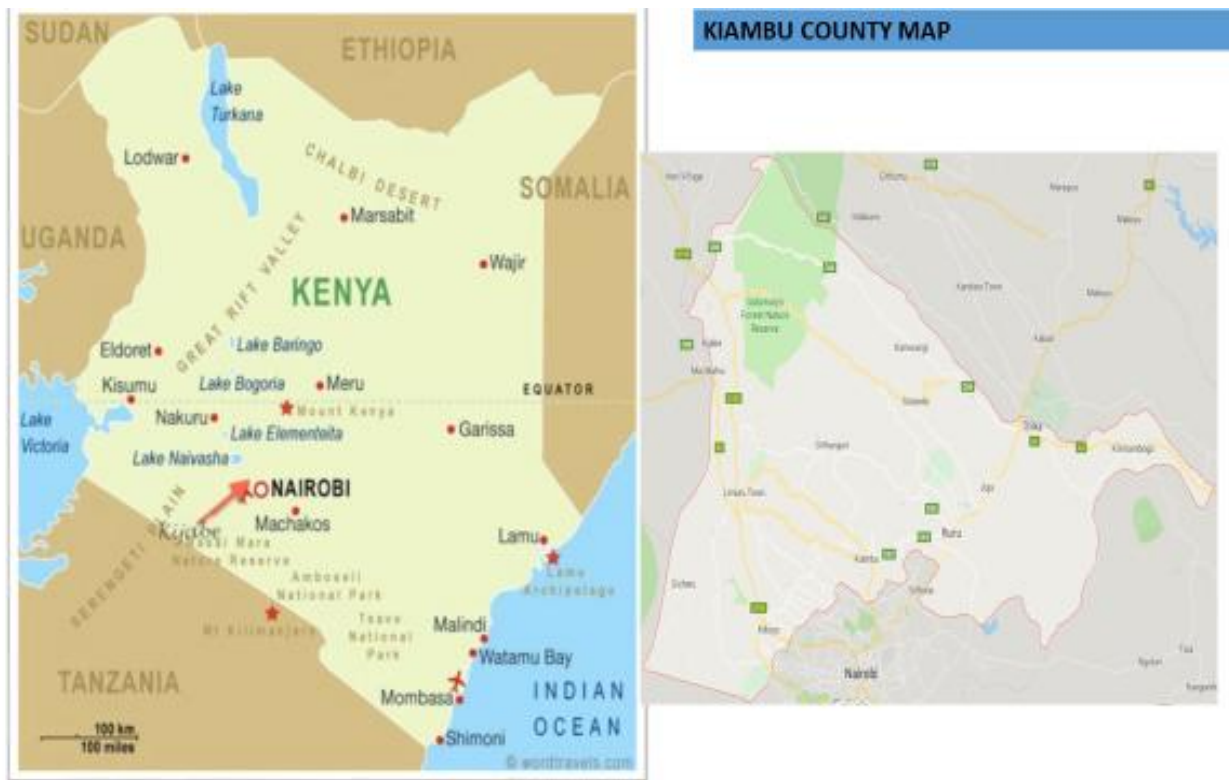


Figure 3.1. The map of Kenya depicting the site of AIC Kijabe Hospital and its catchment area of Eastern Africa and the Horn of Africa

3.4 The study population

The study targeted archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded prostate tissue blocks previously collected from routine simple prostatectomy specimen. The study population included all archived blocks previously diagnosed to have prostatic adenocarcinoma.

3.4.1 Inclusion criteria

Only well-preserved archived 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks from PCa patients who had undergone simple prostatectomy and whose biodata was available were included into the study.

3.4.2 Exclusion criteria

The following was the exclusion criteria;

- 1) Archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks for simple prostatectomy specimen and were diagnosed as any other type of prostate carcinoma other than prostatic adenocarcinoma.
- 2) Archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks for prostatic adenocarcinomas with prior chemotherapy or radiotherapy treatment.
- 3) Archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks for simple prostatectomy specimen that had been diagnosed as prostatic adenocarcinoma but upon retrieval and reexamination microscopically were found to be negative for prostate adenocarcinoma.
- 4) Archival 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks from prostate adenocarcinoma patients that were found to be in poor condition on gross evaluation.

3.5 Sampling technique and sample size determination

3.5.1 Sampling techniques

Purposive sampling technique as per the outlined inclusion criteria and exclusion criteria was used.

3.5.2 Sample size determination

Sample size was calculated based on the number of prostate cancer specimens received at AIC Kijabe Hospital, annually. In 2012-2014, the pathology department received 200-450 prostate cancer prostatectomy specimens annually. Therefore, a population size of 450 archival tissue blocks was used to determine the sample size.

Cochran's formula for categorical data for an alpha rank a priori at 0.05 was used to get minimum sample size (Cochran, 1977).

$$N_0 = [(t^2)(p)(q)] / (d^2)$$

Where,

N_0 = minimum sample size required

$$t = 1.96$$

p = maximum possible proportion. A value of 0.5 was used as the frequency of PCa adenocarcinoma at AIC Kijabe was unknown in 2012-2014.

$$q = 1 - p = 0.5$$

d = acceptable margin of error for the proportion being estimated = 0.05.

Substituting these values in the formula above, gives:

$$N_0 = [(1.96^2) (0.5) (0.5)] / (0.05^2)$$

$$N_0 = [(3.842) (0.5) (0.5)] / (0.003)$$

$$N_0 = 384$$

Therefore, the minimum sample size = 384

$$N_1 = N_0 / (1 + N_0 / \text{population})$$

Where,

N_1 = final sample size

$$N_0 = 384$$

Therefore, final sample size = $384 / (1 + 384/450) = 207$

Adding 1.5% for possible spoilage of tissue blocks = $101.5/100 \times 207 = 210.1$

Therefore, the final sample size for the study was 210 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks.

3.6 Recording of demographic and clinical information

This information was gotten from the pathology laboratory information system at the pathology department (File maker pro-software, Apple Inc, USA). Information on the patient's age, ethnic origin (Bantu, Nilote, Cushite) and gland weight in grams was retrieved and directly entered into excel spreadsheets (Microsoft® Excel 2016). Unique accession numbers were recorded to aid in retrieval of the paraffin tissue blocks.

3.7 Laboratory procedures and research instruments

3.7.1 Retrieval of 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded archival tissue blocks

The acquired demographic and clinical data was used to help in retrieving 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded archival tissue blocks as per the inclusion criteria. Pathologist assistants retrieved the tissue blocks from the archives using the unique accession numbers. The tissue blocks were assessed grossly and those in good condition included. The best tissue block per case was selected.

3.7.2 Histopathology procedures

One histopathology section per case was cut out of the retrieved 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks using a microtome as per microtomy protocol (*Appendix II*). Each tissue section was then stained using routine histological Hematoxylin and Eosin stains as per protocol (*Appendix III*). These stained slides were then mounted in mounting media and coverslipped. They were then microscopically evaluated by two independent histopathologists. Histopathological features including tumor type, Gleason score / ISUP grade group, perineural invasion, lymphovascular invasion, tumor bulk; tumor margins and stage were determined. Cases with discordant results underwent a consensus review at a multithreaded microscope with a third histopathologist. The PCa histopathological characteristic findings were entered into a specially designed data collection form (*Appendix I*).

3.7.3 Immunohistochemistry procedures

Three PCa tumor-rich sections per case were selected and cut from the retrieved tissue blocks using a microtome as per microtomy protocol (*Appendix II*) for immunohistochemical staining for androgen receptor, HER₂/neu receptor expression and p53 protein expression. Immunohistochemistry was performed using standard IHC protocols for AR, HER2 and p53 (*Appendix IV, VI, VIII respectively*). The IHC staining for AR, HER2 and p53 was interpreted as per manufacturer's guidelines (*Appendix V, VII, IX respectively*). The IHC status was analysed by two pathologists. Cases with discordant results underwent a consensus review at a multithreaded microscope with a third pathologist. The PCa AR, HER₂/neu and p53 immunohistopathological findings were entered into the specially designed data collection form (*Appendix I*).

3.8 Data analysis and presentation

3.8.1 Histopathological and immunohistopathological characteristics

The data collected was inputted and sanitized in excel spreadsheets (MS[®] Office) and exported into IBM[®] SPSS Statistics 21.0 (SPSS Inc. Chicago, USA) for coding and statistical analyses. Data obtained from the histopathological and immunohistopathological characteristics of each retrieved PCa tumor block was captured in a consolidated raw registration form and entries done into Excel spreadsheet package workbook (Microsoft office version 2010) by assigning characteristics as either categorical or continuous variables. Data entry was also duplicated by importing Excel captured data onto SPSS statistical application software programme (IBM SPSS Inc. application software version 21.0.1, 2010). Cleaned computed and coded relevant data was assessed to eliminate non-specific information. Continuous data (age and gland

weight) summarized as means and range, and dichotomous measures (ethnicity, histopathological and immunohistochemical profiles) summarized as frequencies (n, %) were tabulated. PNI, LVI, and Gleason score / ISUP grade groups and immunohistochemistry were presented as plates. AR, Her₂/neu and p53 expression rates in PCa summarized as % positivity were presented in bar graphs.

3.8.2 Associations of histopathological with immunohistopathological characteristics

Data analysis was done using several analytical model platforms to test for significance in associations and relationships between the dependent and the independent variables. Results are portrayed as odds ratios (OR) and 95% confidence intervals (CI), and β coefficient for each of the models. Summarized results were tabulated and *P* values of <0.05 were considered statistically significant.

3.8.2.1 Association of high grade Gleason score (≥ 8) with PNI, level of gland involvement and AR expression

Binary logistic regress models were performed to identify the histopathological and immunohistochemical indicators of the high Gleason scores (≥ 8 Gleason scores). In these regression analyses, the Gleason score (≥ 8) was entered as the dependent variable and Gleason score <8 entered as the reference group. The indicator variables comprised of presence of PNI, with absence of PNI as the reference; tumor size of at least 50% with tumor size of less than 50% as the reference group; and positive AR expression with negative AR expression as the reference group. The confounding effect of

ethnicity, age, prostate gland mass and tumor type were controlled for in these regression models.

3.8.2.2 Association of pathological stage III with PNI, LVI, level of gland involvement and AR expression

Binary logistic regress models were performed to identify the histopathological and immunohistochemical indicators of the pathological stage. In these regression analyses, the pathological stage were entered as the dependent variable with stage II entered as the reference group and stage III as the predictor group. The indicator variables comprised presence of PNI, with absence of PNI as the reference; tumor size of at least 50% with tumor size of less than 50% as the reference group. The confounding effect of ethnicity, age, prostate gland mass and tumor type were controlled for in these regression models.

3.8.2.3 Association of AR expression with PNI, Gleason score ≥ 8 , ISUP grade and pathological stage III

Binary logistic regress models were performed to identify the histopathological and immunohistochemical indicators of AR expression. In these regression analyses, the AR expressions were entered as the dependent variable with negative AR expression entered as the reference group and positive AR expression as the predictor group. The indicator variables comprised presence of PNI, with absence of PNI as the reference; A Gleason score of ≥ 8 , with a Gleason score of < 8 as the reference and PCa pathological stage III with PCa pathological stage II as the reference group. The confounding effect of ethnicity, age, prostate gland mass and tumor type were controlled for in these regression models.

