DEMOGRAPHIC, BIOCHEMICAL AND HISTOPATHOLOGICAL FEATURES OF PROSTATE ADENOCARCINOMA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOUTH AFRICA.

Karishma Chandraser

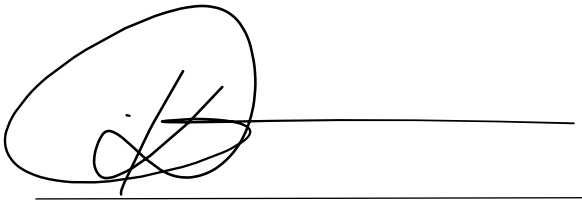
Student number: 302859

Supervisor: Dr R.D. Mohanlal

Degree: For the purpose of partial fulfilment of the requirements for the degree of Master in Medicine in the branch of Anatomical Pathology, University of the Witwatersrand.

DECLARATION

I, Karishma Chandraser, declare that this research report is my own work. It is being submitted in fulfilment for the degree of Master of Medicine in Anatomical Pathology. It has not been submitted before for any degree or examination at this or any other university.



Karishma Chandraser

Signed at Johannesburg on the 28th day of January 2022

DEDICATION

I dedicate this MMED project to my dear husband Mayuir Bhairoparsad who has supported me unconditionally throughout the long hours and late nights and to my precious son Zai who has brought so much meaning to my life.

Also, I dedicate this to my Mum Shamila and my late Dad Raj Chandraser.

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ABSTRACT

Background

One of the main causes of cancer related deaths in men is prostate cancer (PCa) and PCa is also the commonest cancer diagnosed in South African men. There is a lack of research pertaining to PCa in this country. The aim of this study is to describe the demographic, biochemical and histopathological features of prostate carcinoma diagnosed at the Chris Hani Baragwanath Academic Hospital Histopathology Laboratory.

Methods

We embarked on an observational retrospective study collating information from the histopathology reports of all prostate needle core biopsies submitted to the laboratory within a 13-month period (from 1 January 2018 to 31 January 2019). The age, race (where available), serum prostate specific antigen (PSA) level, previous biopsy results, number of cores, diagnostic category, tumour grade, tumour volume, presence or absence of perineural invasion, presence or absence of lymphovascular space invasion, Gleason grade, presence of metastasis and the results of p63 immunohistochemistry were captured from the relevant reports.

Results

A total of 784 cases were reviewed. Of these, 317 cases (40.4%) were assigned a benign diagnosis and 467 (59.6%) were assigned a malignant diagnosis. The mean age of patients diagnosed with malignancy on core biopsy was 66.65 years, which was significantly older than the mean age of 65.19 years in patients with benign diagnoses (p=0.014). Data on race was available for only 289 patients (36.86%) and 36.2% of tumour cases were noted in African men. The median PSA for the cohort was 15.2ug/L and PSA levels were significantly higher in patients with malignancy (median 23.35ug/L) than in patients with benign diagnoses (median 9.60ug/L), p=<0.005. In addition, 60% of patients with a raised PSA (defined as more than 4ug/L) were diagnosed with carcinoma on biopsy. A significant

difference in PSA levels across Grade groups was noted (p=<0.005) and PSA levels in patients with perineural invasion were significantly higher than for those without perineural invasion (p=<0.005). A strong positive correlation was demonstrated between the PSA level and tumour volume (p=<0.005). The median tumour volume was 40%.

Conclusion

This study showed similar findings to other published studies on PCa within the South African population. It has been shown that South African patients present later than those in more western parts of the world and this may explain why in our cohort most cases were diagnosed as PCa, patients were noted to have higher PSA levels as well as a median tumour volume of 40%.

NOMENCLATURE

AMACR – Alpha methylacyl CoA racemase

AR – Androgen receptor

ASAP – Atypical small acinar proliferation

BPH – Benign prostatic hyperplasia

BRCA1 - Breast cancer type 1

BRCA2 - Breast cancer type 2

CHBAH – Chris Hani Baragwanath Academic Hospital

DRE – Digital rectal examination

ERG – Erythroblast transformation specific (ETS) related gene

ESR1 - Estrogen receptor 1

ETS - Erythroblast Transformation Specific

GSTpi - Glutathione S-transferase pi

H&E – Haematoxylin and eosin

HGPIN – High grade prostatic intraepithelial neoplasia

HPC1 - Hereditary prostate cancer 1

HPCX - Hereditary prostate cancer, X linked

HSPG2 - Heparan Sulfate proteoglycan 2

LVI – Lymphovascular space invasion

mRNA – Messenger Ribonucleic Acid

MYC – MYC proto-oncogene, BHLH transcription factor

NCR – National cancer registry

NKX2-5 - NK2 Homeobox 5

P53 - Tumour suppressor TP53

PCa – Prostate carcinoma

PCAP - Predisposing for prostate cancer

PIN – Prostatic intraepithelial neoplasia

PNI – Perineural invasion

PSA – Prostate specific antigen

PTEN - Phosphatase and tensin homolog

RASSF1A - Ras association domain-containing protein 1A

RB – Retinoblastoma gene

SA – South Africa

SPSS – Statistical Package for the Social Sciences (Statistics software)

TMPRSS2 - Transmembrane protease, serine 2

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1.1. INTRODUCTION

Prostate carcinoma (PCa) is one of the leading causes of cancer related mortality in males (Arap 2010). PCa is the most common cancer in men in South Africa (Roux *et al* 2015). In 2007, the incidence rate was 29.4 per 100 000. This increased to 67.9 per 100 000 in 2012 (Roux *et al* 2015). Even though PCa is known to have a slower rate of disease progression than many other cancers, it still is the leading cause of death due to cancer in men (Chen *et al* 2017). There is a lack of statistics pertaining to PCa in Africa and more importantly in South Africa and therefore the aim of this study was to describe the demographic, biochemical and histopathological features of prostate carcinoma at Chris Hani Baragwanath Academic Hospital (CHBAH). The study was a retrospective descriptive study. The prostate core biopsy and serum prostate specific antigen levels of patients at CHBAH were reviewed over a 13-month period.

1.2. PURPOSE OF THE RESEARCH

The aim of this study was to describe the features of histologically diagnosed prostate pathology with emphasis on PCa in patients seen at CHBAH, Johannesburg, South Africa.

1.3. SIGNIFICANCE OF THE RESEARCH

Currently there is a lack of knowledge with regards to PCa amongst elderly African men. Most studies have been conducted in European and American populations. Research has shown that PCa is more prevalent amongst African men (Kgatle *et al* 2016) and may also be more aggressive (Moul *et al* 2015). The reason for this study was to provide more insight into PCa within a cohort of South African patients. CHBAH is one of the largest hospitals in the world. A description of prostate pathology seen here will shed more light on the burden of disease. This may ultimately assist with resource allocation and help to address the lack of early screening and prevention strategies in elderly males in our communities.

1.4. OBJECTIVES OF THE RESEARCH

To describe the demographics of patients on whom prostate biopsies were performed and those histologically diagnosed with PCa.

To assess the prostate specific antigen (PSA) levels across the different diagnostic categories.

To assess for any correlation between the histological (tumour grade and volume) and clinical variables (age, PSA level).

1.5. RESEARCH QUESTION

What are the demographic, biochemical and histopathological features of prostate adenocarcinoma at Chris Hani Baragwanath Academic Hospital, South Africa?

CHAPTER 2: LITERATURE REVIEW

2.1. PROSTATE GLAND

The prostate gland is a small male reproductive organ that is approximately the size of a walnut (Kgatle *et al* 2016). It is situated between the bladder and penis and surrounds the urethra. It does grow over time however its weight does not usually exceed 40 grams. The prostate gland secretes prostatic fluid. Prostate fluid functions as a protective mechanism for sperm. The prostate gland comprises tubulo-alveolar glands lined by cuboidal to columnar epithelium. The glands are surrounded by a basal cell layer. The surrounding stroma comprises both smooth muscle and connective tissue (McNeal 1988). Many benign conditions can affect the prostate namely benign prostatic hyperplasia or hypertrophy (BPH), acute and chronic prostatitis as well as prostate infarction (Kgatle *et al* 2016). PCa may develop when prostate gland cells are stimulated to multiply uncontrollably.

2.2. PROSTATE SPECIFIC ANTIGEN (PSA) SERUM LEVELS AND SCREENING

PSA is an androgen regulated, chymotrypsin-like, serine protease. It is a product of prostatic epithelium that is normally secreted in semen and is found within benign and neoplastic prostate secretory epithelium (Egawa 2001). PSA cleaves and liquefies semen following ejaculation. Only minute amounts of PSA are noted in the serum normally. Localised and advanced PCa cause raised levels of PSA. The PSA level is a serum marker that is organ specific however it is not cancer specific as it can be raised in many conditions (Bunting 1995, Foley *et al* 2004). These conditions may relate to the prostate gland such as PCA, prostatic intraepithelial neoplasia (PIN), atrophy, BPH, prostatitis and ischemia. Extra prostatic sources of PSA include the parotid gland and peri urethral glands. Acute renal failure, bypass surgery, radiation therapy, prostatic surgery as well as clinical manipulations such as digital rectal examinations (DRE), transrectal ultrasounds and prostate biopsies may all cause an increase in PSA (Bunting 1995).

According to the South African prostate cancer screening guidelines, men of Black African descent and men with a first degree relative diagnosed with breast or prostate cancer

should undergo a DRE of the prostate and a serum total PSA level from the age of 40. All other men should begin their screening at the age of 45 (Anderson *et al* 2017, Segone *et al* 2013). High PSA levels or a trend of increasing levels (even if at a low rate) will necessitate a prostate biopsy. As a routine the biopsies are performed as needle cores and a total of 8-12 cores are submitted for histopathological examination (Chen *et al* 2017). PSA testing, first introduced in 1986, has dramatically improved the detection rates of PCA. The use of PSA levels in combination with a DRE were shown to double the detection rate of PCa (Egawa 2001).

Defining a clear cut-off for PSA values has been controversial as levels tend to vary with age, race, and prostate volume (Egawa 2001, Kgatle *et al* 2016, Laguna *et al* 2000, Montironi *et al* 2000). Routinely a value of 4ng/ml has been used. A PSA value of 4ng/ml or less has a sensitivity of 80% and a specificity of 90% (Laguna *et al* 2000). Recent studies have shown that in younger individuals a lower threshold should be applied (Egawa 2001, Laguna *et al* 2000) and that PSA value thresholds should be set according to age. Using a PSA value of 4ng/ml as the upper limit of normal in all men may run the risk of missing younger men with early PCa. In addition, using a single cut off value will also run the risk of unnecessary biopsies in men that are slightly older. PSA values increase on average by 3.2% per year with age as well as with prostate volume (Laguna *et al* 2000). Age specific PSA reference ranges have been well studied and proposed for Caucasian men. According to Montironi *et al* (2000) men with PCa younger than 50 years old had PSA values less than 2.5ng/ml, those between 50 and 60 years had PSA values below 3.5ng/ml, those younger than 70 had PSA values less than 4.5ng/ml and men younger than 80 had PSA values less than 6.5ng/ml. This study demonstrated why age specific values should be used as the upper limit of normal when evaluating PSA results.

Race and ethnicity have also been shown to influence PSA levels with higher levels being found in men of African descent. Even when PSA levels were corrected for age and tumour volume a difference was still identified in men of African descent (Kgatle *et al* 2016).

Despite the emphasis on raised PSA levels (usually above 4ng/ml) being a marker for PCa (Foley *et al* 2004), other studies have shown that patients with PCa may in fact sometimes present with significantly lower PSA values (Punglia *et al* 2006, Zapala *et al* 2018). Punglia *et al* (2006) showed that men younger than 60 years of age with PCa alone presented with a mean PSA level of 2.05ng/ml and men older than 60 years had a mean PSA value of 2.66ng/ml. Patients diagnosed with BPH and PCa were shown to have slightly higher mean PSA values with men <60 years having a mean PSA of 2.56ng/ml and men >60 years, a mean PSA of 3.90ng/ml. PSA levels for men with BPH alone were 0.97ng/ml amongst men <60 years old and 1.75ng/ml in men >60 years. Men with no prostatic disease showed PSA levels of 0.78ng/ml and 1.23ng/ml in men younger than 60 and older than 60 respectively. The authors further emphasize that age specific reference ranges for serum PSA are important to detect all PCa cases especially during early stages of disease. In turn, this will increase the rate of remission and decrease morbidity and mortality for patients diagnosed with PCa. This cohort included men of all races within Orlando Florida, America. Men of Caucasian descent were included if they were at least 50 years old. For men of African descent, they were included from the age of 40.

Maphayi *et al* (2021) in their study (based in Johannesburg) of 20 365 PSA tests showed that only 2% of all patients went on to have a prostate biopsy. Although Black African patients in the cohort had a higher median PSA of 7.40ug/L they had significantly fewer biopsies ($p<0.005$). Interestingly, only 8% of patients with PSA >4ug/L actually went on to have prostate biopsies. The method of assessing when and where patients had prostate biopsies was not elucidated. Subsequent analysis of the cut-off of 4ug/L (used across NHLS laboratories in South Africa) showed low specificity (37.1%) and sensitivity (53.3%).

BPH has been shown to account for approximately 60% of elevated PSA levels. The raised levels in this setting are thought to due to the proliferation of benign prostate gland cells (Montironi *et al* 2000). Patients with BPH have usually have PSA levels between 4 and 10 ng/ml with their levels seldom exceeding 20ng/ml (Laguna *et al* 2000).

Studies have failed to show a correlation between PSA levels and histologic grade of tumour (Janbaziroudsari *et al* 2016) or between stage of disease, differentiation of tumour and PSA levels (Zapala *et al* 2018).

2.3. PROSTATE CARCINOMA

PCa usually develops slowly taking 10 years or more to progress from its precursor lesion, PIN which can be classified into low and high grades depending on the morphological characteristics seen on histological examination (Kgatle *et al* 2016). If PCa is untreated it may metastasize to organs such as bones and lymph nodes. As PCa advances, patients may experience urinary symptoms such as difficulty or pain during micturition and nocturia (Kgatle *et al* 2016). These symptoms may also occur in BPH and therefore a physical DRE together with a serum total PSA test is advised. Zapala *et al* (2018) proved in their study conducted within a European population that early screening of men with lower urinary tract symptoms as well as those men that are asymptomatic improved PCa detection rates significantly. PCa has a good remission rate if diagnosed early. On the contrary, if left too late the disease progresses by metastasizing and there is currently no known cure for this (Zapala *et al* 2018). The main mode of metastasis is haematolymphoid particularly via the lymphatics. The obturator lymph nodes are usually the first nodes to be involved by metastatic PCa and thereafter the para-aortic lymph nodes become involved. The axial skeleton becomes involved via haematogenous spread. Metastasis to bone manifests as osteoblastic lesions (Jiang *et al* 2018).

2.3.1. Incidence and Mortality

PCa is the most common cancer amongst males and is known to contribute to a significant number of deaths (Aref-Eshghi *et al* 2018). In 2013, a worldwide incidence of 1.4 million was noted (Chen *et al* 2017). According to GLOBOCAN 2020, the incidence rate of PCa is three-fold higher in developing countries compared to developed countries. Babb *et al* (2014) showed a higher incidence amongst African Americans when compared to the Caucasian population.

Chen *et al* (2017) reported a worldwide mortality of 293 000 deaths in 2013. WHO GLOBOCAN 2020 reported 375 000 deaths as a result of PCa. Sub Saharan Africa has one of the highest mortality rates (22 per 100 000). Other countries with high mortality rates include the Caribbean (27.9 per 100 000), Middle Africa (24.8 per 100 000), Western Africa (20.2 per 100 000) and Polynesia (18.8 per 100 000). Countries with the lowest mortality rates include South Central Asia (3.1 per 100 000), Eastern Asia (4.7 per 100 000), Southern Europe (7.8 per 100 000), Northern Africa (8.2 per 100 000) and North America (8.3 per 100 000) (Sung *et al* 2021). The incidence and mortality rates of PCa from the GLOBOCAN 2020 study are shown in figure 1.

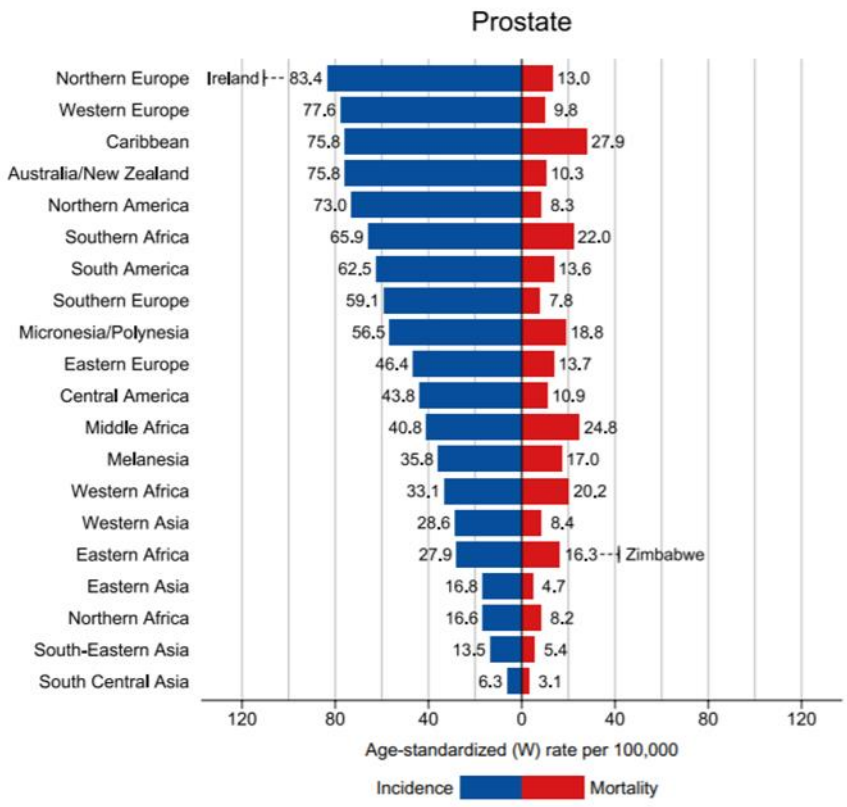


Figure 1: Estimated incidence and mortality rates of prostate cancer worldwide 2020. *Source: GLOBOCAN 2020.* http://globocan.iarc.fr/old/FactSheets/cancers/prostate-new.asp

PCa is a public health burden in Africa and this is due to a lack of screening methods and health promotion programmes (Adeloye *et al* 2016). Southern Africa has one of the highest

incidence rates of PCa (65.9 per 100 000) together with Northern and Western Europe, Australia, New Zealand, Caribbean, and North America. North Africa and Asia reported the lowest incidence rates (Sung *et al* 2021). Overall, Sub Saharan Africa has shown the greatest rate of increase in the number of diagnosed cancers. This could be explained by the larger older population in this region. Over the period 2008-2012, review of cancers in Sub Saharan Africa demonstrated that 9000 new cancers were diagnosed in older adults. Of these cancers, the most diagnosed in elderly males were those of the oesophagus and prostate. In 2012, 238 000 new cancer cases were recorded in patients older than the age of 60. Of these, the most prevalent cancer in elderly males was PCa. A 6-7% increase in the incidence rate of PCa was described during the study period which was also the greatest rate of increase in older male patients (Pilleron *et al* 2019).

PCa is the most common urological cancer seen amongst elderly men in South Africa and is one of the top four most common cancers in this country (Alleyne *et al* 2013). According to the South African National Cancer Registry (NCR), in 2013 the incidence of PCa was 44.3% and it accounted for 18.86% of all cancers amongst the male population. Approximately 1 in 8 South African men will develop PCa in their lifetime. In 2018, South Africa had an age specific incidence rate of 68 per 100 000, an increase from the incidence rate recorded in 2006 of 44.9 per 100 000. A possible reason cited for this increased incidence was improved screening and awareness in 2018 compared to 2006 (Cassim *et al* 2021).

2.3.2. Risk factors

Risk factors of PCa can be divided into two groups: Non modifiable and modifiable risk factors (Kgatle *et al* 2016). Non modifiable risk factors include age, race, family history and genetic alterations. There are currently no known modifiable risk factors.

2.3.2.1. Non-Modifiable risk factors

2.3.2.1.1. Age

PCa incidence rates increase as age increases (Rawla 2019). A higher incidence rate is observed in men older than 50 years old (Kgatle *et al* 2016) and an even higher incidence rate is reported in men over the age of 65 (Rawla 2019). Del'Atti (2016) showed a mean age of 61.3 years within their cohort. Men over the age of 65 years account for 68% of cases of prostate cancer and show 92% mortality. This is relevant as due to increased longevity; a substantial proportion of our population comprises elderly individuals. PCa incidence has been seen to increase dramatically in patients in the 7th and 8th decades of life. Although a high-risk age group does exist, no correlation has been shown between age and histologic grade of tumour in a needle core biopsy (Janbaziroudsari *et al* 2016, Stangelberger *et al* 2008). Current data shows that approximately 25% of men diagnosed with PCa are over 75 years old but clinical trials analysing conservative versus radical treatment of PCa fail to include men over the age of 75 years old within their study cohorts (Pettersson *et al* 2018).

Statistics from the United States of America have shown that life expectancy has increased since the year 2000. Currently, it is expected that a 70-year-old man will live for approximately 13 years more (Bechis *et al* 2011). Considering that the incidence of PCa increases with age, an increase in the incidence of PCa can be expected in the coming years. Considering this, studies on PCa in older men are needed as to date most studies exclude men above the age of 75 years. Postmortem studies of elderly men have shown that men with severe comorbid disease have often died with concomitant PCa. It is thought that elderly men are more likely to die with PCa than because of it (Rawla 2019). An older age has also been shown to be associated with certain genetic alterations such as increased promoter methylation of Glutathione S-transferase pi (GSTpi), Ras association domain-containing protein 1A (RASSF1A), Estrogen receptor 1 (ESR1), NK2 Homeobox 5 (NKX 2-5) (Hall *et al* 2005, Kgatle *et al* 2016).

Bechis *et al* (2011) showed that older men present with more aggressive disease and have an overall lower survival rate compared to younger men. The lower survival rates may be

explained by this age group not receiving localised treatment. Lower probabilities of cure have also been reported in patients of older ages with studies showing a lower survival rate in older individuals particularly those above 73 years old (Hall *et al* 2005).

Older age at diagnosis has been associated with increased risk of mortality from PCa despite tumour characteristics (Pettersson *et al* 2018). The poorer survival rates seen in men of advanced age may be due to them being less likely to receive curative treatment (Bechis *et al* 2011).

The question that arises then is this: Are the poorer survival rates due to a lack of curative treatment or is it due to the disease being inherently more aggressive in elderly men? Interestingly, Bratt *et al* (1998) reported a younger age being associated with a more aggressive tumour. Bechis *et al* (2011) have shown improved survival rates amongst elderly men when treated with curative modalities such as radical prostatectomy for localized disease. Pettersson *et al* (2018), however, showed a strong correlation between older age and increased mortality rates in men who received radical prostatectomies. No association was demonstrated between older age at diagnosis and an increase in the mortality rates when compared with men who received curative radiotherapy. Possible explanations for this are that radical prostatectomy is not an effective means of treatment within this group and that men that undergo radical prostatectomies receive a less extensive preoperative workup compared to men who receive radiotherapy. Men who underwent radical prostatectomies were also noted to have more extensive and aggressive PCa as compared to the group that received radiotherapy and this could explain the differences in treatment outcomes between these two groups of patients. In view of this, it is proposed that treatment decisions should not be based on age alone and that clinicians should consider life expectancy and severity of disease with prognosis when formulating treatment decisions (Bechis *et al* 2011).

The relationship between age, disease progression and survival rates in PCa have not been well studied and further definitive studies are needed. Due to the conflicting data, currently tumour stage and grade are the only reliable variables in terms of tumour prognosis. With our current aging population and evidence of a relationship between older age and more aggressive disease, PCa in elderly patients may warrant a different treatment plan (Hall *et al* 2005).

2.3.2.1.2. Race

African American men have been found to have higher PSA values compared to Caucasian men (Hayes *et al* 2018). They also show more aggressive disease, more risk for metastases, faster PSA doubling times, higher tumour grade and volume as well as a younger age at diagnosis (Berger *et al* 2006, Bigler *et al* 2011, Heath *et al* 2008, Moul *et al* 1995, Powell *et al* 2010, Sundi *et al* 2014, Tewari *et al* 2005). African American men were also noted to seek treatment later than Caucasian American men. There were many factors attributing to this difference such as poor help seeking behaviour, financial burden, and lack of health insurance (Shenoy *et al* 2016).

Tindall *et al* (2014) found a link to African ancestry in their study comparing Caucasian American men and African American men. African American men were noted to be more susceptible to PCa as well as at an increased risk of more severe disease. Furthermore, when comparing Caucasian American and African American men it was shown that African American men were approximately 1.7 times more likely to have PCa, were also approximately 2.5 times more at risk of mortality from the disease and suffered from more aggressive disease.

African American men were also of a younger age at diagnosis compared to Caucasian American men (Tindall *et al* 2014). Interestingly, men of African descent were found to have chromosomal alterations on a specific locus on chromosome 8 (Tindall *et al* 2013).

Limited data exists with regards to the epidemiology of PCa in South Africa and more specifically within Gauteng. A few studies have been published however they were limited to specific health facilities. In South Africa, Black men are affected by PCa more than Caucasian men. Methylation profiles are noted to be different between these two population groups and this may be the reason for the difference in incidence rates (Kgatle *et al* 2016).

A recent study published by Cassim *et al* (2021) assessed PCa incidence in Gauteng, South Africa. It showed a significant difference in age at presentation amongst the racial groups seen in the province, namely Indian/Asian, Black Africans, Coloureds (mixed race) and Caucasians. African males presented at a slightly younger age compared to patients within the other racial groups and were also noted to have a higher incidence of PCa (Cassim *et al* 2021).

Dewar *et al* (2018) showed that Black men in South Africa have a worse clinical and pathological prognosis compared to non-Black men. Unfortunately, the exact cause for the more aggressive disease in Black South African men has to date not been elucidated. It has also been shown that Black South African men were more likely to present with a higher grade of tumour, particularly Gleason scores of 8 or greater (Cassim *et al* 2021).

Tindall *et al* (2014) observed that South African Black men present with higher Gleason scores. They were noted to have tumours with a Gleason score of 7 or higher as compared to their Caucasian counterparts. During the South African Prostate Cancer Study, 35.5% of Black South African patients had tumours with a Gleason score of 7 or more. Within this group, 26% of cases showed poorly differentiated characteristics on histopathological examination which translated to a more aggressive tumour (Tindall *et al* 2014). African American men also showed higher Gleason scores particularly above 7 as compared to Caucasian men in the United States of America. Similar findings were observed between

African men in the United Kingdom and White men in the United Kingdom (Tindall *et al* 2014).

Different ethnic groups have been shown to be susceptible to PCa based on genetic and epigenetic factors (Rebbeck *et al* 2017). Access to early screening as well as treatment of PCa have been shown to be great influencers however even amongst patients with similar socioeconomic backgrounds, a great difference has been seen in men of African descent who seem to be more at risk of PCa as well as more at risk of aggressive disease. Men of African descent are also reported to have a poorer prognosis (Rebbeck *et al* 2017).

A study observing trends within the Southern Bantu clan showed that this population presented with more aggressive disease histologically characterized by a Gleason score of greater than 7 as well as a higher PSA value at presentation (Peterson *et al* 2013, Schuster *et al* 2010, Stefflova *et al* 2011). Within Gauteng and more specifically Johannesburg, it was noted that more aggressive tumours were identified within rural communities as compared to urban residents however no differences in PSA values were seen (Hayes *et al* 2018, Tindall *et al* 2014). Men in rural communities were noted to present on average three years later than men from urban areas and South African men were seen to present five years later compared to men living in America (Hayes *et al* 2018). This difference may be due to lack of screening as well as lack of PCa awareness amongst South African men particularly those living within rural localities. In western countries, early PSA testing has been a routine practice for many years however the same cannot be said for South Africa and other African countries. Another possible explanation for the late presentation amongst men in rural communities was that they consulted traditional health practitioners first thereby delaying hospital presentation (Tindall *et al* 2014). The vast under representation of male related diseases within the South African health care system as well as across media platforms may also be a factor (Hayes *et al* 2018). In South Africa, vast importance and funding have been placed on women's health particularly breast and cervical cancer with established cancer registries for both. This is not the case for PCa even though it is the number one cancer affecting our male population (Alleyne *et al* 2013). In addition, the Cancer Association of

South Africa mobile health clinics are effective at reaching women within rural areas for early screening and prevention (www.cansa.org.za) however there is no campaign or initiative to reach out to the male population within such communities (Hayes *et al* 2018).

When comparing the rural and urban populations, differences in grade and differentiation of the tumour were identified. Men in rural communities present with a higher Gleason score and more poorly differentiated tumours as compared to those in urban communities (Tindall *et al* 2014).