3.8.2.4 Association of p53 over-expression with PNI, and pathological stage III

Binary logistic regress models were performed to identify the histopathological and immunohistochemical indicators of p53 expression. In these regression analyses, the p53 expressions were entered as the dependent variable with negative p53 expression entered as the reference group and positive p53 expression as the predictor group. The indicator variables comprised presence of PNI, with absence of PNI as the reference and PCa pathological stage III with PCa pathological stage II as the reference group. The confounding effect of ethnicity, age, prostate gland mass and tumor type were controlled for in these regression models.

3.9 Ethical considerations

This study was undertaken using 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded archival tissue blocks. Therefore, informed consent from the patients was not obligatory since ethical approval was obtained from the AIC Kijabe Hospital research ethics committee (*Appendix XIV*). Ethical approval was also obtained from National Commission for Science Technology and Innovations (NACOSTI) (*Appendix XVI*) and a permit granted (*Appendix XVII*). Non-maleficence exposing risk or harm to the patients was non-existent in this study since it was performed on 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded archival tissue specimens. Confidentiality was maintained throughout the study by masking all patient identifying information. This was achieved by anonymously coding all the retrieved 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded tissue blocks and utilizing these anonymous codes throughout the data collection, analysis and presentation. Justice was met by ensuring that all the study subjects who qualified to be included in this study had

an equal chance of being sampled. All the tissue blocks sampled for the study underwent similar handling and laboratory procedures. Beneficence was achieved by submitting a copy of the findings of this study to the AIC Kijabe Hospital management.

CHAPTER FOUR: RESULTS

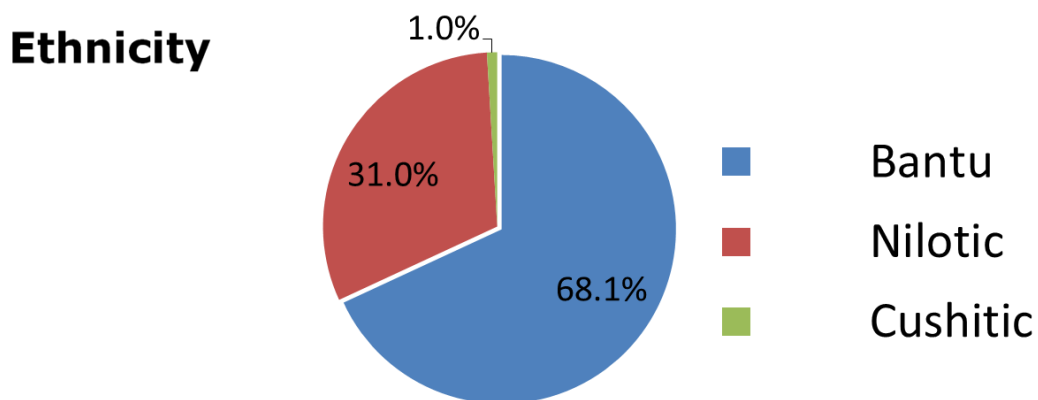
4.1 Introduction

In this chapter, the following results have been presented: Patient demographics and prostatectomy specimen gross characteristics; Histopathological features of the prostatectomy specimens; The evaluation of the AR, HER₂ /neu and p53 protein IHC expression and the association between different histopathological and IHC staining characteristics

4.2 Patient demographics and prostatectomy specimen gross characteristics

A total of two hundred and ten (210) prostatectomy archival paraffin tissue blocks were studied. The demographics of the patients and the gross pathological features of the simple prostatectomy specimen are demonstrated in Table 4.1. The mean (interquartile) age for the patients was 74.0 (45.0-99.0) years. The patients' ethnic extraction was as follows; Bantu (68%), Nilotic (31.0%) and Cushitic (1.0%) groups. The mean (interquartile) prostate gland weight (g) was 36.0 (4.0-239.0).

Table 4.1: Demographics and gross specimen weight. Data presented as means and range for age and gland mass; and as number (n) and proportion (%) of subjects for ethnicity.



Gland weight, grams 36.0 (4.0-239.0)

4.3: The histopathological characteristics of the prostatectomy specimens

The histopathological features of the archival prostate tissue blocks analyzed in this study are presented in Table 4.2. The microscopic examination of the prostate specimens revealed that 97.6% were of acinar prostatic adenocarcinoma type while 2.4% were of ductal prostatic adenocarcinoma type. Tumor involving more than 50% of the gland was identified in 19.5% of the specimens while 80.5% of the specimens had less than 50% of the gland involved by tumor. The rate of high grade Gleason prognostic groups (≥ 8) was 56.6% and low grade Gleason prognostic group (< 8) was 43.4% (Plate, Figure 4.1). In addition, ISUP grade groups were as follows: ISUP 1, 23.3%, ISUP 2, 16.2%, ISUP 3, 17.1%, ISUP 4, 10.5% and ISUP 5, 32.9%. PNI (Plate 4.2) was present in 56.7% of the specimens and LVI (Plate 4.3) was present in 25.2% of the specimens. Positive surgical margins (pathological stage III) were present in 27.1% of the specimens and negative surgical margins (pathological stage II) were 72.9%.

Table 4.2: Histopathological characteristics of PCa in archival tissue blocks. Data Presented as number and proportion (%) of subjects after microscopic evaluation. PNI, perineural invasion. LVI, lymphovascular invasion. ISUP, international society of urological pathologists. Pathological stage II, Prostate adenocarcinoma with negative surgical margins. Pathological stage III, Prostate adenocarcinoma with positive surgical margins.

Histopathological characteristics	Number of subject specimen, n (%)
Type of cancer	
Acinar	205/210 (97.6)
Ductal	5/210 (2.4)
Tumor size, (% of gland involvement)	
≥50	41/210 (19.5)
<50	169/210 (80.5)
PNI	
Yes	119/210 (56.7)
No	91/210 (43.3)
LVI	
Yes	53/210 (25.2)
No	157/210 (74.8)
Gleason prognostic grade	
<8 (Low grade)	119/210 (56.6)
≥8 (High grade)	91/210 (43.4)
ISUP Grade groups	
Grade 1	49/210 (23.3)
Grade 2	34/210 (16.2)
Grade 3	36/210 (17.1)
Grade 4	22/210 (10.5)
Grade 5	69/210 (32.9)
Surgical margins positivity	
Yes	57/210 (27.1)
No	153/210 (72.9)
Pathological stage	
II	153/210 (72.9)
III	57/210 (27.1)

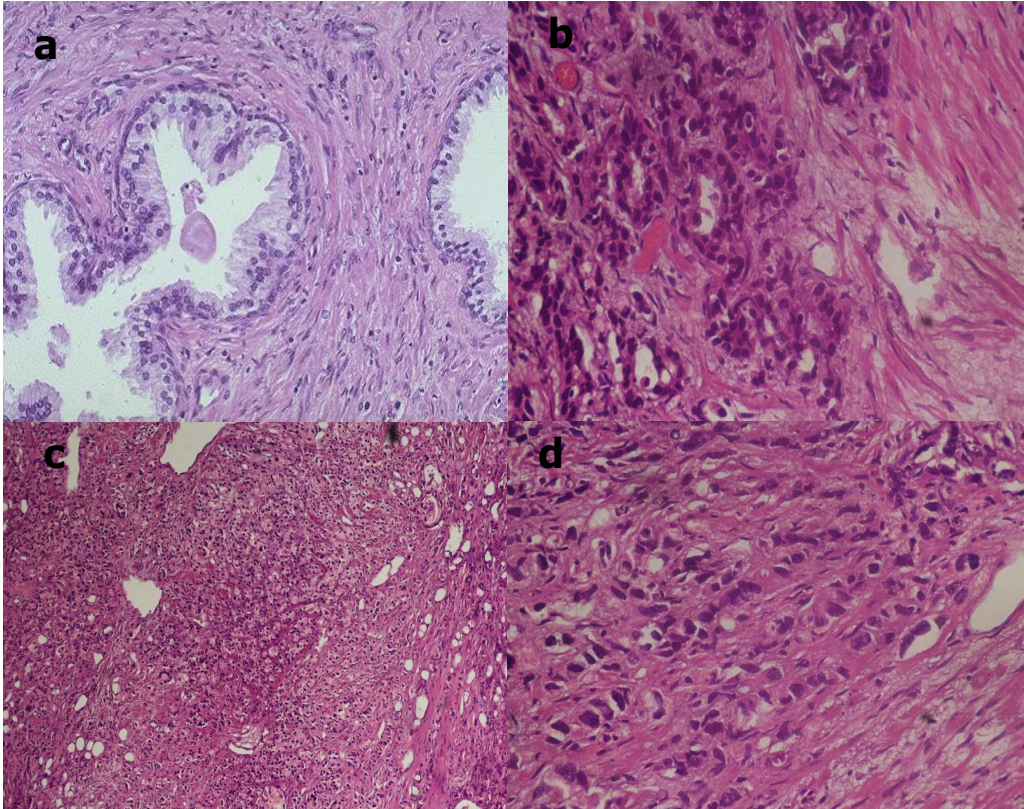


Figure 4.1: Plate showing prostate cancer grades (x40, H&E)

(a) Benign prostatic tissue, (b) PCa Gleason scores 3 + 4 = 7/10, ISUP grade group 2, (c) PCa Gleason scores 4 + 4 = 8/10, ISUP grade group 4, (d) PCa Gleason scores 5 + 5 = 10/10, ISUP grade group 5.