2.3.2.1.3. Family history

An increased risk is noted in patients who have a first degree relative diagnosed with PCa (Kgatle *et al* 2016). Mutations and/or abnormal gene expression of tumour suppressor TP53 (p53); Breast cancer type 1 (BRCA1); Breast cancer type 2 (BRCA2) and Phosphatase and tensin homolog (PTEN) may be seen within families affected by PCa (Kgatle *et al* 2016). Rebbeck *et al* (2017) identified many different genes in their family-based studies on men of European descent. Hereditary prostate cancer 1 (HPC1); Predisposing for prostate cancer (PCAP) and Heparan Sulfate proteoglycan 2 (HSPG2) are genes located on chromosome 1 that have been shown to be associated with PCa. In addition, Hereditary prostate cancer, X linked (HPCX) on chromosome X was associated with prostate cancer (Rebbeck *et al* 2017).

2.3.2.1.4. Genetic alterations

Common genetic alterations seen in PCa include MYC proto-oncogene, BHLH Transcription Factor (MYC) oncogene amplification, PTEN tumour suppressor gene deletions, loss of p53 by either deletions or mutations, deletions of Retinoblastoma (RB) and amplification of the androgen receptor (AR) gene. Studies have shown that epigenetics play a role in prostate carcinogenesis and the most common epigenetic alteration involves hypermethylation of Glutathione S-transferase pi (GSTpi) (Rebbeck *et al* 2017). Kgatle *et al* (2016) have shown the following epigenetic alterations in patients diagnosed with prostate carcinoma: histone modifications, DNA methylation and non-coding microRNAs (miRNA). These changes target and silence genes involved in cell signalling. This in turn leads to tumorigenesis.

In men of non-European descent, the following susceptibility loci were identified: 12q24; 1q24-25; 2p16; 2p21 and specifically 1p36 in African American men. African American men specifically also show the following susceptibility genes associated with PCa: 2p21, 11q22, 17p11, 22q12, and Xq21 (Rebbeck *et al* 2017).

Somatic and acquired mutations in conjunction with epigenetic modifications are implicated in PCa.

2.3.2.2. Modifiable risk factors

To date, there are no definitive modifiable risk factors for PCa, however a few specific lifestyle and environmental factors are associated with the development of PCa (Tindall *et al* 2013).

Modifiable risk factors possibly associated with PCa include exposure to polycylic aromatic hydrocarbons, herbicides, pesticides and polychlorinated biphenyls (Kgatle *et al* 2016, Leitzmann *et al* 2012). Dietary factors increasing the risk of PCa include the consumption of high fat and processed foods. Protective dietary factors have been postulated including ingestion of iron, zinc, curcumin and transfatty acids (Kgatle *et al* 2016). Lifestyle factors associated with the incidence of PCa include sexual activity patterns, a family history of cancer, diabetes mellitus, specific balding patterns, erectile dysfunction, and aspirin usage (Tindall *et al* 2013).

2.3.3. Molecular markers

Although many molecular markers have been suggested to play a role in PCa pathogenesis, not all are clinically significant. AR is one of the more important markers and many patients develop resistance to androgen. This translates to them becoming resistant to available treatments. Hormone resistance in turn impacts mortality and morbidity (Edwards *et al* 2003).

Genome-wide association studies (GWAS) have discovered numerous single nucleotide polymorphisms (SNPs) related to the development of PCa. Some common molecular alterations include mutations within chromosome 8 and the kallikrein genes of chromosome 19. Next generation sequencing has identified germline mutations in BRCA2 to be associated with a predisposition to PCa. Men with BRCA2 mutations are 5 times more likely to develop PCa than men without a BRCA2 mutation (Faisal *et al* 2019). BRCA2 mutations have also been associated with an earlier age of onset and more aggressive disease. One of the commonest somatic mutations involve the Transmembrane protease, serine 2 (TMPRSS2), which is a member of the Erythroblast Transformation Specific (ETS) family of transcription factors. Loss of PTEN has been identified in some patients with PCa and is associated with metastatic disease at resection (Faisal *et al* 2019).

2.3.4. Prostate biopsy

The decision on whether to biopsy a patient or not is individualised and is usually performed if: the PSA is raised, there is a clinical suspicion for cancer, or the DRE is abnormal (nodular, asymmetrical or diffusely firm prostate). Traditionally, biopsies are performed using transrectal ultrasound, but more recently magnetic resonance imaging (MRI) guided biopsies are performed with assignment of a prostate imaging and reporting data system (PI-RADS) score (Streicher *et al* 2019).

The optimal number of cores that should be obtained is controversial. Not only do more cores increase the detection rate of PCa but they also improve the accuracy of the Gleason score which is a major determinant of the type of therapy selected. Coogan *et al* (2006) have shown in their study of 404 patients that the accuracy of the Gleason score is improved when 10 (versus 8 or 6, $p < 0.005$) prostate cores are submitted when they compared the scores assigned on core biopsy to the ones determined on radical prostatectomy specimens. The issue of how many cores are sufficient and from where these should be obtained has been hotly debated. Prostate biopsies were traditionally sextant, with sampling of the base, mid-gland and apex of both lobes halfway between the

midline and lateral border of the gland. Recently, there have been modifications. Pretsi (2003) advises at least 10 cores, with focus on the lateral aspect and the apex to ensure that the periphery of the gland, which is most often involved by prostate carcinoma, is targeted.

2.3.5. Histology

2.3.5.1. Prostatic intraepithelial neoplasia

High grade PIN (HGPIN) is recognised as large branching glands. These glands are lined by atypical epithelium with prominent nucleoli. The cytological features of PIN and PCa are identical, but basal cells are retained in the PIN. PIN is a precursor lesion to PCa. Evidence linking PIN and PCa include:

- Both are located peripherally in the prostate and they both tend to spare the other zones of the prostate
- PIN is noted to be more extensive and seen more frequently in prostate biopsies showing invasive carcinoma
- PIN and PCa share similar molecular alterations (such as ETS gene rearrangements)

In literature cited by Meng *et al* (2003), HGPIN shows variations with regard to age and ethnic groups similar to PCa e.g., autopsy studies showed that African American men had more extensive involvement by PIN at a younger age than their Caucasian counterparts.

Antonelli *et al* (2011) in their study of 546 patients with HGPIN demonstrated that 174 (31.8%) were subsequently diagnosed with carcinoma. Twenty-one point one percent (21.1%) were diagnosed at first re-biopsy and 10.6% after a median of 2 biopsies. Interestingly, they noted that an abnormal PSA of >7ng/ml and an abnormal DRE were related to the finding of PCa on the first re-biopsy. The presence of HGPIN on 4 or more cores correlated to finding carcinoma on further/subsequent biopsies. There is, however, no consensus in the literature at large, regarding the role of PSA, transrectal ultrasound and DRE in predicting the likelihood of PCa in patients initially diagnosed with HGPIN (Epstein &

Potter 2001), therefore close follow-up is advised in patients diagnosed with HGPIN (Meng *et al* 2003). In a review, HGPIN was noted to have an incidence of up to 24% with a median incidence of 5-6% (Epstein & Potter 2001)

2.3.5.2. Atypical small acinar proliferation

Atypical small acinar proliferation (ASAP) is defined as the stage between healthy prostatic tissue and PCa. This diagnosis should be made when prostate epithelial cells display atypia which is not normal, however, definitive malignancy cannot be proven. In this scenario, clinical correlation and repeat serum PSA levels with or without repeat biopsies are advised (Adamczyk *et al* 2014). Histopathologists may attempt to resolve atypical proliferation using immunohistochemistry and serial levels. The incidence of ASAP is between 2 and 5% and 34-60% of biopsies diagnosed with ASAP will show PCa on repeat biopsy. ASAP should not be regarded as a diagnostic entity but rather as a category that reflects a pathologist's difficulty in making an unequivocal diagnosis of carcinoma (Iczkowski & Bostwick 2014).

2.3.5.3. Macroscopy

The main zones that are affected by PCa are the peripheral and posterior zones - this is the reason it can be easily palpated on DRE. On macroscopic examination, prostate tumours are generally poorly defined and peripheral zone tumours are easier to visualize. (Lindh *et al* 2018). A firm tumour with a gritty appearance may be seen (Jiang *et al* 2018). The tumour may appear white, tan, yellow or even orange (especially transitional zone lesions). The difficulty identifying the tumours macroscopically is thought to be due to formalin fixation that distorts the prostate gland. Bisection of the prostate gland in radical prostatectomy specimens prior to formalin fixation is advised (Lindh *et al* 2018)

Local extension preferentially involves the peri-prostatic tissue but there may also be extension to the seminal vesicles and urinary bladder (Jiang *et al* 2018).

2.3.5.4. Microscopy

Microscopically, PCa displays a wide range of morphological appearances from well to very poorly differentiated neoplasms. Four major patterns of adenocarcinoma in the prostate

have been described namely: Medium sized glands; small glands, diffuse infiltration and cribriform glands. The malignant glands are lined by a single layer of epithelium that is low columnar in type. The glands are crowded, may seem fused and are not branching or papillary in appearance (as may be seen in benign glands). The basal layer of cells, found in benign glands, is absent. The neoplastic cells display large nuclei with irregular nuclear borders, nuclear hyperchromasia and prominent nucleoli (Grignon 2018). Mitotic activity within PCa is usually low to absent.

Other less common types of PCa include atrophic, pseudohyperplastic, foamy gland, mucinous and others (Li *et al* 2016). Intraductal carcinoma is characterized by the presence of malignant epithelial cells lining prostatic glands and acini however the basal cell layer is retained (Sehn *et al* 2018).

2.3.5.5. Histological mimics of prostate carcinoma

Numerous benign mimics of PCa have been reported. These can be divided into categories based on architecture. Pathologies that may be difficult to differentiate from PCa with small gland architecture include lesions originating from prostate epithelium as well as extraprostatic sources (Hameed *et al* 2010).

Lesions originating from the prostate:

- Atrophy
- Atypical adenomatous hyperplasia
- Crowded benign glands
- Sclerosing adenosis
- Basal cell hyperplasia (see figure 2)
- Radiation induced changes
- Reactive atypia
- Verumontanum mucosal gland hyperplasia

Extraprostatic sources:

- Seminal vesicle (see figure 2)
- Ejaculatory duct
- Cowper glands
- Mesonephric remnants
- Colon glands
- Nephrogenic adenoma

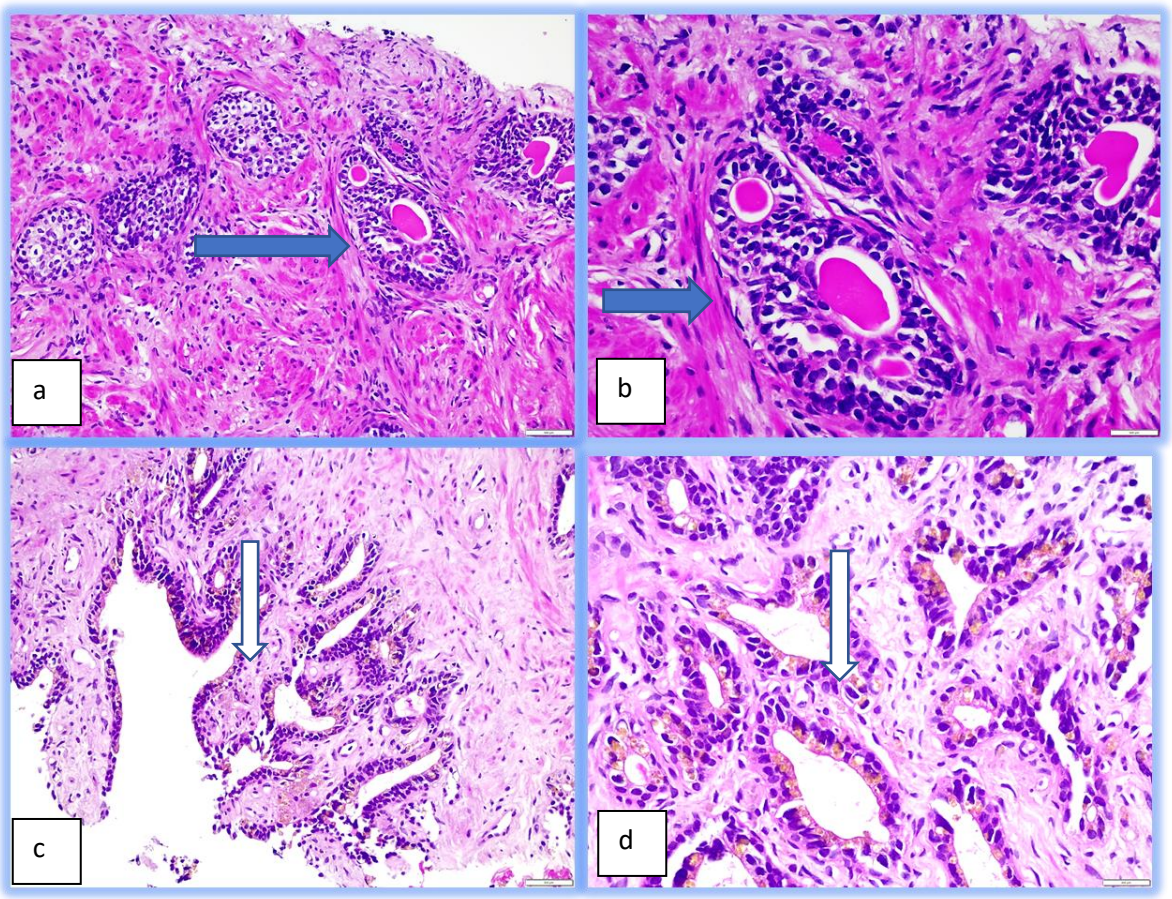


Figure 2: H&E images of basal cell hyperplasia and seminal vesicle. Basal cell hyperplasia at 20x objective (a) and 40x objective (b) highlighted by blue arrows. Seminal vesicle at 20x objective (c) and 40x objective (d) highlighted by white arrows.

Conditions that may be confused with PCa displaying large and cribriform glands include clear cell cribriform hyperplasia, cribriform basal cell hyperplasia and large hyperplastic glands. Thorson *et al* (2003) reported an incidence rate of 10.2% for cases of basal cell

hyperplasia. Granulomatous prostatitis, prostatic xanthoma, paraganglia and signet ring-like changes may be difficult to distinguish from PCa showing a solid and non-glandular growth pattern (Hameed *et al* 2010).

Giunchi *et al* (2017) showed that there is a high percentage of interobserver variability amongst general pathologists. Agreement was highest for PCa (80%) and lowest (49%) for proliferative atrophic lesions. This highlights difficulties in diagnosing the spectrum of atrophic lesions.

Beltran *et al* (2019) reported that the most common misdiagnoses on needle core biopsies related to adenosis being misinterpreted as cancer together with atrophy, PIN, seminal vesicle epithelium and crowded hyperplastic glands.

Adenosis is usually located within the transitional zone and comprises a well-defined nodule comprising tightly packed and small prostate glands. The challenge here, is that a nodule may not be appreciated on a needle core biopsy. Glands within adenosis may also show evidence of atypia but the basal cell layer, even though patchy, is retained on immunohistochemical staining.