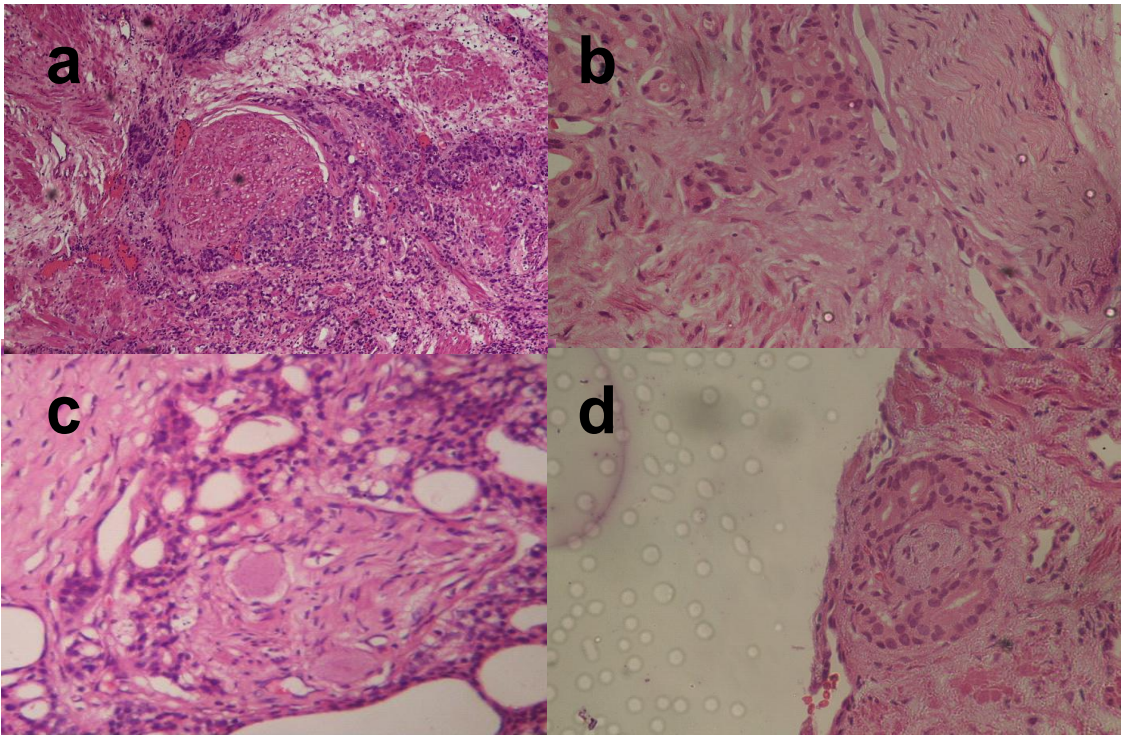


Figure 4.2: Plate showing different forms of PCa PNI (x40, H&E)
 (a) Semi circumferential PNI, (b) Longitudinal PNI, (c) Complete circumferential PNI, (d) PNI around a small nerve.

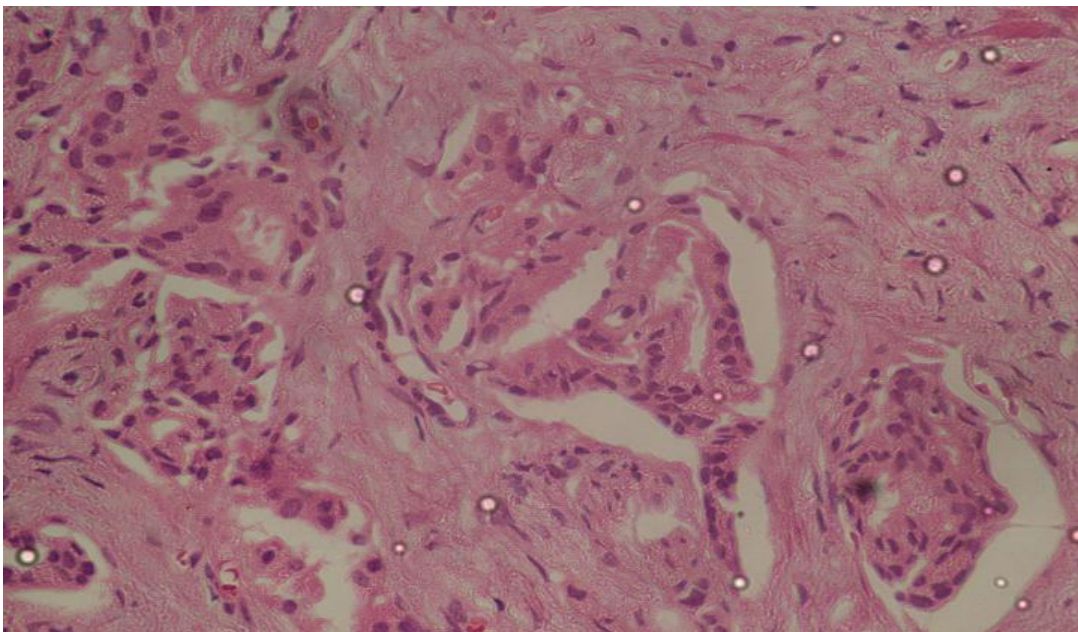


Figure 4.3: Plate showing LVI (x40, H&E). Shows tumor emboli within several small sized endothelial lined lymphovascular channels.

4.4: Immunohistochemical staining characteristics of the PCa

The immunohistochemical staining for AR showed that 65.2% of the tumors had strong nuclear positive staining. The AR staining findings are presented as bar graph in figure 4.4 and as immunohistochemical plate in figure 4.5.

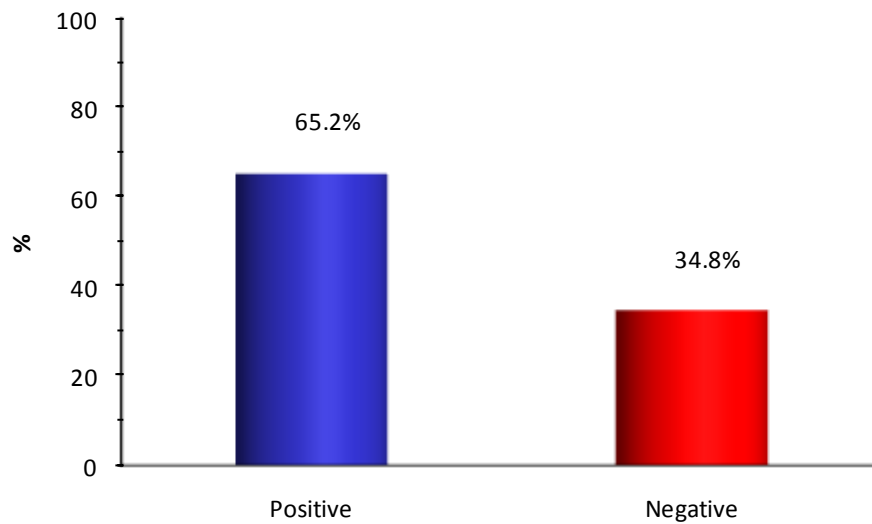


Figure 4.4: Bar graph demonstrating AR expression in PCa on archival tissue blocks. Data presented as proportion (%) of subjects (n=210).

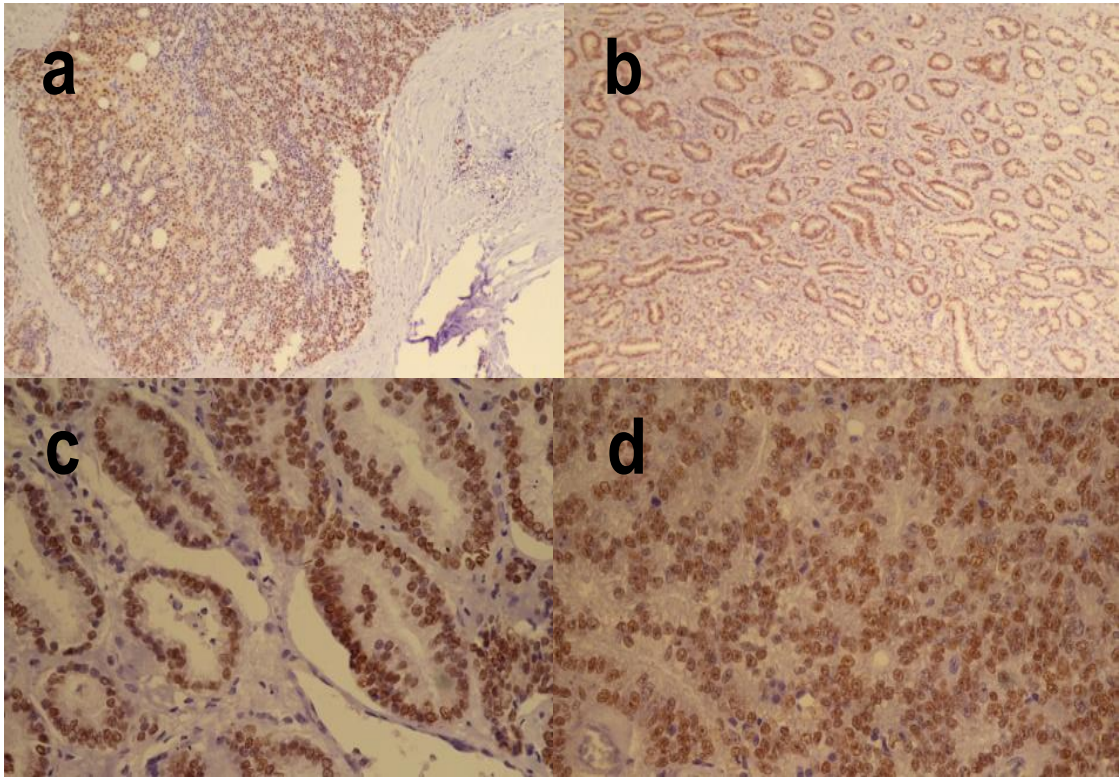


Figure 4.5: Plate showing positive AR IHC staining(x40). Strong nuclear staining for AR in different PCa grades; (a) PCa Gleason score 3 + 4 = 7, ISUP grade group 2, (b) PCa Gleason score 3 + 3 = 6, ISUP grade group 1, (c) PCa Gleason score 3 + 3 = 6, ISUP grade group 1, (d) PCa Gleason score 5 + 5 = 10, ISUP grade group 5.

The immunohistochemical staining for HER₂/neu showed that only 4.5% of the tumors had positive membrane staining. The membrane staining showed weak intensity. The HER₂/neu staining findings are presented as bar graph in Figure 4.6 and as immunohistochemical plate in Figure 4.7.

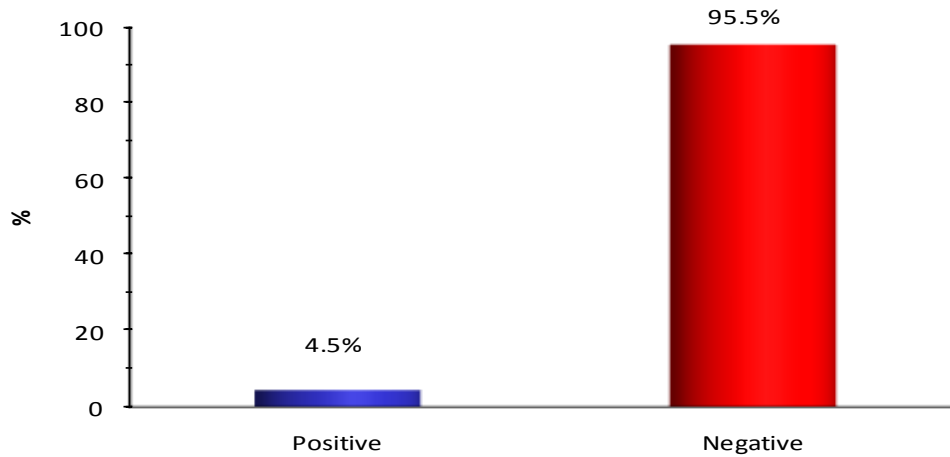
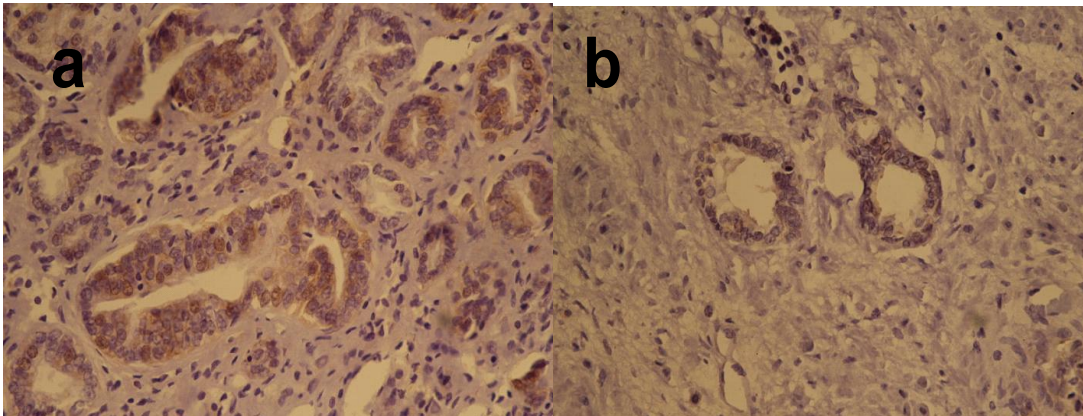


Figure 4.6: Bar graph showing proportion of PCa HER₂/neu expression Data presented as proportion (%) of subjects (n=210).



**Figure 4.7: Plate showing HER₂/neu IHC staining (x40)
The plate shows, (a) Weak intensity membrane staining for HER₂/neu receptor. (b) Negative membrane staining for HER₂/neu receptor.**

The immunohistochemical staining for p53 protein showed that 19.7% of the PCa had strong nuclear positive staining. The p53 protein staining findings are presented as bar graph in Figure 4.8 and as immunohistochemical plate in Figure 4.9.

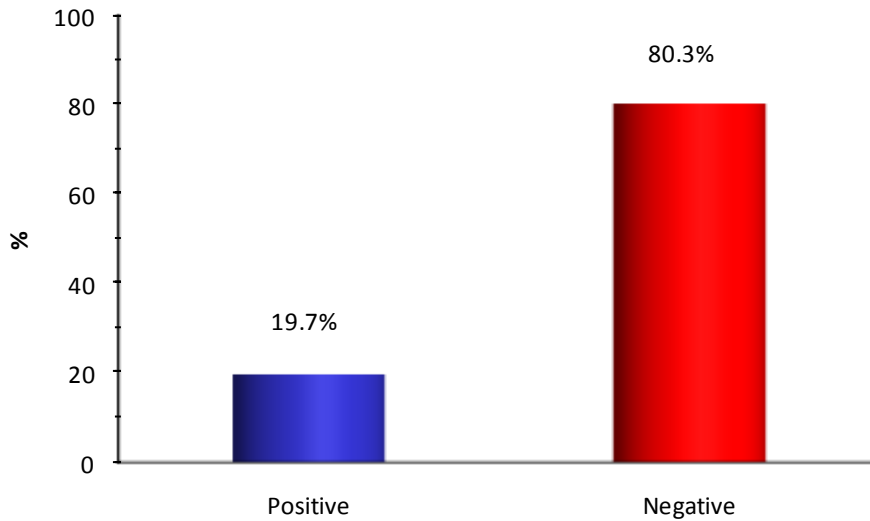


Figure 4.8: Bar graph showing proportion of PCa p53 protein over expression
Data are presented as proportion (%) of subjects (n=210).

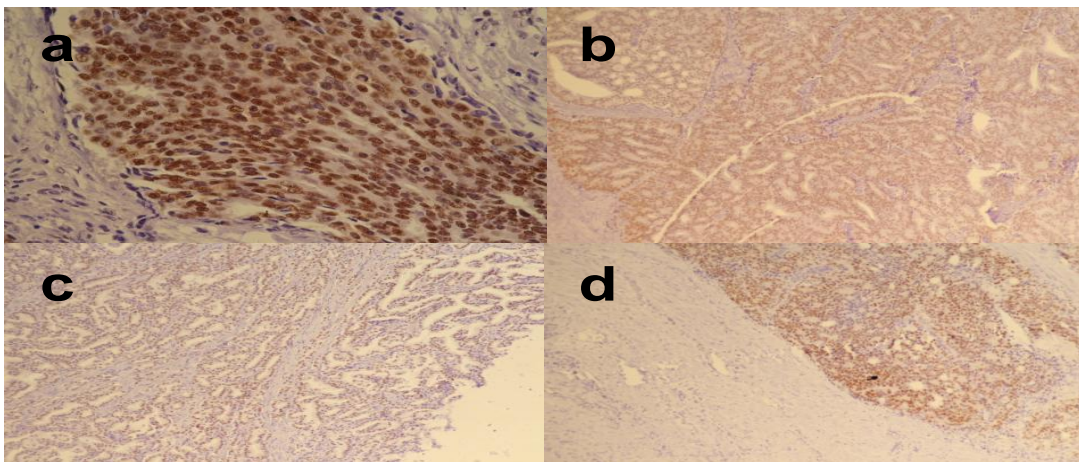


Figure 4.9: Plate demonstrating strong p53 nuclear staining. Strong p53 nuclear staining in different PCa grades (a) PCa Gleason scores 5 + 5 =10, ISUP grade group 5, (b) PCa Gleason scores 3 + 4 = 7, ISUP grade group 2, (c) PCa Gleason scores 3 + 3 = 6, ISUP grade group 1, (d) PCa Gleason scores 4 + 3 = 7, ISUP grade group 3.

4.5 The association between different HPCs and IHC staining characteristics on archival PCa tissue at the AIC Kijabe Hospital

Several histopathological and immunohistochemical characteristics were selected evaluated for associations. This was performed using binary logistic regression models.

4.5.1. Association of high grade Gleason score (≥ 8) with PNI, level of gland involvement and AR expression

Evaluation of the link between the high grade Gleason score (≥ 8) and the histopathological and immunohistochemical characteristics of the PCa (Table 4.3) showed a likelihood of PNI ($\beta = -3.699$; OR, 40.407; 95% CI, 11.036-147.949; $P < 0.0001$) and tumor size $> 50\%$ ($\beta = 3.416$; OR, 30.447; 95% CI, 7.220-128.397; $P < 0.0001$) and unlikelihood of AR expression ($\beta = -2.215$; OR, 0.109; 95% CI, 0.019-0.621; $P = 0.013$).

Table 4.3: Association of high grade Gleason score (≥ 8) with PNI, % gland involvement and AR expression. Data shown as odds ratios (OR) and 95% confidence interval (CI). β coefficient for the model. P value less than 0.05, significance level. Ref, reference group. PNI, perineural invasion. AR, androgen receptor.

<i>Indicator</i>	Gleason score ≥ 8		
	β	<i>OR (95% CI)</i>	<i>P</i>
PNI			
<i>Absent</i>	Ref		
<i>Present</i>	3.699	40.407 (11.036-147.949)	<0.0001
% Gland involved			
<i><50%</i>	Ref		
<i>$\geq 50\%$</i>	3.416	30.447 (7.220-128.397)	<0.0001
AR expression			
<i>Absent</i>	Ref		
<i>Present</i>	-2.215	0.109 (0.019-0.621)	0.013

4.5.2. Association of pathological stage III with PNI, LVI, level of gland involvement and AR expression

PCa samples exhibiting pathological stage III (Table 4.4) were more likely to have PNI ($\beta=3.630$; OR, 37.710; 95% CI, 8.762-162.288; $P<0.0001$); LVI ($\beta=3.512$; OR, 33.531; 95% CI, 13.757-81.729; $P<0.0001$); and tumor size $>50\%$ ($\beta=0.829$; OR, 2.291; 95% CI, 1.040-50.50; $P=0.040$) but negative AR expression ($\beta=-0.980$; OR, 0.375; 95% CI, 0.091-1.551; $P=0.176$).

Table 4.4: Association of Pathological stage III with PNI, LVI, % gland involvement and AR expression. Data shown as odds ratios (OR) and 95% confidence interval (CI). β coefficient for the model. P value less than 0.05, significance level. Ref, reference group. PNI, perineural invasion. LVI, lymphovascular invasion. AR, Androgen receptor.

PCa pathological stage III			
<i>Indicator</i>	β	OR (95% CI)	P
Perineural invasion			
<i>Lacking</i>	Ref		
<i>Evident</i>	3.630	37.710 (8.762-162.288)	<0.0001
Lymphovascular invasion			
<i>Lacking</i>	Ref		
<i>Evident</i>	3.512	33.531 (13.757-81.729)	<0.0001
%Gland involvement			
<i><50%</i>	Ref		
<i>$\geq 50\%$</i>	0.829	2.291 (1.040-50.500)	0.040
AR expression			
<i>Lacking</i>	Ref		
<i>Evident</i>	-0.980	0.375 (0.091-1.551)	0.176

4.5.3: Association of AR receptor expression with PNI, Gleason score ≥ 8 , ISUP grades and pathological stage III

Evaluation of histopathological predictors of AR expression (Table 4.5) indicated that patients exhibiting PNI ($\beta=-1.434$; OR, 0.238; 95% CI, 0.064-0.883; $P=0.032$); Gleason score ≥ 8 ($\beta=-2.864$; OR, 0.057; 95% CI, 0.009-0.383; $P=0.003$); and Epstein

grades 2 ($\beta=-2.641$; OR, 0.071; 95% CI, 0.007-0.707; $P=0.024$), 3 ($\beta=-3.543$; OR, 0.029; 95% CI, 0.002-0.436; $P=0.010$), 4 ($\beta=-3.541$; OR, 0.029; 95% CI, 0.002-0.360; $P=0.006$), and 5 ($\beta=-6.333$; OR, 0.002; 95% CI, 0.000-0.071; $P=0.001$) were considerably less likely to express the AR. In addition, patients presenting with pathological stage III were non-significantly less likely to express the AR ($\beta=-1.253$; OR, 0.286; 95% CI, 0.067-1.212; $P=0.089$; Table 4.5).

Table 4.5: Association of AR receptor expression with PNI, Gleason score ≥ 8 , ISUP grades and pathological stage III. Data demonstrated as odds ratios (OR) and as 95% confidence interval (CI). β coefficient for the model. P value less than 0.05, significance level. AR, Androgen receptor. Ref, reference group. PNI, perineural invasion, ISUP, International society of urological pathology grade groups. Stage III, Positive margins.