Beltran *et al* (2019) reported atrophy as the second most common cause of a misdiagnosis of PCa in their study. Atrophic glands are usually small and crowded. They have a lobular architecture on low power magnification. The cells may have abundant cytoplasm but they do not have prominent nucleoli. Again, patchy basal cells are noted. The incidence of atrophy is quite high in several previous studies. Postma *et al* (2005) reported an incidence of 91%, Billis *et al* (2010) showed an incidence of 83.7% and Kryvenko *et al* (2012) showed an incidence rate of 88-100%.

2.3.5.6. Immunohistochemistry

P63 is a homologue of P53 and is expressed in basal cells lining prostate glands (Signoretti *et al* 2000). P63 immunohistochemistry can be requested on needle core biopsies showing features worrisome for carcinoma. P63 will stain basal cells in benign glands and will be negative in carcinoma as the malignant glands lack a basal cell layer. In certain needle core biopsies, the differentiation between malignancy and its mimics such as atrophy is difficult. P63 immunohistochemistry is helpful in these situations (Beltran *et al* 2018, Shah *et al* 2017, Srinivasen *et al* 2011). Bonert *et al* (2020) reported that 60% of cases reviewed within their cohort had immunohistochemical stains done to help with diagnosis. Watson *et al* (2013) reported that approximately 40% of their prostate cases warranted immunohistochemistry.

Alpha methylacycl coenzyme A racemase (AMACR) is upregulated in prostate cancer and can also be utilised as an immunohistochemical marker with positive staining being noted in carcinoma. Studies have not shown if one of the above markers is more superior than another. The efficient use of immunohistochemical stains will limit overdiagnosis of benign conditions that may mimic PCa (Shah *et al* 2017).

Erythroblast transformation specific (ETS) related gene (ERG) immunohistochemistry is a new immunohistochemical stain. ERG immunohistochemical staining has shown a strong correlation with overexpression of ERG mRNA and has been shown to be specific for the detection of PCa on needle core biopsies. Shah (2013) showed that ERG immunohistochemistry was most useful in biopsies where more than 2 cores were involved by tumour or in biopsies showing more than 50% involvement of the submitted tissue. Park *et al* (2010) showed that ERG immunohistochemistry had a 96% sensitivity and 97% specificity using the C terminus clone EPR 3864.

There are well recognised pitfalls in interpretation of immunohistochemical staining. In the context of staining performed on prostate biopsies, these include loss of P63 staining within benign entities such as adenosis and partial atrophy (Bonert *et al* 2020, Brimo *et al* 2011). In HGPIN one may also see negative or patchy staining of the basal cell layer. False positive

staining of basal cell immunohistochemical markers such as P63 may occur in PCa, especially tumours with a Gleason score of 3. PCa may show aberrant diffuse expression of P63 (Brimo *et al* 2011). It is therefore important to assess the patients PSA value, histology findings as well as immunohistochemical staining pattern together. Bonert *et al* (2020) also advise the use of more than one immunohistochemical stain and suggests a combination of P63, 34BE12 and AMACR when faced with cases showing features that are worrisome for PCa on the Haematoxylin & Eosin (H&E) stain.

A small subset of PCa can show expression of P63. This is a rare phenomenon and these P63 positive tumours have been shown to be molecularly distinct from acinar type adenocarcinoma of the prostate. These tumours are usually located peripherally, are infiltrative and are composed of small glands. The tumour cells are predominantly secretory cells with nuclear atypia and accompanying atrophy is usually a feature. (Tan *et al* 2015). The tumour cells have a more basaloid appearance as compared to conventional acinar adenocarcinoma (Wu *et al* 2013). In terms of immunohistochemistry, this tumour is positive for PSA, P63 and AMACR. It is negative for high molecular weight cytokeratins and has a low proliferation index on Ki67 staining (Wu *et al* 2013). The differential diagnosis for this tumour includes basal cell hyperplasia and basal cell carcinoma (Tan *et al* 2015).

Another recognised pitfall of P63 immunohistochemistry is that in some cases of atrophy or adenosis, isolated glands may be negative for P63 and therefore individual glands should not be assessed and rather the prostate biopsy in its entirety should be evaluated. If most of the glands within a group retain basal cells, then the isolated P63 negative glands may be interpreted as benign (Beltran *et al* 2019). AMACR may also be positive in adenosis and atrophy which is another pitfall of immunohistochemistry.

2.3.5.7. Perineural invasion

Perineural invasion (PNI) is defined as the presence of tumour cells tracking around and attached to a nerve. It is an important independent predictor of recurrence of PCa (Zhang *et al* 2018) and is a well-established mode of metastases for many tumours. Other variables

considered to be risk factors for biochemical recurrence in PCa include extension to seminal vesicle, positive surgical margins and extra prostatic extension (Zhang *et al* 2018). PNI is now becoming an important predictor of recurrence as previously used variables have been proven to be unreliable (Zhang *et al* 2018). The high risk of recurrence that PNI confers is despite the modality of treatment (radical prostatectomy versus radiotherapy) used (Zhang *et al* 2018). Dell'Atti (2016) has shown that PNI in biopsy specimens is associated with progression of disease in patients with a statistically significant (p value < 0.001) correlation between the presence of PNI and biochemical recurrence. Also, a correlation between PNI on biopsy and extraprostatic extension on radical prostatectomy as well as positive lymph node involvement and spread to seminal vesicles has been identified (Dell'Atti 2016). However, a statistically significant correlation between perineural invasion and positive surgical margins was not noted (Dell'Atti 2016). Contrary to these findings, Kraus *et al* (2019) found that PNI alone was not a risk factor for biochemical recurrence. They concluded that its presence is usually associated with more aggressive histology, higher Gleason scores and higher Grade Groups and argued that it does not necessarily confer an increased risk of recurrence. Saadat *et al* (2012) also showed a strong association between high Gleason scores and PNI in prostate needle core biopsies. Del'Atti (2016) showed a significant association between PNI and the degree of tumour aggressiveness ($p<0.001$) and pathological stage of tumour ($p<0.002$).

2.3.5.8. Lymphovascular space invasion

Lymphovascular invasion (LVI) is defined as the presence of tumour cells within lymphatic and vascular channels and is a common mode of metastases for many tumours including PCa.

Jiang *et al* (2018) found that the presence of LVI could be used as an indicator of biochemical recurrence. Although numerous conflicting studies exist, a meta-analysis showed that it is associated with more aggressive tumour pathology (Jiang *et al* 2018). Baydar *et al* (2008) and Brooks *et al* (2006) showed an association between LVI and a higher Gleason score as well as seminal vesicle invasion.

Studies have shown that LVI is associated with a high risk of biochemical recurrence (Brooks *et al* 2006, Jiang *et al* 2018, Kang *et al* 2017) and as such it should be regarded as an independent risk factor for recurrence in patients with PCa (Jiang *et al* 2018).

Kang *et al* (2017) noted a strong association between LVI and the presence of lymph node metastases ($p<0.001$). In addition, patients with LVI at presentation were noted to have a higher rate of 10-year bone metastases and cancer mortality compared to patients without LVI ($p=0.001$). As noted by Freeman (2009), LVI is rarely seen on prostate biopsy. He proposed that pathologists may be under-reporting this and that retraction artefact (where the stroma pulls away from tumour this creating a space) is a confounding factor. Baydar *et al* (2008) reported that a contributing factor to under reporting of LVI in needle core biopsies could be due to lack of identification by pathologists. Furthermore, the application of immunohistochemical stains such as CD31 to highlight LVI is hindered by the focus being cut way on subsequent levels.

2.3.5.9. Tumour volume

The assessment of tumour volume in prostate needle core biopsies is important as it may predict the possibility of biochemical recurrence especially in those patients with aggressive disease (Hong *et al* 2011, Tanaka *et al* 2012). A higher tumour volume has been shown to be associated with adverse clinicopathologic findings such as extraprostatic extension, positive surgical margins and lymph node involvement (Knoedler *et al* 2014). It has also been shown to provide valuable information regarding biochemical recurrence as well as predict morbidity and mortality of patients with prostate adenocarcinoma (Knoedler *et al* 2014). Most studies assessing tumour volume have focused on radical prostatectomy specimens rather than needle core biopsies.

In a systematic review published in 2011, Epstein addressed some of the issues in estimating tumour volume. A visual assessment may actually overestimate the tumour volume. There is

also currently debate on how to calculate volume if there are discontinuous foci of tumour in a single core and he is of the opinion that it should be regarded as a single focus and the benign stroma included. He acknowledges that quantification of the volume using an ocular micrometer is time consuming. The fraction of positive cores has been shown by others to have a significant predictive value. He recommends that the number of involved cores and either the length of tumour or the percentage involvement should be provided as a minimum. In surveys, cited in this review, pathologists report volume differently. The number of involved cores, the percentage of cancer per core, the overall percentage of cancer, millimeters of cancer per core, the overall millimetres of cancer per case or a subjective measure (eg. minute, focal) were used in reporting (Epstein 2011).

2.3.5.10. Grading and staging

The grading and staging of PCa are important as they are the most used prognostic indicators. The Gleason scoring system divides prostate cancer into patterns 1-5 based on the architectural features on histology (Humphrey 2004). Dr Donald F Gleason spent many years developing a scoring system to prognosticate PCa (Kgatle *et al* 2016). The scoring system named The Gleason score helps clinicians identify patients with aggressive tumours. The Gleason score is divided into major and minor categories. The tumour is histologically assigned a major score (this is a score based on what most of the tumour looks like histologically i.e., how well or poorly differentiated it is) and a minor score (what the second most common pattern is or what the worst pattern of tumour is).

Gleason scores should be assigned based on evaluation at 4x objective and in certain circumstances 10x objective (Gordetsky *et al* 2016). They are reported as an equation with the sum of the major and minor components in the biopsy. Gleason scores of 1 and 2 are no longer used in the reporting of needle core biopsies. A Gleason pattern of 3 refers to glands that are well formed and individual. They may show various shapes and sizes including areas demonstrating a branching pattern however fusion of glands is not allowed in pattern 3 (Gordetsky *et al* 2016). A Gleason pattern of 4 is assigned to those biopsies demonstrating irregular and fused glands. The glands in pattern 4 are poorly formed and may exhibit a

cribriform growth pattern. Gleason pattern 4 also includes hypernephroid and glomeruloid growth patterns of the neoplastic cells. Pattern 5 is assigned to those tumours with a solid growth pattern or demonstrating the presence of single cell infiltration (Gordetsky *et al* 2016). Gleason pattern 5 includes tumours displaying comedonecrosis as well as signet ring cell morphology.

In terms of Gleason scores, three risk categories are described namely low risk, intermediate risk and high risk depending on the sum of the major and minor Gleason scores (Epstein *et al* 2016[b]). A low-risk category is awarded to a Gleason score between 2 and 6, intermediate risk is for a Gleason score of 7 and a Gleason score between 8 and 10 confers high-risk disease (Epstein *et al* 2016[b]).

In 2014 the International Society of Urological Pathology (ISUP) reviewed and improved this scoring system with a Grade Group index (Epstein *et al* 2016[a]). Together the Gleason score and Grade group index provide important prognostic information and is still in use to date with no better prognostic index having been developed. The Gleason scoring system despite its wide application and use did have limitations. The system was complex and was not easy for patients to understand when using it to discuss prognosis. Therefore, a new grading system proposed by a group from John Hopkins was introduced (Gordetsky *et al* 2016). The Grade Groups have proven to be more well correlated with patient prognosis (Sehn *et al* 2018). There are five Grade Groups designated as follows (Gordetsky *et al* 2016):

- Grade Group 1 = Gleason score less than or equal to 6
- Grade Group 2 = Gleason score 7 (3+4)
- Grade Group 3 = Gleason score 7 (4+3)
- Grade Group 4 = Gleason score 8
- Grade Group 5 = Gleason score 9 and 10

Grade group 1 tumours constitute the lowest grade and have the best prognosis. The grade groups are a far simpler way to prognosticate and are noted to be more accurate in relation to the tumour biology (Gordetsky *et al* 2016). It is recommended that the Grade Groups system be used in combination with the Gleason scoring system. Grade Groups are now being used more widely as a strong predictor of cancer related mortality in patients with PCa with the risk of mortality increasing as the Grade group increases (Milonas *et al* 2021).

"The World Health Organization (WHO) 2016 Pathology and genetics: Tumours of the urinary system and male genital organs" have also accepted the Grade Groups as an important prognostic indicator of PCa.

Staging is important in the selection of therapy. The Union for International Cancer Control (UICC) 8th edition is used to stage prostate carcinomas according to the TNM (tumour size, node involvement and presence of metastases) criteria.

2.3.5.11. Association between coexisting benign pathology and prostate carcinoma

Benign prostatic hyperplasia (BPH) is a condition during which prostate gland cells replicate and grow causing the prostate to increase in size (Laguna *et al* 2000). As the name implies it is a benign condition. It generally occurs in elderly males, the same population that is at an increased risk for PCa (Laguna *et al* 2000). PSA levels can be increased in this condition and therefore the only means of making a definitive diagnosis of BPH and exclusion of PCa is by histologic examination of needle core biopsies of the prostate gland (Tornblom *et al* 1999). Tindall *et al* (2013) showed that BPH was not associated with an increased risk of PCa and they postulated that this may be because BPH affects the transitional zone of the prostate, and most cases of PCa target the peripheral zones.

Literature regarding the relationship between prostatitis and PCa are conflicting (Sfanos *et al* 2012). Jiang *et al* (2013) reported a significant correlation between prostatitis and PCa with an odds ratio of 1.64 and a confidence interval 95%. Hung *et al* (2013) showed and

increased risk of PCa in patients with both BPH and prostatitis (adjusted odds ratio 49.2 with a 95% confidence interval). This is similar to other studies by Cai *et al* (2019), Davidsson *et al* (2011) and Perletti *et al* (2017). Reasons for this link between prostatitis and PCa include:

1. Many genes involved in innate immunity are also involved in PCa (Hughes *et al* 2005).
2. Inflammation results in release of inflammatory mediators which promotes carcinogenesis (Hughes *et al* 2005, Jiang *et al* 2013).
3. There is a loss of tumour suppressor genes.
4. Inflammation causes damage to the prostate gland that leads to carcinogenesis and PCa (Davidsson *et al* 2011 and Perletti *et al* 2017)

However, Roberts *et al* (2004) and Rybicki *et al* (2016) were unable to prove an association. It was felt that the higher detection rate of PCa is due to the increased screening in patients with prostatitis (so called selection bias) and not due to a direct correlation between the two (Roberts *et al* 2004, Langston *et al* 2019).