Indicator	AR expression		
	β	OR (95% CI)	P
Perineural invasion			
<i>Absent</i>	Ref		
<i>Present</i>	-1.434	0.238 (0.064-0.883)	0.032
Gleason score			
<8	Ref		
≥ 8	-2.864	0.057 (0.009-0.383)	0.003
ISUP grade			
<i>I</i>	Ref		
<i>2</i>	-2.641	0.071 (0.007-0.707)	0.024
<i>3</i>	-3.543	0.029 (0.002-0.436)	0.010
<i>4</i>	-3.541	0.029 (0.002-0.360)	0.006
<i>5</i>	-6.333	0.002 (0.000-0.071)	0.001
Pathological stage			
<i>II</i>	Ref		
<i>III</i>	-1.253	0.286 (0.067-1.212)	0.089

4.5.4: Association of p53 over-expression with PNI and pathological stage III

Evaluation of histopathological predictors of p53 protein over expression (Table 4.6) illustrated that PNI was significantly less likely to be associated with p53 expression

($\beta=-1.852$; OR, 0.157; 95% CI, 0.032-0.778; $P=0.023$) but not pathological stage III ($\beta=-0.163$; OR, 0.850; 95% CI, 0.205-3.513; $P=0.822$).

Table 4.6: Association of p53 protein over-expression with PNI and pathological stage III. Data demonstrated as odds ratios (OR) and as 95% confidence interval (CI). β coefficient for the model. P value less than 0.05, significance level. Ref, reference group. PNI, perineural invasion, Stage III, Positive margins.

Predictor	p53 protein		
	β	OR (95% CI)	P
Perineural invasion			
<i>Absent</i>	Ref		
<i>Present</i>	-1.852	0.157 (0.032-0.778)	0.023
Pathological stage			
<i>II</i>	Ref		
<i>III</i>	-0.163	0.850 (0.205-3.513)	0.822

CHAPTER FIVE: DISCUSSION

5.1. Patient demographics

The mean age of the subjects with prostate cancer was 74 years. This is 5 years older compared to a mean age of PCa diagnosis of 69 years in the United States and 69.5 years in a Ugandan study (Okuku et al., 2016). These differences might be due to the high age-standardized incidence of prostate cancer (per 100,000) in USA (124.8) and in Uganda (38) compared to Kenya's (16.6) (Haas, Delongchamps, Brawley, Wang & Roza, 2008). The difference with the USA mean age can also be attributed to the intensity of screening and diagnostic efforts that leads to detection of occult prostate cancer disease among Americans. The youngest subject was 45 years old. This might point to a form of familial prostate cancer risk as some research has demonstrated that an affirmative familial history of prostate cancer is a hazard for acquiring PCa in younger men (Henrik, Damber, L. & Damber, J., 2006).

Regarding ethnicity of the subjects, 68% were Bantu, 31% Nilotes and 1% of Cushitic extraction. These proportions roughly correspond to the Kenyan national population of these different ethnic groups (Kenyan National Bureau of Statistics, 2010). However, the geographical habitation and the origin of these distinct ethnic groups are different hence their genetic make-up and risk for developing prostate cancer might be different.

5.2. Gross simple prostatectomy weight

The mean prostate gland weight in the study was 36.0 g. The glands were generally slightly enlarged compared to the mean normal prostate weight of 20g but not as

enlarged as prostate glands with benign prostatic hyperplasia whose mean weight is 100g (Sarma & Wei, 2012). Clinically, the size of the prostate gland is assessed using digital rectal examination and through transurethral ultrasonography. These two modalities plus elevated PSA levels are used to detect prostate cancer in patients. Currently, in resource endowed centers most prostate cancers are diagnosed on needle biopsy, rarely in transurethral resection specimens. However, in resource poor settings a number of prostate cancers are still diagnosed on prostatectomy specimen.

5.3. Histopathological findings in PCa

Upon histopathological evaluation, 97.6% of the PCa were of acinar adenocarcinoma type while the rest were of ductal adenocarcinoma type. This is comparable to published data showing that 95 to 99.6 % of PCa are of acinar type while 0.4 to 5% is of ductal type (Bock & Bostwick, 1999). Ductal type prostatic adenocarcinomas have been demonstrated to have worse outcome compared to the acinar type (Todd, Christopher, Funda, Daniel, & Jonathan, 2010). However, the number of ductal adenocarcinoma type in this study was too few (5/210) to have any statistical significance on analysis.

The tumor bulk in most of the specimen was voluminous with 80.5% of the cases having more than 5% cancer volume compared to 19.5 % that had minute tumor (less or equal to 5%). However, the utility of estimating tumor volume in prostate cancer is contentious notwithstanding a prior consensus on the role of cancer size as a staging criterion for several types of solid neoplasms. This is because prostate cancers demonstrate a more uneven growth pattern (Epstein, 2011).

Evaluation for PNI showed that 56.7% of the cases had PNI while 43.3% showed no PNI. Prostatic perineural invasion is thought to be mediated by tumor expression of nerve cell adhesion molecule (Li, Wheeler, Dai & Ayala, 2003). PNI in PCa is a poor prognostic factor since it has been shown that these tumors acquire a growth advantage compared with PCa without perineural invasion (Fromont et al., 2012). It is a less appreciated metastatic pathway for a number of malignancies including prostatic carcinoma, pancreatic carcinoma, colorectal carcinoma, head and neck malignancies, biliary tract malignancies and gastric carcinomas. In most of these malignancies, PNI has been demonstrated to be an indicator of worse prognosis and signifies shorter survival (Liebig et al., 2009). D'Amico et al. (2001) demonstrated PNI to be an autonomous projecting factor for PCa reappearance following radical prostatectomy. However, Reeves et al. (2015) in their study claimed that PNI on radical prostatectomy specimen is not an autonomous forecaster of biochemical cancer reappearance and hence they don't advocate for routine reporting of PNI.

A study by DeLancey et al (2013) established that PNI is autonomously connected to unfavorable pathologic characteristics and poor survival outcomes following radical prostatectomy. Therefore, PNI in prostatic biopsy specimens is recommended to be factored in prostate cancer management assessment planning and clinical care plan. Work based on the action by the histomorphology-based extrapolative characteristic working group of the 2004 WHO-facilitated International Consortium on handling and reporting of prostate cancer protocol (Epstein et al., 2005) recommends that PNI status be included in all prostate cancer reports. Further studies are recommended

targeting the biology of PNI to increase the understanding of its function in prostate cancer disease progression.

LVI is defined as the spread of a cancer cells into the blood vessels and lymphatic vessels. It is identified morphologically in the peritumoural area. It is a crucial step in the infiltration-metastasis progression of malignancies and is considered a marker of metastatic capability strongly linked with a worse outcome in many solid neoplasms. Malignant cells have the potential to interrelate with the endothelium, permeate the lymphovascular wall and tolerate the intravascular forces through different molecular mechanisms. LVI is the commonest route of metastases for malignancies. The other routes are direct spread and transcoelomic spread. When the tumor cells invade the lymphatics or blood vessels, the chance of this malignancy to spread to regional and distant parts of the body is very high (Cheng et al., 2005). In this study, LVI was demonstrated in 25.2% of the prostate cancer cases. This is comparable to findings by Yong et al. (2016) that showed LVI incidence of 20% and 21.5% respectively.

However, other studies by Mitsuzuka et al., (2015) and Matthias et al., (2006) showed significantly lower incidences of LVI of 10.4% and (10.2%). This can be attributable to the fact that these studies were done in populations where prostate cancer screening is intensive and hence the study population has generally low stage prostate cancers. LVI has been demonstrated to be a very important predictive feature in prostate cancer since it is significantly linked to higher presurgical serum PSA, high pathological stage, higher Gleason grade score, positive surgical excision margins, extra prostatic spread, seminal vesicle infiltration, nodal spread and

perineural infiltration (Yong et al., 2016; Mitsuzuka et al., 2015; Matthias et al., 2006). This is also backed by the histomorphology-based predictive characteristics committee of the 2004 World Health Organization-facilitated International Consortium on handling and reporting of prostate cancer protocol (Epstein et al., 2005).

In this study, low grade prostatic adenocarcinoma (Gleason score ≤ 7) constituted 56.6% while high grade prostatic adenocarcinoma (Gleason score ≥ 8) constituted 43.4%. This is dissimilar to findings by Schreiber et al., 2016 that indicated that low grade carcinoma constituted 90.2% while high grade carcinoma constituted only 9.8%. This indicates that compared with other study populations, prostate cancer patients at AIC Kijabe Hospital tend to present with higher grade disease. This might be due to late diagnosis.

In this study, the proportions of the different ISUP grades groups were as follows; ISUP Grade group 1 (23.3%), Grade group 2 (16.2%), Grade group 3 (17.1%), Grade group 4 (10.5%) and Grade group 5 (32.9%). This contrasts with findings by Samaratunga & Delahunt (2015). In their study, they found ISUP Grade group 1 (13.5%), Grade group 2 (49.6%), Grade group 3 (17.6%) Grade group 4 (3.7%) and Grade group 5 (15.6%), respectively. Their study showed that only 36.9% of the cases were \geq ISUP grade 3 while this study showed that 60.5% of the cases were \geq ISUP grade 3. These findings indicate that PCa patients in this study had higher ISUP grade prostate carcinoma compared with the western population.

Cancer staging scheme is a universal way for the malignancy care teams to illustrate the extend of malignancy spread. The commonly used cancer staging system for PCa is the American Joint Committee on Cancer (AJCC) that utilizes Tumor characteristics, Nodal spread, and distant Metastases (TNM) system. Prostate cancer patients undergo two types of staging; the clinical cancer stage is the clinician's best approximation of the degree of prostate cancer disease, using the results of the digital rectal examination, laboratory tests, prostatic biopsy and any imaging studies performed. After surgery, the pathologists establish the pathologic stage based on the clinical stage and the histomorphological features of the submitted specimen. Pathological stage is superior to the clinical staging (Buyyounouski et al., 2017).

Deciding the cancer stage gives a methodical way of describing the quantity and the scope of a neoplasm at a specific point in time. In this study, 27.1% of the cases had positive surgical margins and hence were stage III prostate cancers. This compares with findings by Liang et al. (1999) who found that margin positivity rate for prostate cancer was 29%. They also found that surgical margin status was a sovereign forecaster of evolution post radical prostatectomy.

5.4. Immunohistochemical findings in PCa

Androgens are male sex hormones including testosterone, dehydroepiandrosterone, androstenedione, androstenediol, androsterone, and dihydrotestosterone (DHT). Determination of the androgen receptor (AR) status therefore can assist in forecasting androgen hormone reaction and disease progression in prostate cancer. Hormonal treatment either medical or surgical castration is still the foundation of systemic

management of prostate cancer (Alia et al., 2014; Grossmann et al., 2001). This study showed that 34.8% of the prostate cancers were AR negative. This percentage of AR negative prostate cancer is double the findings by Pertschuk et al. (1995) who found that only 16.7% of the prostate cancers were AR negative. In their study, the AR negative patients represented a subset of prostate cancer patients with poor prognosis evidenced by a 2.5 times greater death risk compared with AR positive patients. Takeda et al. (1996) demonstrated that PCa with a lower Gleason score had appreciably more androgen receptor substance compared to those with a higher Gleason score while the reverse is true for PCa with high Gleason scores (Takeda et al., 1996).