2.3.6. NEW BIOMARKERS

Many new biomarkers have been developed with a specific focus on detecting tumour aggressiveness as well as response to treatment modalities (Zapala *et al* 2018). Although a few new biomarkers are available, limitations with regards to cost and availability preclude their current use. It is for this reason that total PSA serum levels are still used as the initial screening test. However, total PSA levels are not able to predict the grade of the tumour neither can they predict response to treatment.

PSA changes during tumour development with different forms being secreted at different stages of oncogenesis (Zapala *et al* 2018). The different variants that are secreted are dependent upon the cells that are mutated (Zapala *et al* 2018). As a result of these developments, the traditional method of measuring the total serum PSA level is becoming

outdated and therefore further studies looking at the different forms of PSA and how they are impacted during oncogenesis may play a vital role in more effective management of this disease. It is also important to consider different serum markers to reduce the over detection and over treatment that is seen with measurements of total serum PSA levels (Zapala *et al* 2018).

Other PSA values besides the total serum PSA level such as PSA velocity, PSA density and free PSA levels have been studied but to date do not seem to be superior to the total serum PSA levels (Nordstrom *et al* 2015). However, in patients on active surveillance, the PSA density has been shown to be more beneficial than total serum PSA levels alone (Stattin *et al* 2015).

2.3.6.1. PSA density

The PSA density considers the volume of the prostate gland whereas total PSA does not (Stattin *et al* 2015). PSA density is calculated by using the total serum PSA level and the prostate volume. Prostate volume is calculated by means of a transrectal ultrasound. PSA density will therefore be beneficial in patients with concomitant BPH or atrophy. PSA density levels have been categorized into three groups namely normal; intermediate and raised/pathological (Montironi *et al* 2000). Patients with PCa were shown to have a lower prostate volume and higher PSA density whereas patients with BPH had a higher prostate volume and lower PSA density (Laguna *et al* 2000).

There have been discrepancies in study findings with regards to PSA density with some showing that it is superior to total PSA levels when differentiating between benign and malignant conditions (Bazinet *et al* 1994) and others showing no significant differences between the 2 modalities (Montironi *et al* 2000). Differences in findings on PSA density may be related to the population groups studied or differences in technique of prostate biopsies and transrectal ultrasounds used to determine the prostate volume (Montironi *et al* 2000).

It has been shown that the use of different serum forms of PSA, correlation to prostate volume (PSA density) and correlation to the different prostate zones have been beneficial in differentiating BPH from PCa (Laguna *et al* 2000).

2.3.6.2. Ratio of free PSA to total PSA

The ratio of free PSA to total PSA has been found to be lower in patients with PCa and this ratio may be helpful in patients with borderline total PSA values (Zapala *et al* 2018). The free PSA molecule is very unstable though which may negatively affect the analytical outcomes of this variable. In men with normal DRE and total PSA values between 2.5 and 4ng/ml, the percentage of free PSA can be beneficial to detect PCa (Zapala *et al* 2018). In patients with localized PCa, a lower ratio of free PSA to total PSA has been linked with extracapsular extension of PCa (Zapala *et al* 2018).

2.3.6.3. New modality: Transmembrane serine protease isoform 2-TMPRSS2 fused with ERG-ETS transcription factor family member gene (TMPRSS2-ERG)

TMPRSS2-ERG is an AR fusion gene that has been identified in approximately 50% of cases of PCa and 20% of cases of HGPIN (Leyten *et al* 2014, Shah 2013). To date, this gene alteration is the most common genetic anomaly seen in PCa (Leyten *et al* 2014). Shah (2013) further described that ERG gene fusions were negative in benign prostate as well as benign mimics of PCa. TMPRSS2-ERG is a result of failure of the repair genes, and this has a negative result on signalling pathways. This fusion gene is also noted to be a key player in metastatic disease (Sabaliauskaite *et al* 2012). TMPRSS2-ERG can be used as a biomarker and has been detected through reverse transcriptase polymerase chain reaction (PCR) techniques on urine samples (Leyten *et al* 2014). Detection rates of TMPRSS2-ERG may be better on biopsy material as compared to urine samples (Leyten *et al* 2014). On its own, the detection of this fusion gene does not appear to be helpful but in combination with currently used biomarkers and risk stratification variables, the TMPRSS2-ERG fusion gene may be beneficial to detect aggressive disease as well as recurrence of PCa (Leyten *et al* 2014).

To date total PSA serum level testing is the only available biomarker that we have available to us in South Africa. The European Association of Urology advises the use of serum total PSA levels together with DRE and assessment of the age, race, and family history of PCa to risk stratify patients (Heijnsdijk *et al* 2016). It is also recommended that life expectancy should be used as an additional risk stratification variable (McGrath *et al* 2016).

2.3.7. TREATMENT

Currently there are approximately three modalities of treatment for PCa namely radical prostatectomy, radiation therapy and active surveillance (Li *et al* 2012). Three variables considered when treatment plans are being devised include the age of the patient, the stage of the cancer and the grade of the tumour (Li *et al* 2012).

Recent data have revealed that localized and moderately differentiated tumours predominate however these statistics may vary depending on the age of the patient as well as the patient's time of presentation (Li *et al* 2012).

Treatment plans and strategies based solely on tumour stage and grade may become outdated soon. Even though data shows more aggressive disease in elderly men, this does not necessarily translate to more aggressive treatment strategies in this population (Hall *et al* 2005). Life expectancy as well as quality of life needs to be addressed when deciding on treatment strategies (Hall *et al* 2005). PCa is often an indolent disease with a life expectancy of more than 10 years in many cases, therefore, before opting for aggressive treatment regimens, clinicians need to assess a patient's life expectancy and decide if an aggressive treatment plan is the right choice (Hall *et al* 2005). Patients should also be well informed of all treatment options as well as the impact of these on their quality of life so that they are well equipped to make informed decisions regarding their treatment plans (Hall *et al* 2005).

CHAPTER 3: RESEARCH METHODOLOGY

3.1. THE STUDY DESIGN

This study is an observational retrospective descriptive study that analysed prostate needle core biopsies over a 13-month period. All needle core biopsies that were submitted to the National Health Laboratory Service (NHLS) CHBAH, Johannesburg, South Africa from 1 January 2018 to 31 January 2019 were reviewed. CHBAH is the third largest hospital in the world and has approximately 3700 beds. The hospital is based in Soweto, Johannesburg South Africa and is one of the major teaching hospitals affiliated with the University of the Witwatersrand.

3.2. TARGET POPULATION

The target population included all prostate needle core biopsies performed at CHBAH during the period 1 January 2018 to 31 January 2019.

3.3. SAMPLING

Data was extracted with the assistance of the Central Data Warehouse (CDW) using the specific Systematized Nomenclature of Medicine (SNOMED) tissue code T-92000 (prostate).

3.4. ETHICAL CONSIDERATIONS

Ethics approval was granted by the University of the Witwatersrand, Human Research Ethics Committee, Medical (see appendix).

Permission was granted by the Head of the Department, Dr Yvonne Perner and the NHLS Academic Affairs to access the NHLS laboratory information system (see appendix).

All cases were anonymised and assigned a unique study number.

The spreadsheets containing the results are stored on the desktop computer of the principal investigator. This computer is password protected.

No information other than that specified on the data collection sheet (see appendix) was collected.

3.5. INCLUSION AND EXLUSION CRITERIA

All prostate needle core biopsies performed during the period 1 January 2018 to 31 January 2019 and submitted to the Histopathology Laboratory at CHBAH were included in this retrospective observational study. Prostate chip biopsies and radical prostatectomy specimens were excluded. Prostate needle core biopsies that were insufficient for adequate histological examination (too little tissue for assessment or no representation of prostate glands) were also excluded from this study.

3.6. DATA COLLECTION

The histopathological reports were provided by the NHLS Corporate Data Warehouse and data on the following variables were extracted from histopathological reports where available:

Age of patient,
Race (Black, Caucasian, Indian/Asian, Coloured and not specified),
Prostate specific antigen level (recorded in ug/L),
Previous biopsy results,
Number of cores at macroscopy,
Number of prostate cores at microscopy,
Diagnostic category (adenocarcinoma, high grade prostate intraepithelial neoplasia, atypical small acinar proliferation, acute prostatitis, atrophy, basal cell hyperplasia, chronic inflammation, normal)

The tumour volume (as the overall percentage of tumour within the specimen),
The number of cores involved by tumour,

Perineural invasion, (present or absent)

Lymphovascular space invasion, (present or absent)

Gleason score (major and minor pattens),

Major and Minor patterns (please see “Grading and Staging” section of the literature review, page 26),

Grade group category (please see “Grading and staging section of the literature review, page 26),

Presence of metastasis (as documented in the clinical history section of the report),

P63 immunohistochemistry (positive or negative).

If more than one PSA level was available, the most recent serum level was used. PSA levels were assigned one of two categories. For the purposes of this study, as per international guidelines (Laguna et al 2000), a PSA value of less than or equal to 4ug/l was reported as normal and a PSA level of greater than 4ug/l was reported as elevated or raised.

The data that was collected was entered onto a Microsoft Excel 2010® Excel spreadsheet.

3.7. DATA ANALYSIS METHODS

Statistical analysis software - Statistical Package for the Social Sciences (SPSS) was used to analyse the data with the assistance of a statistician. Descriptive statistical analysis was conducted. Numerical data (e.g. age, PSA, number of cores) have been represented as means and standard deviations (SD) where normally distributed. Where not normally distributed, median and interquartile ranges have been reported as well as mean ranks. Categorical data (such as diagnostic category, tumour grade) have been expressed as frequencies.

The median PSA levels are shown for each diagnostic category and have been assessed to deduce if there are statistically significant differences using a [1]Chi Square test and [2]Kruskal Wallis test.

The PSA levels have been compared to see if there is any correlation between:

(a) PSA levels and Grade group using Kruskal Wallis test followed by Mann Whitney test. A Welch test (adjustment to ANOVA) with Games-Howell post hoc test was also used.

(b) PSA levels and tumour volume using Spearman's correlation.

3.8. RELIABILITY AND VALIDITY

Internal validity in this study was assured by the researcher adopting a study design that fulfilled research requirements.

The researcher ensured reliability in this study by confirming that the design of data collection sheet yielded the same results for all histopathological reports that were reviewed. The results were double checked after entry.

The reliability and internal validity of the data and integrity are crucial in managing bias.

External validity was ensured by careful consideration of the sample population selection process as well as use of a standardized data collection sheet for both the malignant/tumour and benign cases.

[1] A Chi Square test is used to compare observed results in a study with expected results. This test analyses data and decides if a true relationship exists between variables or if it is due to chance.
[2] The Kruskal Wallis test is a nonparametric test. It is used when testing two or more independent variables

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. SAMPLE SIZE

Over the study period (1 January 2018 to 31 January 2019), there were 18 337 cases received at CHBAH Histopathology laboratory. Prostate samples accounted for 874 (4.8%) of these samples. Of these, 90 prostate cases were excluded. The excluded cases were either inadequate/unsatisfactory, prostate chip biopsies or radical prostatectomy specimens.

A total of 784 cases were included in the cohort and were subsequently reviewed. Of these, 317 cases (40.4%) were assigned a benign diagnosis and 467 (59.6%) were assigned a PCa diagnosis as represented in figure 3.

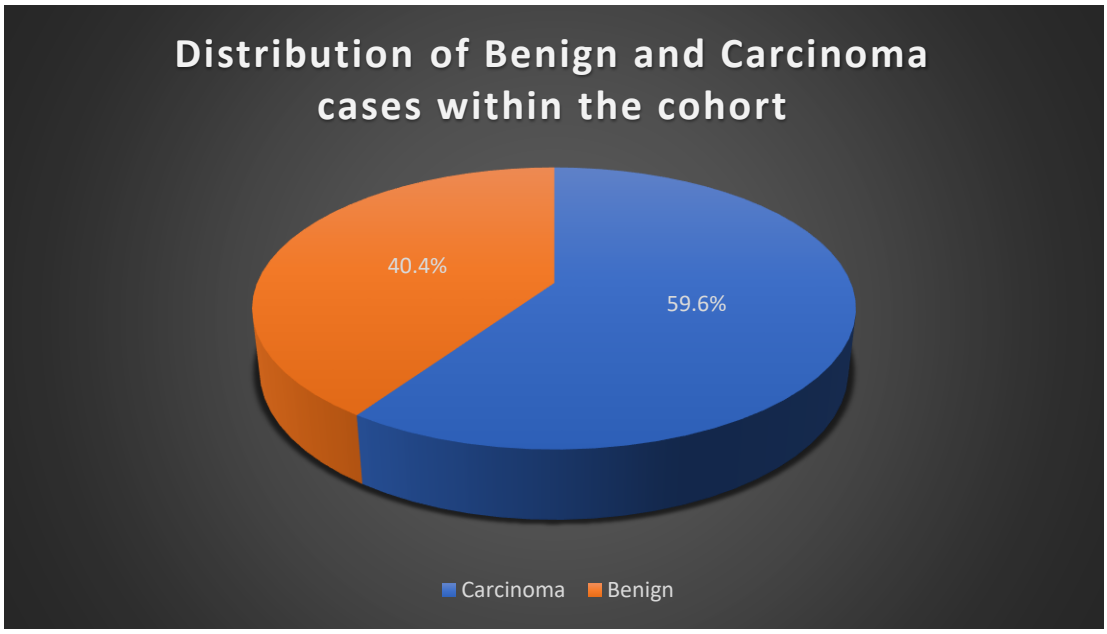
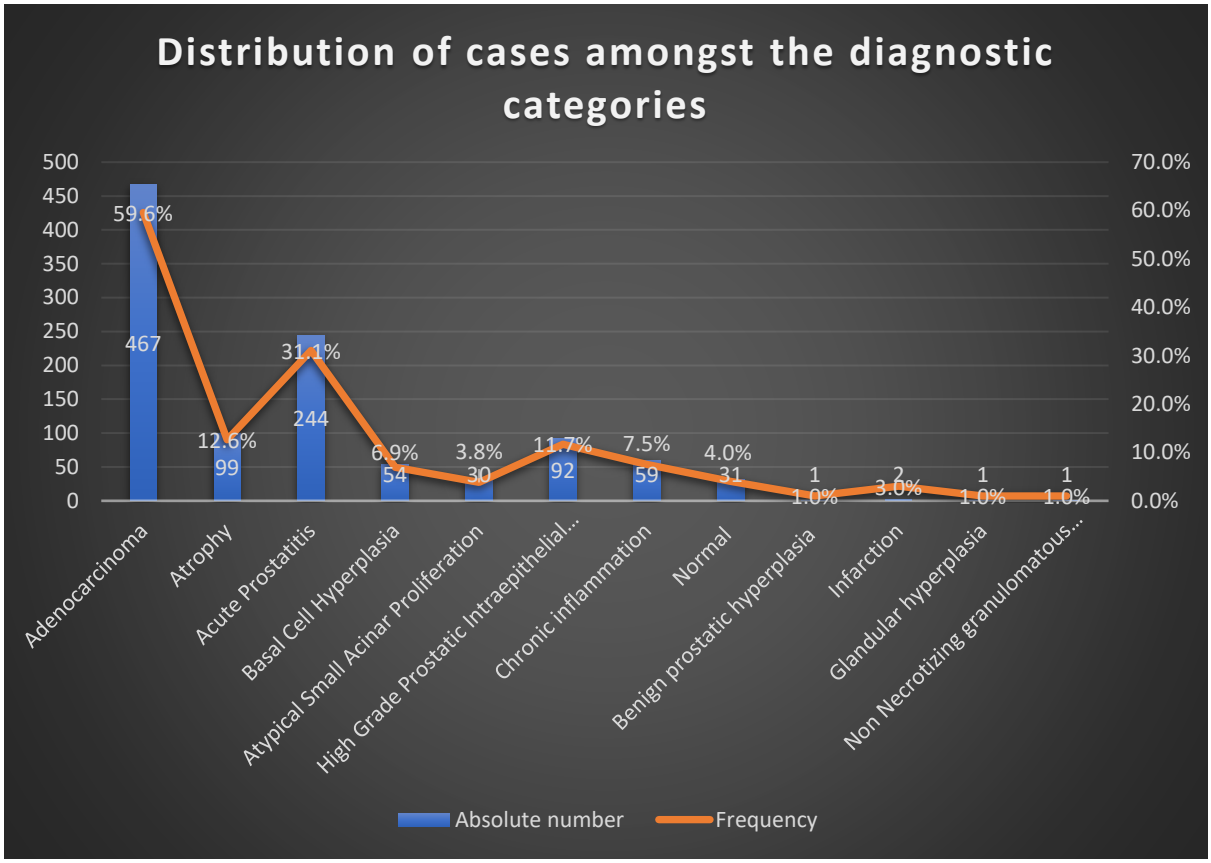