Heinlein and Chang (2004) established that the AR expression is sustained all through prostate cancer carcinogenesis, and most of androgen-autonomous or hormone noncompliant prostate cancers still articulate AR. Transformation of AR, especially transformations leading to a loosening of the AR ligand specificity, may help in the evolution of prostate cancer and the malfunction of endocrine treatment by permitting AR transcriptional inauguration in response to antiandrogens or other endogenous hormones. Likewise, alterations in the comparative appearance of AR coregulators have been shown to occur with prostate cancer evolution and may add to differences in AR ligand specificity or transcriptional action (Shi et al., 2001).

Studies by Noah et al. (1999) have shown androgen-independent prostate cancers articulate superior levels of the HER₂/neu tyrosine kinase receptor compared to their androgen-reliant peers. HER₂/neu is a proto-oncogene that encodes a transmembrane

receptor from the epidermal growth factor receptors family. Growing evidence shows that HER₂/neu over expression may participate in androgen hormone non response in prostate cancer (Grignon et al., 1997). Therefore, HER₂/neu receptor over expression can be utilised to be a surrogate marker of Androgen independent or insensate prostate cancers. In this study only 4.5% of the PCa cases showed HER₂/neu over expression indicating that most of the cancers are still androgen dependent and hence androgen ablation is still an option for the management of these prostate adenocarcinomas.

The p53 gene is among the commonly mutated tumor suppressor genes in several human cancers. Research has demonstrated that p53 mutations are uncommon in prostate cancer but are linked to progressed disease usually metastatic disease indicating that p53 mutations is a belated occurrence in PCa pathogenesis (Shi et al., 2001). Usually, amplified expression is linked to point mutations in one p53 gene allele with loss of the other allele. Over expression of p53 in a tiny proportion of prostate cancers is associated with a worse outcome in terms of disease evolution and mortality (Grignon et al., 1997; Thomas et al., 1993). Buildup of the protein product can be demonstrated using immunohistochemical technics. In this study, 19.7% of the cases showed p53 over expression. These findings are similar to the findings by Thomas et al. (1993) that showed 13% of the PCa over expressed the p53 protein.

5.5. Relationship between histopathological and immunohistopathological characteristics

The Gleason grading and scoring system is one of the best predictive parameter for prostate cancer. This study showed that there is a positive association of high grade Gleason score (≥ 8) with PNI ($\beta = -3.699$; OR, 40.407; 95% CI, 11.036-147.949; $P < 0.0001$) and tumor involving $> 50\%$ of the prostate gland ($\beta = 3.416$; OR, 30.447; 95% CI, 7.220-128.397; $P < 0.0001$). These findings are consistent with studies showing that high Gleason score, PNI and high tumor bulk are poor prognostic factors. DeLancey et al. (2013) demonstrated that PNI is independently linked with unfavorable pathologic characteristics among them, a high Gleason score. In addition, previous studies showed that PNI and Gleason score to important presurgical determinants of evolution of PCa after surgery (Sebo et al., 2002), while tumor volume correlates with the Gleason grade (Schmid & McNeal, 1992).

The higher the PCa tumor volume, the higher the Gleason score. However, it is questionable whether measurement of tumor volume provides any additional prognostic value more than that provided by the Gleason score. The results of this study showing a negative association of high grade Gleason prognostic group (≥ 8) with AR expression ($\beta = -2.215$; OR, 0.109; 95% CI, 0.019-0.621; $P = 0.013$) parallel data showing that prostate carcinomas with a lower Gleason score show a considerably higher androgen receptor substance than those with a higher Gleason score while the reverse is true for PCa with high Gleason scores (Takeda et al., 1996). Both high Gleason score and negative AR on immunohistochemistry are poor prognostic markers for PCa. Mutations in the AR gene have been picked in high stage

and metastatic PCa and postulated to be the reason why some metastatic PCa are androgen independent (Taplin et al., 1995; Pertschuk et al., 1995).

Pathologic stage is the ultimate indicator of tumour extent and thus it is the most accurate prognostic factor in PCa (Buyyounouski et al., 2017). This study showed a strong positive association between PCa pathological stage III and PNI ($\beta=3.630$; OR, 37.710; 95% CI, 8.762-162.288; $P<0.0001$), LVI ($\beta=3.512$; OR, 33.531; 95% CI, 13.757-81.729; $P<0.0001$); and tumor size $>50\%$ ($\beta=0.829$; OR, 2.291; 95% CI, 1.040-50.50; $P=0.040$). These results are similar to previous study indicating a strong relationship between the level of tumor infiltration (PNI, LVI) and the cancer volume and the rate of cancer recurrence (Wheeler et al., 1998). However, PCa pathological stage III had a negative association with AR expression ($\beta=-0.980$; OR, 0.375; 95% CI, 0.091-1.551; $P=0.176$). This is in keeping with the fact that PCa pathological stage III is advanced PCa and hence it tends to be AR negative due to some late mutations of the AR gene. Some of these PCa are androgen independent (Taplin et al., 1995.; Pertschuk, et al., 1995).

AR receptor expression showed a negative association with PNI ($\beta=-1.434$; OR, 0.238; 95% CI, 0.064-0.883; $P=0.032$); Gleason score ≥ 8 ($\beta=-2.864$; OR, 0.057; 95% CI, 0.009-0.383; $P=0.003$); and Epstein grades 4 ($\beta=-3.541$; OR, 0.029; 95% CI, 0.002-0.360; $P=0.006$), and 5 ($\beta=-6.333$; OR, 0.002; 95% CI, 0.000-0.071; $P=0.001$). In addition, cases presenting with pathological stage III were non-significantly less likely to express the AR ($\beta=-1.253$; OR, 0.286; 95% CI, 0.067-1.212; $P=0.089$). These findings are keeping with the fact that advanced PCa tends to lose AR. Research has

shown mutations of the AR gene in advanced and metastatic PCa and postulated to be the reason why some metastatic PCa are androgen independent (Taplin et al., 1995, Pertschuk et al., 1995).

Over expression of p53 was significantly less likely to be associated with PNI ($\beta=-1.852$; OR, 0.157; 95% CI, 0.032-0.778; $P=0.023$) but not pathological stage III ($\beta=-0.163$; OR, 0.850; 95% CI, 0.205-3.513; $P=0.822$). Although the p53 cancer suppressor gene is transformed in a subset of advanced stage PCa (Thomas, 1993), the value of this finding might not be significantly independent from PCa grade and stage.

5.6 Limitations of the study

This study had limited funds. Therefore, the number of immunohistochemical panels done was limited to just 3 due to financial constraints. The ethnicity of the study subjects was based purely on what the patient or care giver had indicated as the patient's ethnicity. Cases of mixed ethnicity or mistaken ethnicity could have existed. Risk factors including sexual behavior, genital infections and family history of prostate cancer could not be elucidated since this was a cross-sectional study using archival prostatectomy specimens. Other diagnostic parameters including PSA level, digital rectal examination findings and prior prostate needle core biopsy findings were not factored in this cross-sectional study.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Study conclusions

The mean age of the prostate cancer patients seen at AIC Kijabe was 74 years. This is 5 years older than the mean age in Ugandan and United States studies. Ethnicity of the subjects, were as follows; 68% were Bantu, 31% Nilotic and 1% of Cushitic extraction. These proportions roughly correspond to the national population of these different ethnic groups.

Histopathologically, 97.6% of the prostate cancers were of acinar adenocarcinoma type while the rest were of ductal adenocarcinoma type. The tumor bulk in most of the subjects was voluminous with 80.5% of the cases have more than 5% cancer volume compared to 19.5 % that had minute tumor ($\leq 5\%$). Evaluation for perineural analysis showed that 56.7% of the cases had perineural invasion. Lymphovascular invasion was demonstrated in 25.2% of the PCa cases. High grade prostate cancer (Gleason score ≥ 8) constituted 43.4% of the cases. In our study, the frequencies of the different ISUP grades were as follows; ISUP Grade group1 (23.3%), Grade group 2 (16.2%), Grade group 3 (17.1%), Grade group 4 (10.5%) and Grade group 5 (32.9%). 27.1% of the PCa cases had positive surgical margins and hence were pathological stage III.

This study showed that 34.8% of the PCa were AR negative. This percentage of AR negative prostate cancer is double the findings by Pertschuk et al. (1995) among western populations. Only 4.5% of the PCa cases showed HER₂ over-expression indicating that most of the PCa are still androgen dependent. Over-expression of p53 protein was present in 19.7%.

Regression modelling showed that high Gleason score ≥ 8 and pathological grade III were associated with PNI, LVI and at least 50% prostate gland involvement suggesting advanced disease in these patients.

6.2 Study recommendations

1. There is need for wide spread health education on prostate cancer to ensure men are enlightened on ways of preventing prostate cancer. The government should institute national prostate cancer screening programme to ensure that cases of prostate cancer are picked at early stages.
2. County governments should strive to ensure prostate cancer diagnostic personnel and equipments are put in place to ensure there is a standard way of prostate cancer diagnosis for effective prostate cancer management.
3. Immunohistochemistry and other molecular tests for prostate cancer are recommended to enhance understanding of the pathogenesis of prostate cancer. This will include markers for genetic mutations as well as for environmental factors like infections which have been linked with prostate cancer pathogenesis.
4. The findings of this study if adopted will improve the management of PCa and might constitute standard of care in future. This will benefit humans by providing evidence-based diagnosis and treatment of PCa.

6.3 Recommendation for further research

A larger prospective prostate cancer study is recommended encompassing other diagnostic parameters including clinical signs and symptoms, sexual history, PSA levels and prior prostate core biopsy diagnosis.

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APPENDICES

Appendix I: Data Collection Form.

A. BIODATA

- i. Serial Number.....
- ii. Age.....
- iii. Ethnicity.....

B. Gland weight.....

C. Histopathological characteristics

- i. Tumor type.....
- ii. Tumor bulk.....%
- iii. Gleason grade.....
- iv. WHO/ISUP grade group
- v. Perineural invasion.....PresentAbsent.....
- vi. Lymphovascular invasion.....PresentAbsent.....
- vii. MarginsPositiveNegative
- viii. Tumor stage

D. Immunohistochemical characteristics

i. AR EXPRESSION

Proportion.....
 Intensity.....
 Interpretation: PositiveNegative

ii. HER₂/neu EXPRESSION

Proportion.....
 Intensity.....
 Interpretation: PositiveNegative

iii. P53 PROTEIN EXPRESSION

Proportion.....
 Intensity.....
 Interpretation: PositiveNegative

Appendix II: Microtomy protocol.

Materials and Reagents;

Water bath

Container with ice

Glass microscope slides

Microtome and blade

Oven

Sectioning;

1. Chill paraffin-embedded tissue blocks on ice before sectioning. Cold wax allows thinner sections to be obtained by providing support for harder elements within the tissue specimen. The small amount of moisture that penetrates the block from the melting ice will also make the tissue easier to cut.
2. Fill a water bath with ultrapure water and heat to 40 - 45°C.
3. Place the blade in the holder, ensure it is secure and set the clearance angle. The clearance angle prevents contact between the knife facet and the face of the block. Follow the microtome manufacturer's instructions for guidance on setting the clearance angle. For Leica blades this is normally between 1° and 5° (Figure 1).
4. Insert the paraffin block and orientate so the blade will cut straight across the block.
5. Carefully, approach the block with the blade and cut a few thin sections to ensure the positioning is correct. Adjust if necessary.
6. Trim the block to expose the tissue surface to a level where a representative section can be cut. Trimming is normally done at a thickness of 10-30 µm.
7. Cut sections at a thickness of about 4-5 µm.

8. Using tweezers pick up the ribbons of sections and float them on the surface of the water in the water bath so they flatten out. Use the tweezers to separate the sections.
9. Use microscope slides to pick the sections out of the water bath and store upright in a slide rack.
10. Place the slide rack into an oven and allow sections to dry overnight at 37°C.

Appendix III: Hematoxylin and Eosin staining protocol.

Principle

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm (Dhurba, 2015).

Reagents;

1. Harris Hematoxylin stain
 2. A = 1 gm hematoxylin in 10 ml ethanol
 - B = 20 gm ammonium alum in hot distilled waterMix A & B, boil and add 0.5 gm of mercuric oxide and filter.
3. Eosin solution
4. Yellow eosin = 1 gm
 - Distilled water = 80 ml
 - Ethanol = 320 ml
 - Glacial Acetic Acid = 2 drops
5. 0.5% HCl
6. Dilute ammonia water

Procedure

1. Deparaffinize the section: flame the slide on burner and place in the xylene. Repeat the treatment.
2. Hydration: Hydrate the tissue section by passing through decreasing concentration of alcohol baths and water. (100%, 90%, 80%, 70%)

3. Stain in hematoxylin for 3-5 minutes
4. Wash in running tap water until sections “blue” for 5 minutes or less.
5. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes.
6. Wash in running tap water until the sections are again blue by dipping in an alkaline solution (eg. ammonia water) followed by tap water wash.
7. Stain in 1% Eosin Y for 10 minutes
8. Wash in tap water for 1-5 minutes
9. Dehydrate in increasing concentration of alcohols and clear in xylene
10. Mount in mounting media
11. Observe under microscope

Appendix IV: Androgen Receptor IHC Staining Protocol (www.ihcworld.com)**Description**

The androgen receptor (AR) is a NR3 Steroid Receptor located on the X chromosome.

AR is a phosphoprotein and acts as a steroid hormone-activated transcription factor for androgen-responsive genes. Mutations in the AR gene are associated with androgen insensitivity syndrome (CAIS) or testicular feminization syndrome, Reifenstein syndrome, and Kennedy spinal and bulbar muscular atrophy. They lead to symptoms such as low virilization, reduced sperm production, testicular atrophy, and infertility. Immunohistochemistry: LS-B3326 was validated for use in immunohistochemistry on a panel of 21 10% neutral buffered formaldehyde-fixed, paraffin wax-embedded (FFPE) human tissues after heat induced antigen retrieval.

Primary Antibody

Name: Rabbit Anti-AR Antibody

Clone: Rabbit Polyclonal

Supplier: LifeSpan BioSciences

Catalog Number: LS-B3326

Dilution: 1:200 using IHC-Tek™ Antibody Diluent (Cat# IW-1000 or IW-1001) to reduce background and unspecific staining and serum blocking step is NOT needed.

Incubation Time/Temp: 60 min/room temperature

Antigen Retrieval

Device: IHC-Tek™ Epitope Retrieval Steamer Set (Cat# IW-1102)

Buffer/pH value: IHC-Tek™ Epitope Retrieval Solution (Cat# IW-1100)

Heat/Cool Temperature: 95-100 °C/room temperature

Heat/Cool Time: 20 minutes/20 minutes

Detection Methods

Standard Method: ABC Method or LSAB Method

Enhanced Method: Polymeric Methods

Chromogen Substrate

Reagent: DAB Chromogen Substrate

Incubation Time/Temperature: 1-3 minutes/room temperature

Counterstain

Reagent: Mayer's Hematoxylin

Staining Time: 30 seconds

Results:

Staining Pattern: Nuclear

Additional Information:

Species Reactivity: Human, mouse, rat

Fixation: Formalin fixed paraffin sections

Positive Control: Human prostate

Negative Control: Omit primary antibody, isotype control, absorption control

Blocking: 2-5% normal serum to reduce unspecific background staining;

0.5-3% H₂O₂ to block endogenous peroxidase activity; avidin/biotin to

block endogenous biotin activity if necessary.

Appendix V: Allred scoring system for interpreting AR IHC staining

Diagn Pathol. 2014; 9: 221.

Proportion score A	Positive cells, %	Intensity	Intensity score B
0	0	None	0
1	<1	Weak	1
2	1 to 10	Intermediate	2
3	11 to 33	Strong	3
4	34 to 66		
5	>67		

Final score range (A + B): 0-8

Scores of 0 and 2 are considered negative. Scores of 3 to 8 are considered positive

Appendix VI: HER₂/neu IHC Staining Protocol (www.ihcworld.com)

Description: HER₂/neu (c-erbB-2) is a 185 kDa receptor tyrosine kinase belonging to the epidermal growth factor receptor family, which also includes EGFR (HER₁), HER₃ (c-erbB-3), and HER₄ (c-erbB-4). Amplification of the HER-2 gene with overexpression of the protein occurs in 20-30% of breast cancers, and tumor progression in mammary cancer is associated with elevated levels of tyrosine-phosphorylated neu and erbB-3. Tyr is the phosphorylation site that is most tightly linked to oncogenic transformation and coupling to the ras/MAP kinase signaling pathway. Cases of invasive breast carcinoma with the phosphorylated receptor have displayed aggressive clinicopathological features and adverse prognoses. Activation of HER-2 is frequent in ductal carcinoma *in situ* suggesting that HER-2 signaling plays a critical role in the early stages of breast tumorigenesis

Primary Antibody

Name: c-erbB-2/HER-2/neu (phospho-specific) Ab-18 Antibody

Clone: PN2A, Mouse. Supplier: Lab Vision.

Catalog Number: MS-1072-P

Dilution: 1:20 using IHC-TekTM Antibody Diluent (Cat# IW-1000 or IW-1001) to reduce background and unspecific staining and serum blocking step is NOT needed.

Incubation Time/Temp: 60 minutes/room temperature

Antigen Retrieval

Device: IHC-TekTM Epitope Retrieval Steamer Set (Cat# IW-1102)

Buffer/pH value: IHC-TekTM Epitope Retrieval Solution (Cat# IW-1100)

Heat/Cool Temperature: 95-100 °C/room temperature

Heat/Cool Time: 20 minutes/20 minutes

Detection Methods

Standard Method: ABC Method or LSAB Method

Enhanced Method: Polymeric Methods

Chromogen Substrate

Reagent: DAB

Incubation Time/Temperature: 1-3 minutes/room temperature

Counterstain

Reagent: Mayer's Hematoxylin

Staining Time: 30 seconds

Results: Staining Pattern: Membrane

Additional Information:

Tissue Type: Prostate

Fixation: Formalin fixed paraffin sections

Positive Control: EGF-treated SKBR3 cells. About 5% of breast carcinomas are positive for phospho-*c-erbB-2/HER-2/neu* oncoprotein.

Negative Control: Omit primary antibody, isotype control or absorption control

Blocking: 2-5% normal serum to reduce unspecific background staining;

0.5-3% H₂O₂ to block endogenous peroxidase activity; avidin/biotin to block endogenous biotin activity if necessary.

Blocking: 2-5% normal serum to reduce unspecific background staining;
0.5-3% H₂O₂ to block endogenous peroxidase activity; avidin/biotin to
block endogenous biotin activity if necessary

Appendix VII: Interpretation of HER₂/neu IHC staining:

Dako HercepTest™

Scoring guidelines are given by Dako in the Food and Drug Administration (FDA) approved package insert as follows:

Score	Interpretation	Definition
0	Negative	No staining is observed OR membrane staining is in less than 10% of tumor cells.
1+	Negative	A faint, barely perceptible membrane staining is detected in more than 10% of tumor cells. However, only part of the membrane is staining.
2+	Positive	A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.
3+	Positive	A strong complete membrane staining is observed in more than 10% of tumor cells.

Appendix VIII: p53 IHC Staining Protocol (www.ihcworld.com)

Description: p53 is a DNA-binding, oligomerization domain- and transcription activation domain-containing tumor suppressor that upregulates growth arrest and apoptosis-related genes in response to stress signals, thereby influencing programmed cell death, cell differentiation, and cell cycle control mechanisms. p53 localizes to the nucleus yet can be chaperoned to the cytoplasm by the negative regulator MDM2, an E3 ubiquitin ligase that is upregulated in the presence of active p53, where MDM2 poly-ubiquitinates p53 for proteasome targeting. p53 can assemble into tetramers in the absence of DNA, fluctuates between latent and active (DNA-binding) conformations, and is differentially activated through post-translational modifications including phosphorylation and acetylation. Mutations in the DNA-binding domain (DBD) (amino acids 110-286) of p53 can compromise energetically favorable association with cis elements and are implicated in several human cancers.

Primary Antibody

Name: p53 Antibody

Clone: Mouse anti-Rat

Supplier: Cymbus Biotechnology

Catalog Number: CBL422

Dilution: 1:100 using IHC-Tek™ Antibody Diluent (Cat# IW-1000 or IW-1001) to reduce background and unspecific staining and serum blocking step is NOT needed.

Incubation Time/Temp: 60 min/room temperature

Antigen Retrieval

Device: IHC-Tek™ Epitope Retrieval Steamer Set (Cat# IW-1102)

Buffer/pH value: IHC-Tek™ Epitope Retrieval Solution (Cat# IW-1100)

Heat/Cool Temperature: 95-100 °C/room temperature

Heat/Cool Time: 20 minutes/20 minutes

Detection Methods

Standard Method: ABC Method or LSAB Method

Enhanced Method: Polymeric Methods

Chromogen Substrate

Reagent: DAB

Incubation Time/Temperature: 1-3 minutes/room temperature

Counterstain

Reagent: Mayer's Hematoxylin

Staining Time: 30 seconds

Results:

Staining Pattern: Nuclear

Additional Information:

Species Reactivity: Mouse, rat

Fixation: Formalin fixed paraffin sections

Positive Control: Skin cancer, tumors

Negative Control: Omit primary antibody, isotype control, absorption control

Appendix IX: Allred scoring system for interpreting p53 IHC staining

Diagn Pathol. 2014; 9: 221.

Proportion score A	Positive cells, %	Intensity	Intensity score B
0	0	None	0
1	<1	Weak	1
2	1 to 10	Intermediate	2
3	11 to 33	Strong	3
4	34 to 66		
5	>67		

Final score range (A + B): 0-8

Scores of 0 and 2 are considered negative. Scores of 3 to 8 are considered positive

Appendix X: Abstract(s) for conferences/workshops/seminars.