Figure 3: Pie chart showing the distribution of benign and carcinoma cases

Figure 4 shows the distribution of cases amongst the different diagnostic categories within the study cohort. There was a total of 467 cases of prostate adenocarcinoma (59.6%). The most common benign diagnosis was acute prostatitis (244 cases, 31.1%). This was followed

by atrophy (photo-micrographed in figure 5) and HGPIN. 31 prostate core biopsies (4.0%) were concluded as normal. ASAP was diagnosed in 30 cases (3.8%).



[34]Figure 4: Graph showing the distribution of the different diagnostic categories

[3] We had a total of 784 cases. Figure 4 illustrates the different diagnostic groups. This includes cases with more than 1 diagnostic category (e.g prostate carcinoma with acute prostatitis and basal cell hyperplasia)

[4] We had a total of 176 cases with acute prostatitis alone. Some cases did show dual pathology and therefore figure 4 shows a total of 244 biopsies with acute prostatitis

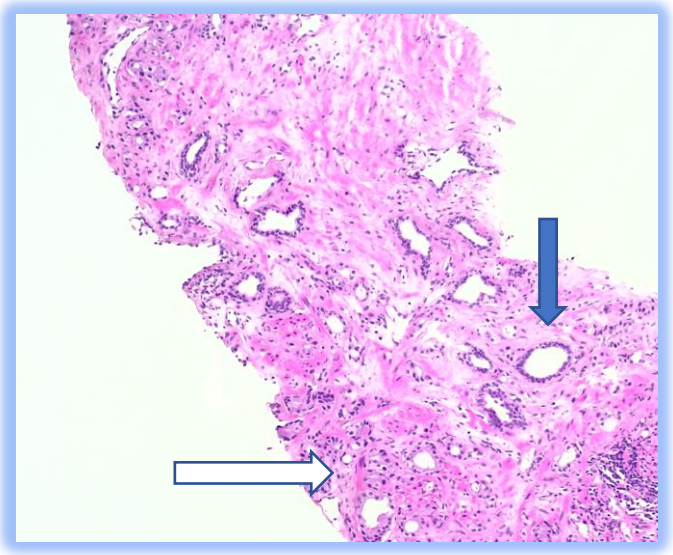


Figure 5: H&E image illustrating atrophy (blue arrow) and prostate carcinoma (white arrow) (10x objective)

4.1.2. AGE

Three cases within the cohort had no patient age recorded on the request form. The age range within the cohort was 36-92 years old. The mean age within the cohort was 66.1 years with a Standard deviation (SD) of 8.2 years.

Age was normally distributed and therefore mean values were used. An independent sample t-test was used. This showed that the mean age in the PCa group was 66.7 years with an age range of 43-89 years old. The mean age in the benign group was 65.2 years with a range of 36-92 years old. These findings are shown in Table 1. Patients in the PCa group were noted to be significantly older than those in the benign group, p=0.014.

Table 1: Age ranges, Means and Standard deviations of patients within the cohort

	Age range (years)	Mean (years)	Standard Deviation (years)
Whole cohort	36-92	66.1	8.2
Benign group	36-92	65.2	8.5
Carcinoma group	43-89	66.7	7.9

4.1.3. RACE

Data on race was available for 289 patients (36.9%) in total. In total, 495 cases (63.1%) did not have a race category assigned to the patient, of which 63.6% were PCa cases and 62.5% were benign cases.

Of the cases that did have an assigned race, the following were noted: 36.2% of PCa cases were found in African men and 0.2% in Coloured men. There were no PCa cases in White men or Indian/Asian men where race was assigned. Of the benign cases, 36.9% were in African men and 0.6% in Coloured men.

4.1.4. PROSTATE SPECIFIC ANTIGEN

A total of 779 cases (99.3%) had serum PSA levels available with 5 cases (0.6%) missing a PSA value. The serum PSA range was 0.1-37460ug/L. The median PSA for the cohort was 15.2ug/L with an Interquartile range (IQR) of 14.6ug/L.

4.1.4.1. Normal PSA

6 patients within the cohort had normal PSA levels (less than or equal to 4ug/L). Of these, 2 patients had PCa (33.3%), and 4 patients were assigned benign diagnoses (66.7%) (see figure 6, below).

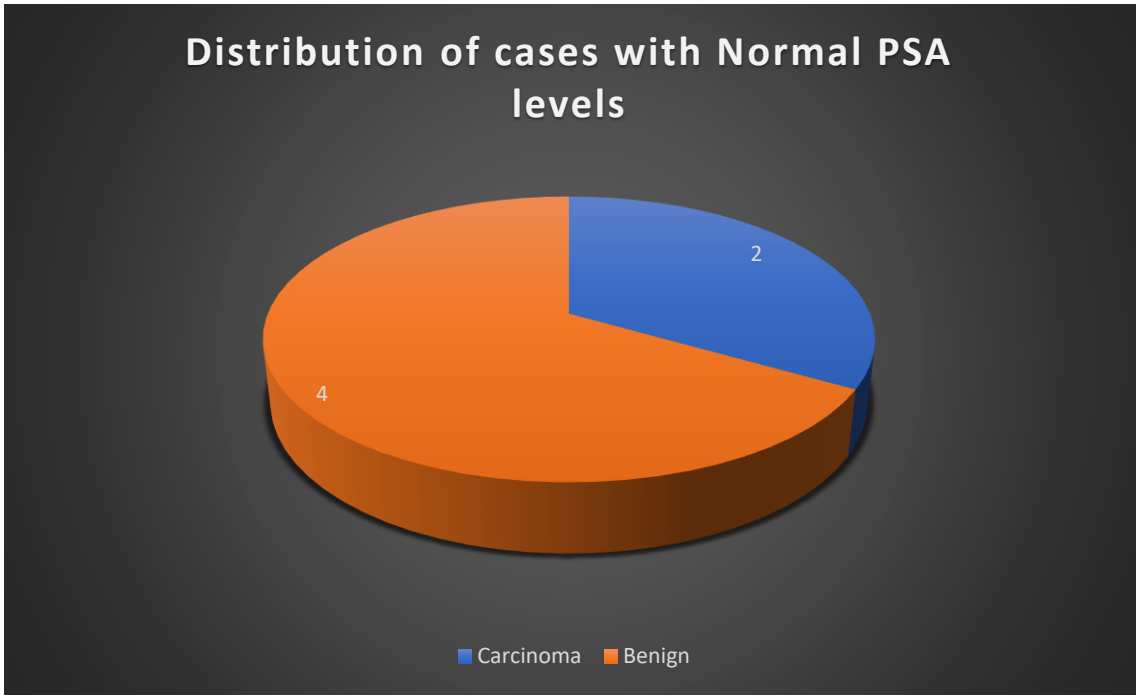


Figure 6: Pie chart showing the distribution of cases with normal PSA levels (less than or equal to 4ug/L)

4.1.4.2. Raised PSA

Figure 7 represents all the patients (n=773) with raised PSA levels (greater than 4ug/L). Five patients had missing PSA values. Of the cases with raised PSA levels, 462 (59.8%) were assigned a PCa diagnosis and 311 (40.2%) were benign.

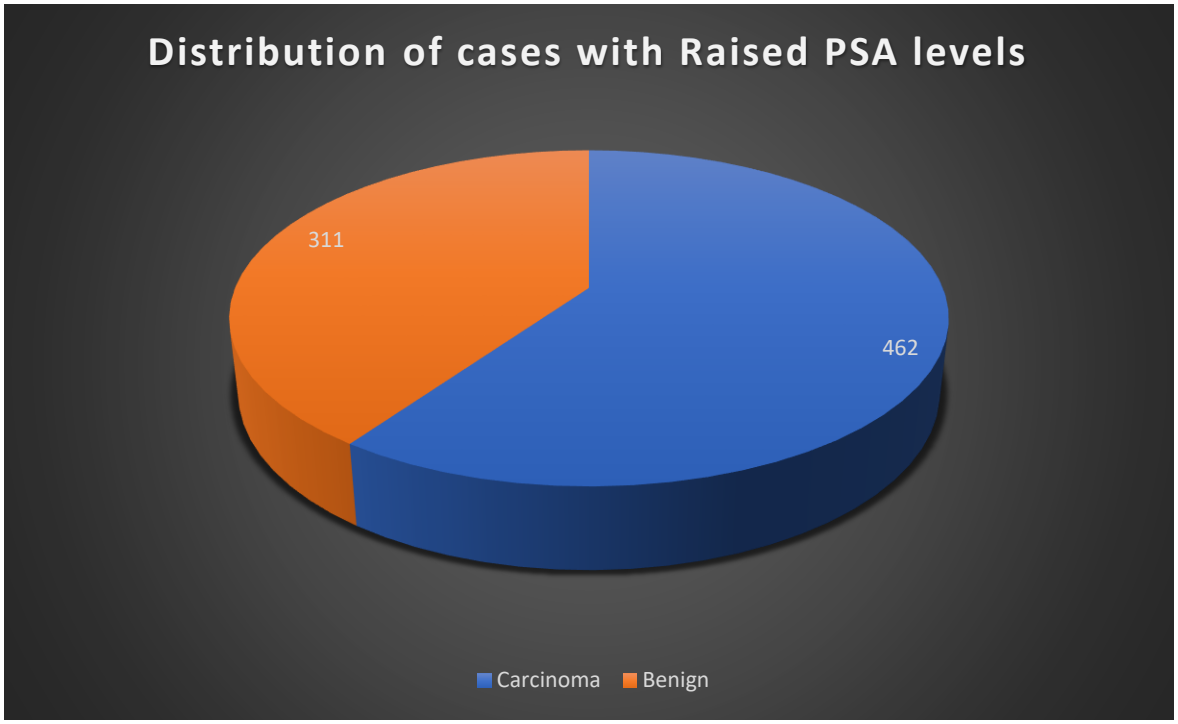


Figure 7: Pie chart showing the distribution of cases with raised PSA levels (greater than 4ug/L)

Table 2 shows the median PSA levels for the benign and carcinoma groups within the entire cohort (patients with raised and normal PSA levels). The benign group had a median PSA of 9.6ug/L and an IQR of 9.9ug/L. The carcinoma group had a median PSA of 23.4ug/L and an IQR of 93.3ug/L.

A Mann-Whitney test showed that PSA levels were significantly higher in patients with carcinoma ($p<0.0005$ and median of 23.4ug/L).

Table 2: Median PSA values within the benign and carcinoma group

	Benign (number and %)	Carcinoma (number and %)
Missing PSA value	2 (0.6%)	3 (0.6%)
Valid PSA value	315 (99.4%)	464 (99.4%)
TOTAL	317	467
Median	9.6ug/L	23.4ug/L
Interquartile range	9.9ug/L	93.3ug/L

Serum PSA values in each diagnostic category were compared as shown in Table 3. The Kruskal Wallis test showed a significant difference in PSA levels across benign and carcinoma categories, $p<.0005$. The Wilcoxon signed ranks test showed that PSA levels for carcinoma were greater than the PSA levels for atrophy, acute prostatitis, ASAP, HGPIN, chronic inflammation, and normal prostate core biopsies. This test also showed that PSA levels in acute prostatitis and HGPIN were greater than the PSA levels seen with normal prostate core biopsies.

Table 3: Median PSA levels for each diagnostic category

Diagnostic Category	Number of cases	Percentage of total cohort (%)	Median PSA (ug/L)	Interquartile range (ug/L)
Adenocarcinoma	464	59.2	23.4	93.3
Atrophy	14	1.8	8.3	5.8
Acute prostatitis	176	22.4	10.0	11.5
Basal cell hyperplasia	9	1.1	15.0	19.7
Atypical small acinar proliferation	30	3.8	9.1	9.4
High grade prostatic intraepithelial neoplasia	22	2.8	10.9	13.9
Chronic inflammation	32	4.1	8.8	10.6
Normal	31	3.9	8.0	3.1
Glandular hyperplasia	1	0.1	12.0	0.0

4.1.4.3. PSA and Grade Groups

PSA levels amongst the Grade Groups of tumours were analysed. A Kruskal Wallis test, followed by Mann Whitney on the groups showed a significant difference in PSA serum levels across the five Grade Groups, $p<.0005$. The significant differences are PSA for Grade Groups 2-5 were greater than (>) the PSA for Grade Group 1; PSA for Grade Groups 3–5 > PSA for Grade Group 2; PSA for Grade Group 5 > PSA for Grade Group 3 and PSA for Grade Group 5 > PSA for Grade Group 4.

When testing the mean values using the Welch test (adjustment to ANOVA) with Games-Howell post hoc tests, results show that PSA for Grade Group 3 is > PSA for Grade Group 1; and PSA for Grade Group 5 > PSA for Grade Groups 1 and 2.

Table 4 shows the median PSA values within each Grade Group. As Grade Group increases, so do the median PSA levels.

Spearman's correlation coefficient is a measure of a monotonic relationship. The results showed that there is a moderate monotonically increasing relationship between PSA and Grade Group, $p<0.0005$. A moderate positive correlation exists between PSA and the Major and Minor Gleason scores ($p<0.0005$ in each case).

Table 4: Median PSA values for each Grade Group

Grade Group	Number of cases	Median PSA (ug/L)	Interquartile range (ug/L)
1	49	8.9	8.2
2	151	13.5	20.0
3	104	24.5	80.6
4	34	55.0	117.1
5	130	144.2	613.1

4.1.4.4. PSA in patients with carcinoma versus acute prostatitis

A comparison of PSA levels in patients with carcinoma versus those with acute prostatitis showed a median PSA of 23.4ug/L in patients with carcinoma with a range of 0.3-37460ug/L. A median PSA of 10.0ug/L and a range of 2.3-221ug/L was seen in patients with acute prostatitis. A Mann Whitney U test showed that PSA levels were significantly higher in patients with carcinoma as compared to patients with acute prostatitis, p=<0.005.

4.1.4.5. PSA and perineural invasion

PNI was noted in a total of 233 cases (49.9%). The median PSA level in cases with PNI was 60.1ug/L (mean rank 287.9) and the median PSA level in cases without PNI was 14.0ug/L

(mean rank 178.8). The PSA level in cases with PNI were significantly higher than the PSA levels in the cases without PNI, p<0.005.

4.1.4.6. PSA and lymphovascular invasion

LVI was identified within 55 cases (11.8%). The median PSA level in cases with LVI was 113.5ug/L (mean rank 333.5). The median PSA level in cases without LVI was 20.6ug/L (mean rank 219.8). PSA levels for cases with LVI were significantly higher than cases without, p<0.005.

4.1.5. NUMBER OF CORES

The range for number of cores for the entire cohort was 1-32 cores. The lowest number of cores submitted was one and the highest number of cores submitted was 32. The median number of cores submitted was 11 with an IQR of 4. The median number of cores for the benign group was 12 (IQR 5) and the median number of cores for the carcinoma group was 10 (IQR 4).

4.1.5.1. Number of cores in benign versus carcinoma groups

A Mann Whitney test showed that the number of cores submitted, both on macroscopy and microscopy was significantly more for the benign group than the carcinoma group, p=0.001 and p<0.0005 respectively.

4.1.5.2. Number of cores and Grade Groups:

Table 5 shows the mean ranks[5] for the number of cores within each Grade Group category. The mean ranks of number of cores within each Grade Group increases as the Grade Group increases i.e., as the Grade Group increases so do the number of cores submitted.

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[1]Mean ranks are average positions within a list. The mean rank is calculated by sorting data into order from lowest to highest and assigning each category on the list a rank number. Mean ranks are used instead of medians when the two variables used have different shapes (www.statstutor.ac.uk).

Table 5: Mean ranks of the different Grade Group categories

Grade Group	Number of cores mean rank
1	84.5
2	204.0
3	250.2
4	255.3
5	301.4

A Kruskal Wallis test followed by Mann-Whitney test showed that there is a significant difference in the number of cores involved depending upon the Grade Group, $p<0.0005$.

Specific differences are as follows (G refers to Grade Group):

Number of cores involved G2, G3, G4, G5 > G1; G3, G5 > G2; G5>G3.

4.1.6. TUMOUR VOLUME

Tumour volume was calculated at the time of histological diagnosis. Tumour volume was calculated by using the total percentage of tumour within the submitted biopsy specimens. The median tumour volume for the study cohort was 40% (IQR 60%). The range was 1-96%. Table 6 shows that a more aggressive tumour/higher Grade Group is associated with a larger tumour volume percentage.

Using Spearman's correlation, a strong positive correlation was shown between the PSA level and tumour volume ($p<0.0005$).

Table 6: Tumour volume mean ranks for each Grade Group category

Grade Group	Tumour volume mean rank
1	66.2
2	192.9
3	239.0
4	242.4
5	337.0

A Kruskal Wallis test followed by Mann-Whitney test showed that there is a significant difference in tumour volume depending upon the Grade Group, $p<0.0005$.

Specific differences are as follows (G refers to Grade Group):

Tumour volume G2, G3, G4, G5 > G1; G3, G4, G5 > G2; G5>G3; G5>G4.

4.1.7. GLEASON SCORE

Figure 8 illustrates the percentage of cases within each Gleason pattern. Within the cohort, a major Gleason pattern 3 was seen in 45.3% (n=212) of biopsies. A major Gleason pattern 4 was seen in 48.1% (n=225) of cases and a major Gleason pattern 5 was observed in 6.6% (n=31) of cases. A minor Gleason 3 pattern was seen in 33.30% (n=156) of cases, minor Gleason pattern 4 was seen in 41.5% (n=194) and a minor Gleason pattern of 5 was observed in 25.2% (n=118).

Spearman's correlation showed a moderate positive correlation between the major and minor Gleason scores and PSA levels ($p<0.005$). Gleason patterns are photo-micrographed in figures 9 and 10.

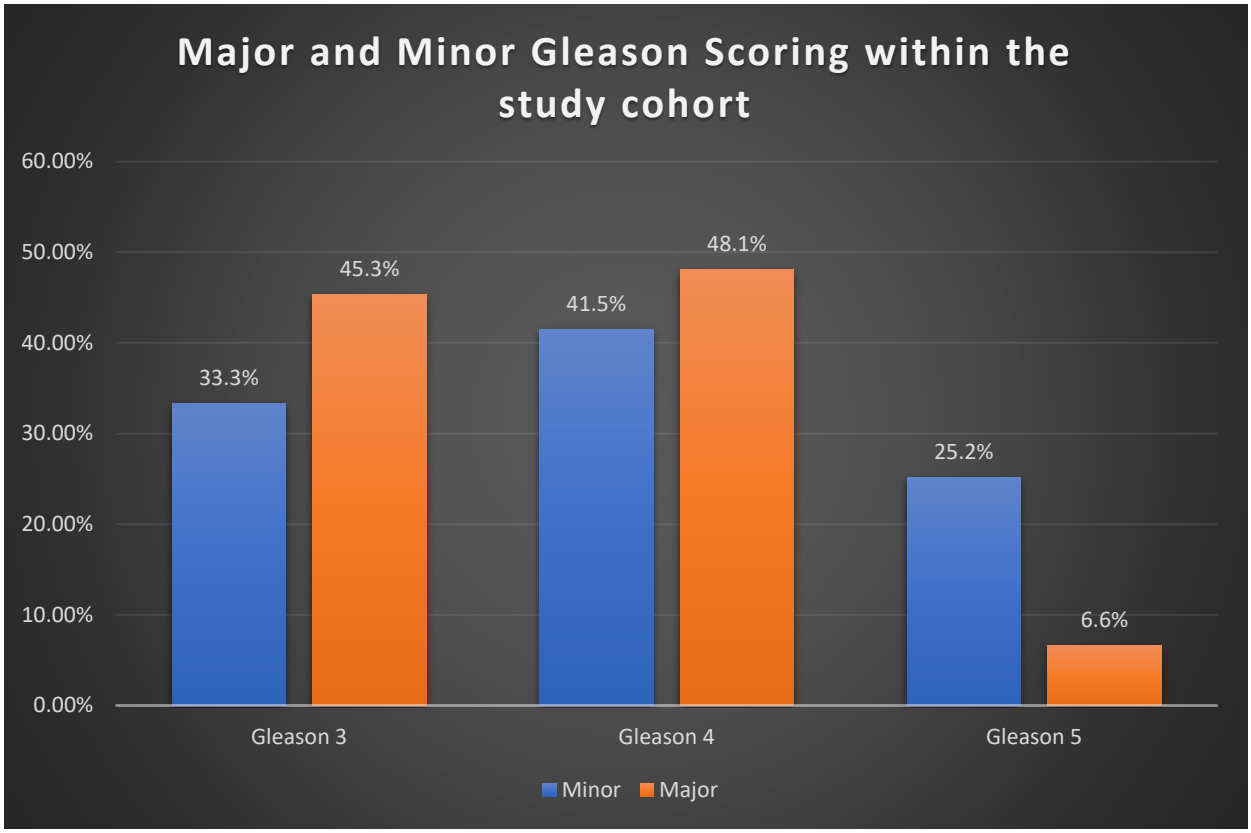

Figure 8: Graph showing the distribution of Gleason pattern 3, 4 and 5

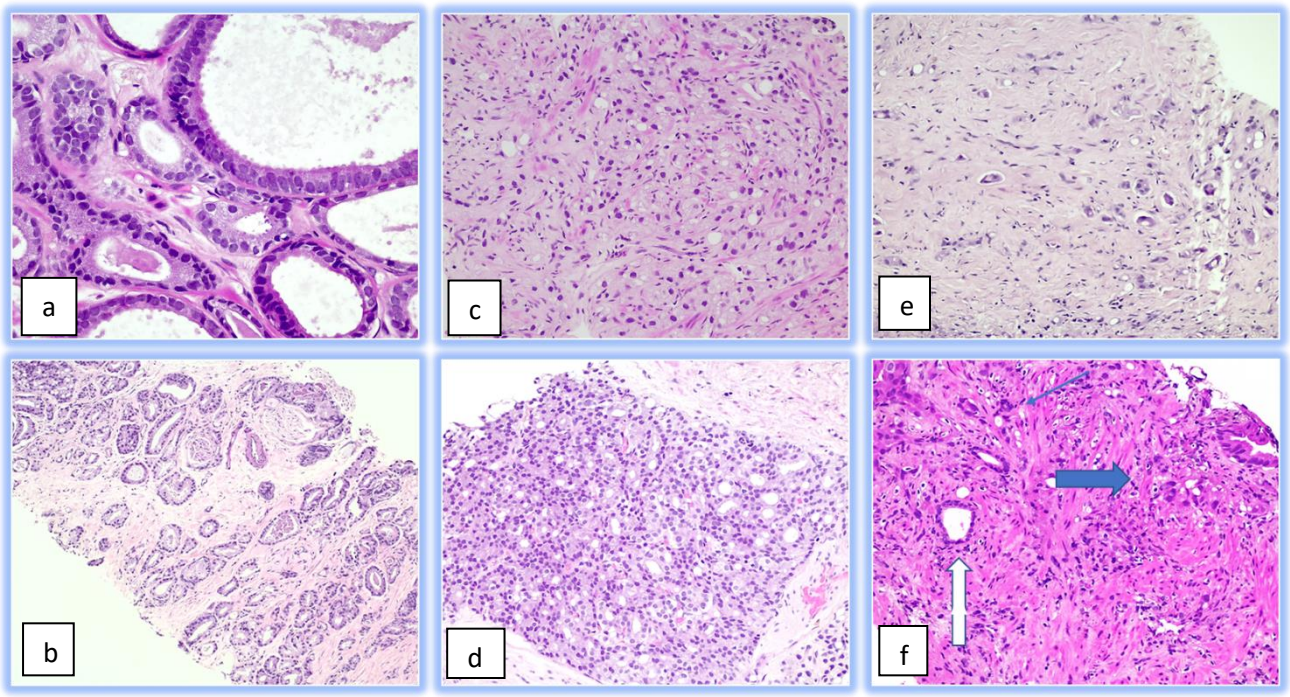

Figure 9: H&E images of Gleason patterns 3 (Figure a at 40x objective and Figure b at 10x objective), 4 (Figure c at 20x objective and Figure d at 10x objective) and 5 (Figure e at 20 x objective and Figure f at 20x objective) of prostate adenocarcinoma

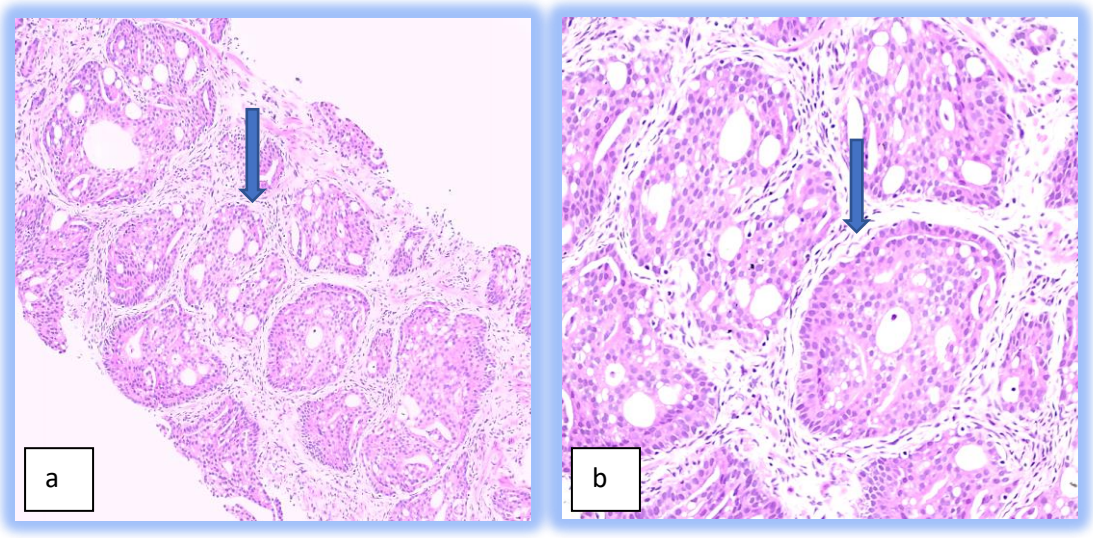

Figure 10: H&E images of Gleason pattern 4, cribriform subtype highlighted by blue arrows. Figure a – 10x objective. Figure b – 20x objective.

4.1.8. GRADE GROUPS

Table 7 shows the absolute number and frequency of cases within each Grade Group category and figure 11 highlights the distribution of cases and shows that a significant number of cases fall within Grade Groups 2,3 and 5 ($p<0.005$). Most cases were assigned to Grade Group 2 (n=151, 32.3%). The least number of cases were assigned a Grade Group 4 category (n=34, 7.3%).

Spearman's correlation is applied since the Grade Groups are ordinal. A strong positive correlation exists between the PSA level and the Grade Group, $p<0.0005$.