**12TH BI-ANNUAL SCIENTIFIC CONFERENCE 15TH -17TH NOVEMBER 2017 AT
TURTLE BAY BEACH IN WATAMU, MALINDI – KENYA.**

**DETERMINATION OF PROSTATE CANCER ISUP GRADE GROUPS ON
ARCHIVAL TISSUE SPECIMEN AT AIC KIJABE HOSPITAL, KENYA**

Authors

¹ Dr Ancent Nzioka, Consultant Surgical Pathologist and Senior Lecturer *Department of Pathology, Mount Kenya University.*

² Prof. Alloys Orago, Department of Pathology School of Medicine, Kenyatta University

³ Dr. Tom Were, Department of Clinical Medicine Masinde Muliro University

Key words: *Prostate cancer, ISUP grade groups*

Abstract

Objective: The aim of this study was to determine the proportion of the different prostate cancer International society of Urological pathology (ISUP) prognostic grade groups.

Setting: These findings were part of a bigger cross sectional study done to determination of the pathological characteristics of prostate cancer on archival tissue specimen at AIC Kijabe Hospital, Kenya.

Methods: Prostate cancer archived tissue blocks for eligible subjects were retrieved, sectioned using a microtome and stained using routine histological Hematoxylin and

Eosin stains. These stained slides were then microscopically evaluated for morphological determination of the ISUP grade group.

Results: A total of 210 prostate cancer subjects' tissue blocks were evaluated. The proportions of the different ISUP grade groups were as follows; ISUP grade group 1(23.3%), grade group 2(16.2%), grade group 3(17.1%) grade group 4(10.5%) and grade group 5(32.9%).

Conclusion: The ISUP grade group is a simplified patient-centered grading system composed of 5 prognostic grade groups for prostate cancer. The findings of this study show that our prostate cancer patients have higher ISUP grade groups (60.5% \geq ISUP grade group 3) compared to western population (36.9% \geq ISUP grade group 3).

Appendix XI: Copies of accepted/published work from this thesis

1. Incidence of perineural invasion in prostate adenocarcinoma: Rural Kenyan population experience.

Author; A Nzioka

Publication date: 2015

Publisher: Kenyatta University

Journal: East and Central Africa Medical Journal 2015; 2(1): 7-9

Abstract

Introduction: Prostate cancer appears to be more aggressive amongst patients of African descent, a condition which could be associated with the presence of perineural invasion and higher Gleason scores of the cancer. Objective: This study evaluated the incidence of perineural invasion in previously diagnosed prostate cancer cases in a rural hospital which receive numerous cases of prostate cancer annually. Setting: This was a retrospective cross sectional study conducted at a busy mission referral hospital in rural Kenya. A total of 151 cases were included in the study. Patients' biodata was retrieved from the laboratory information system. Hematoxylin and eosin stained slides of previously diagnosed cases of prostate cancer over a period of one year (January 2012 to December 2012) were retrieved. These slides were microscopically examined for perineural invasion and the tumour grade determined using Gleason scoring. Results: Specimens from 151 patients were examined for perineural invasion. The patients' ages ranged from 46 to 93, mean age 73.77 (SD 9.474). Tumours in high grade category were 75.5% of the total number of cases while the rest (24.5%) were intermediate/low grade tumours. Out of these, 55.6% of the biopsies had perineural invasion. There was no significant relationship between the

patients' age and the grade of tumour. Data on comparison of the cancers with perineural invasion and those without perineural invasion for grade demonstrated significant association between the tumour grade and the presence of perineural invasion.

Key words: Prostate cancer, Gleason score, Perineural invasion, Rural Kenyan African population

2. DETERMINATION OF PROSTATE CANCER ISUP GRADE GROUPS ON ARCHIVAL TISSUE SPECIMEN AT AIC KIJABE HOSPITAL, KENYA

Authors

¹ Dr Ancent Nzioka, Consultant Surgical Pathologist and Senior Lecturer *Department of Pathology, Mount Kenya University.*

² Prof. Alloys Orago, Department of Pathology School of Medicine, Kenyatta University

³ Dr. Tom Were, Department of Clinical Medicine Masinde Muliro University

Key words: *Prostate cancer, ISUP grade groups, prognosis*

Accepted for publication by East African Pathology Journal (EAPJ)

Abstract

Objective: The aim of this study was to determine the proportion of the different prostate cancer International society of Urological pathology (ISUP) prognostic grade groups.

Setting: These findings were part of a bigger cross sectional study done to determination of the pathological characteristics of prostate cancer on archival tissue specimen at AIC Kijabe Hospital, Kenya.

Methods: Prostate cancer archived tissue blocks for eligible subjects were retrieved, sectioned using a microtome and stained using routine histological Hematoxylin and Eosin stains. These stained slides were then microscopically evaluated for morphological determination of the ISUP grade group.

Results: A total of 210 prostate cancer subjects' tissue blocks were evaluated. The proportions of the different ISUP grade groups were as follows; ISUP grade group

1(23.3%), grade group 2(16.2%), grade group 3(17.1%) grade group 4(10.5%) and grade group 5(32.9%).

Conclusion: The ISUP grade group is a simplified patient-centered grading system composed of 5 prognostic grade groups for prostate cancer. The findings of this study show that our prostate cancer patients have higher ISUP grade groups (60.5% \geq ISUP grade group 3) compared to western population (36.9% \geq ISUP grade group 3).

Appendix XII: PhD admission letter

**KENYATTA UNIVERSITY
OFFICE OF THE REGISTRAR (ACADEMIC)**

P. O. Box 43844,
NAIROBI, Kenya.
Tel. 8710901/811622
Email: admission@ku.ac.ke.

Our Ref. P97/20291/2012

DATE: 25th June 2012

Nzioka Ancent Kituku
P.O. Box 28154 - 00200
NAIROBI
Student's Tel. No. 0722678449

Dear Nzioka

**RE: ADMISSION TO DOCTOR OF PHILOSOPHY - Ph.D COURSE
(PROVISIONAL) - 2012/2013 ACADEMIC YEAR**

Following your application for admission to Kenyatta University to undertake a Doctor of Philosophy degree course, I am pleased to inform you that your admission for the **Doctor of Philosophy (Histopathology)** in the School of Health Sciences has been approved.

Your admission number is **P97/20291/2012**.

This offer is made on the basis of the statement of your qualifications as indicated by you in your application form. It is subject to satisfactory verification of those qualifications by the University authorities.

The degree course will be offered by thesis in the **Department of Pathology**. The duration of the degree programme is **three (3) years**. In special circumstances acceptable to the University, a **one (1) year** extension may be granted.

Your registration will be effective from **27th August, 2012** subject to payment of fees and will be governed by the common regulations for Ph.D. degrees in all the Schools. You should therefore ensure that you are familiar with these regulations. However this registration is provisional until you develop a proposal and register with Graduate School within **eight (8)** months after which you will be given a substantive registration. The study programme will run as per the schedule in the enclosed document (**KU/10**).

Also enclosed are detailed joining instructions **KU/2, KU/3A, KU/4, KU/6 and KU/7** which you should complete and return to the Registrar (Academic).

Appendix XIII: Approval of research proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 1st August, 2015

TO: Mr. Ancent Kituku Nzioka
C/o Pathology Dept.
Kenyatta University

REF: P97/20291/2012

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board at its meeting of 29th July, 2015 approved your Research Proposal for the Ph.D. Degree. Entitled "Determination of the Pathological Characteristics of Prostate Cancer on Archival Tissue Specimen at AIC Kijabe Hospital, Kenya".

You may now proceed with data collection, subject to clearance with the Director General, National Commission for Science, Technology, and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking forms per semester. The form has been developed to replace the progress report forms. The supervision Tracking Forms are available at the University's website under Graduate School webpage downloads.

By copy of this letter, the registrar (Academic) is hereby requested to grant you Substantive registration for your Ph.D studies.

Thank you.


RUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Pathology Department.

Registrar (Academic)

Appendix XIV: Ethical Approval**MEDICAL EDUCATION AND RESEARCH DIVISION**

PO Box 20 Kijabe 00220 Kenya

Tel: 020-324-6637 fax: 020-3246335

E-mail: researchadmin.kh@kijabe.net

21ST NOV 2014

Dear DR. ANCENT NZIOKA,

RE :PROSTATE CANCER PATHOLOGY;A CROSS SECTIONAL ARCHIVAL STUDY FRFEOM A TERTIARY REFERRAL HOSPITAL IN KENYA

The institutional review board having carefully reviewed your above title proposal grants you approval to conduct this study at kijabe hospital.

This approval is for a period of one year from 21/11/14. Kindly note that if you intend to continue this study beyond 23/11/2015 then you will need to apply for approval from the institutional review board.

Kijabe IRB requires you to provide regular updates reports, from the study, for monitoring purposes.

We look forward to receiving the results of the interim analysis

We wish you all the best in the study. Kindly furnish this office with a copy of your results.
Thank you,

Sincerely,

Leland Albright, MD

AIC KIJABE HOSPITAL
MEDICAL EDUCATION DIRECTOR
P. O. Box 20.
KIJABE.

Chair, Kijabe Hospital IRB

“Health Care to God’s Glory”

Appendix XV: Kenyatta University research approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P97/20291/2012

DATE: 1st August, 2015

Director General,
National Commission for Science, Technology
And Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

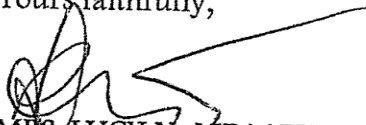
RE: RESEARCH AUTHORIZATION FOR ANCENT KITUKU NZIOKA – REG. NO. P97/20291/2012

I write to introduce Mr. Ancent Kituku Nzioka who is a Postgraduate Student of this University. He is registered for Ph.D degree programme in the Department of Pathology.

Mr. Ancent Kituku intends to conduct research for a Ph.D Proposal entitled, "Determination of the Pathological Characteristics of Prostate Cancer on Archival Tissue Specimen at AIC Kijabe Hospital, Kenya".

Any assistance given will be highly appreciated.

Yours faithfully,


MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

Appendix XVI: Nacosti research authorization

**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

Telephone: +254-20-2213471,
2241349, 3310571, 2219420
Fax: +254-20-318245, 318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/18/4201/21390**

Date: **16th March, 2018**

Dr. Ancent Kituku Nzioka
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on *“Determination Of the pathological characteristics of prostate cancer on archival tissue specimen at AIC Kijabe Hospital, Kenya,”* I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for the period ending **16th March, 2018**.

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Kiambu County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

**GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO**

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.


Appendix XVII: Nacosti research permit


Permit No : NACOSTI/P/18/4201/21390
Date Of Issue : 16th March,2018
Fee Received :Ksh 2000

THIS IS TO CERTIFY THAT:
DR. ANCENT KITUKU NZIOKA
of KENYATTA UNIVERSITY, 0-200
Nairobi, has been permitted to conduct
research in Kiambu County

on the topic: DETERMINATION OF THE
PATHOLOGICAL CHARACTERISTICS OF
PROSTATE CANCER ON ARCHIVAL
TISSUE SPECIMEN AT AIC KIJABE
HOSPITAL, KENYA

for the period ending:
16th March,2018.


.....
Applicant's
Signature


.....
Director General
National Commission for Science,
Technology & Innovation