[6]Table 7: Distribution of the Grade Groups

Grade Groups	Absolute number (n)	Percentage (%)
1	49	10.7
2	151	32.3
3	104	22.2
4	34	7.3
5	130	27.8

[6] One case of mucinous carcinoma has been included in the Grade groups but not assigned as part of total number of conventional prostate adenocarcinoma cases hence the total number of cases in Table 7 is 468 and not 467

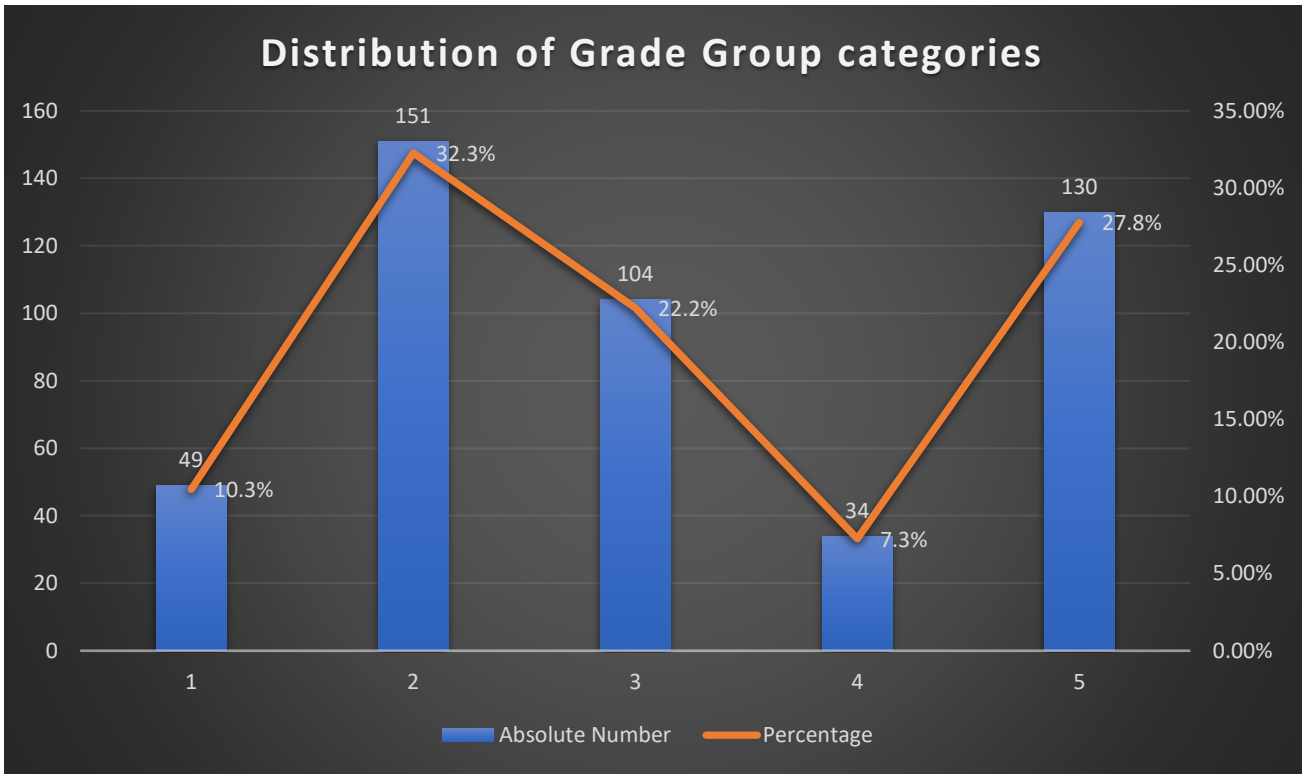

Figure 11: Graph showing the number of cases within each Grade group

4.1.9. PERINEURAL INVASION

PNI was present in 233 cases (49.9%) and 234 cases did not have PNI (50.1%).

4.1.9.1. Perineural invasion and Grade Groups

On analysis of the relationship between the presence of PNI and the Grade Groups of tumours, it is noted that as the Grade Group increases and the tumour becomes more histologically aggressive, the likelihood of finding PNI also increases.

As shown in figure 12, a far greater proportion of cases amongst the Grade Group 1 category did not have PNI whilst amongst cases in Grade Group 5 category, a far greater proportion of cases showed PNI. Cases showing the presence of PNI have been photo-micrographed and are presented in figure 13.

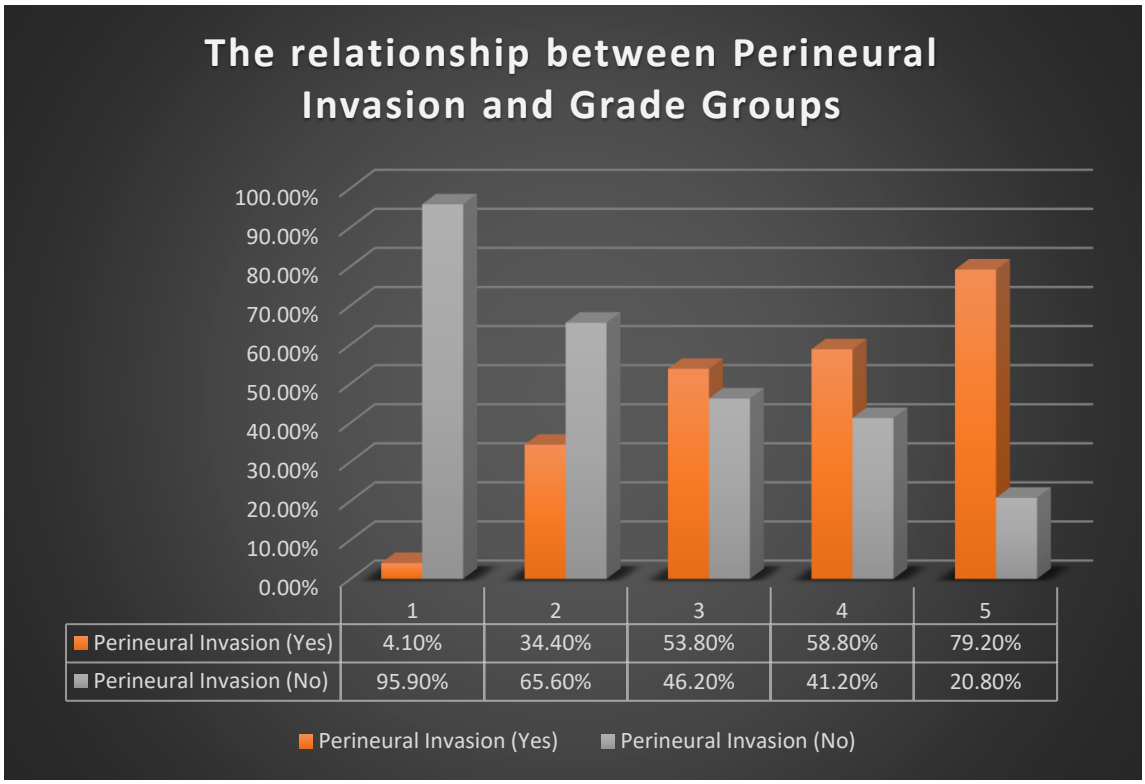

Figure 12: Graph showing the number of cases and percentage of cases with perineural invasion across the different Grade groups

Using a Pearson Chi-Square test, a significant number of cases from Grade Groups 1 (n=47, 95.9%) and 2 (n=99, 65.6%) did not have PNI whilst a significant number of cases (n=103, 79.2%) from Grade Group 5 did have PNI, $p<0.005$.

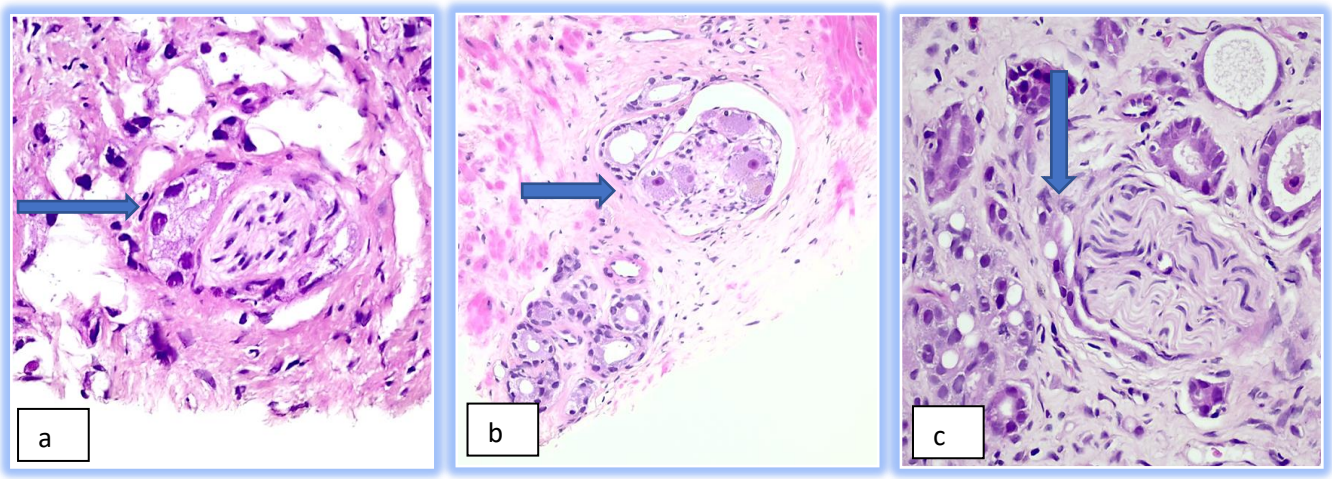

Figure 13: H&E images of perineural invasion by prostate carcinoma highlighted by blue arrows. Figure a – 20x objective. Figure b – Perineural invasion of a ganglionated nerve fibre at 20x objective. Figure c – 40x objective.

4.1.10. LYMPHOVASCULAR SPACE INVASION

LVI was identified in 55 cases (11.8%). A significant proportion (n=413, 88%) of cases of PCa did not show the presence of LVI ($p<0.005$).

4.1.10.1. Lymphovascular space invasion and Grade Groups

The presence and absence of LVI was assessed within the five Grade Groups and the findings thereof are shown in figure 14. Cases with LVI have been photo-micro graphed and are presented in figure 15.

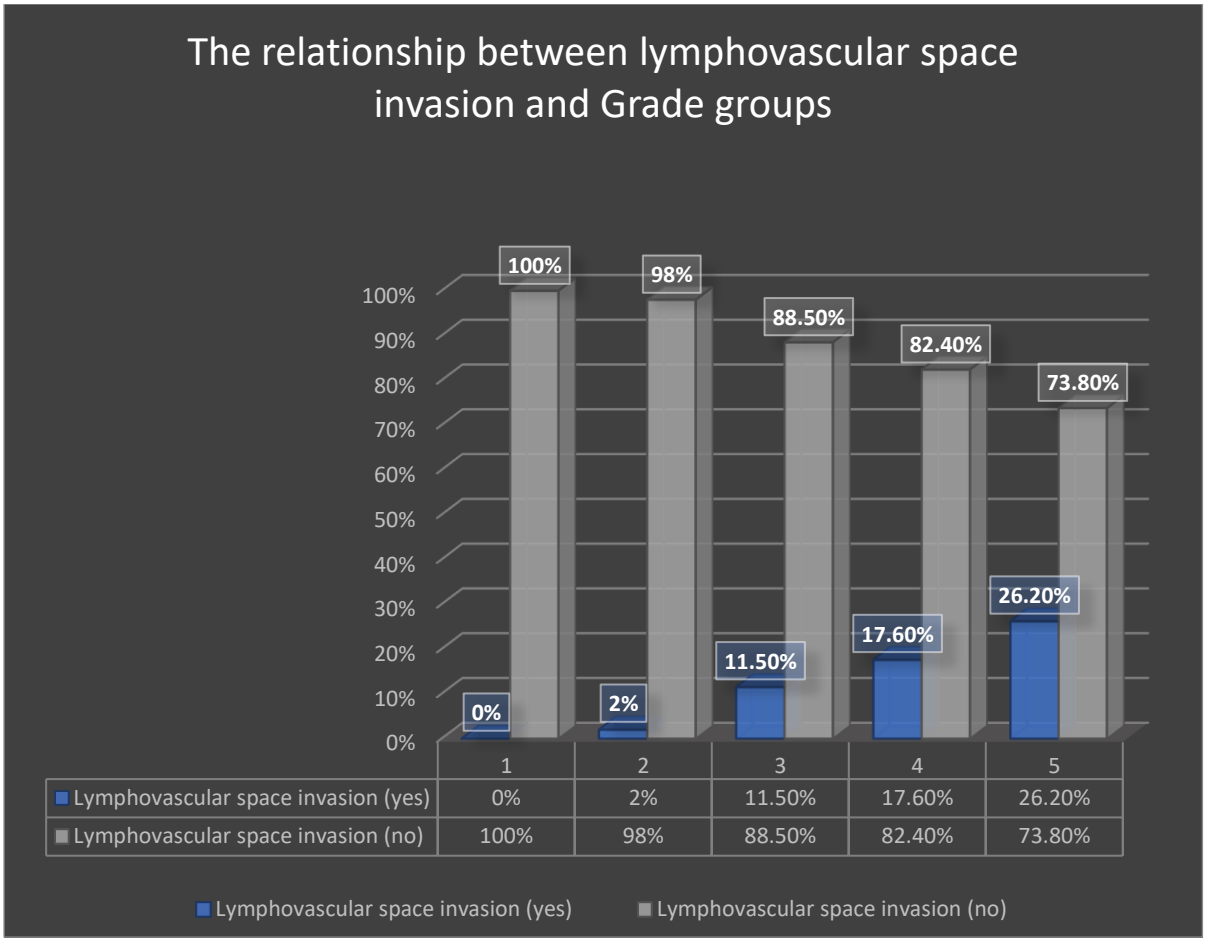

Figure 14: Graph showing the number and percentage of cases with and without lymphovascular space invasion in each Grade group category.

A Pearson Chi-Square test showed that a significant number of cases from Grade Group 2 (98%, n=148) did not have LVI while a significant number from Grade Group 5 (26.2%, n=34) did, $p<0.005$.

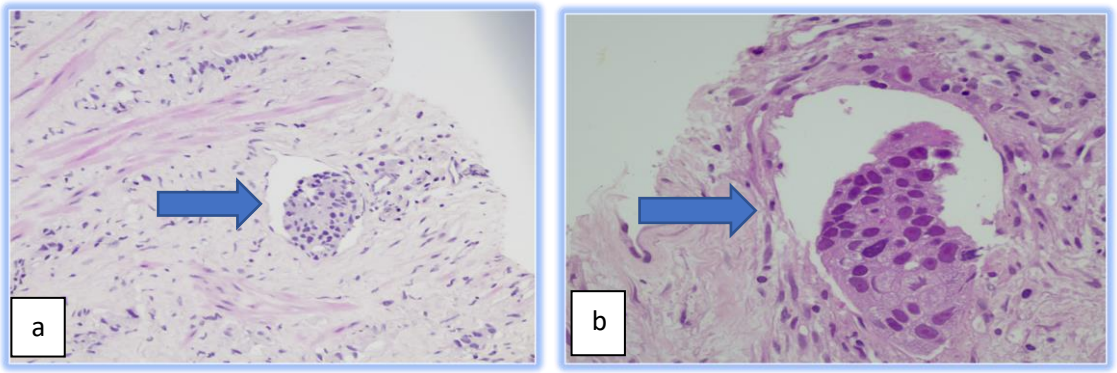

Figure 15: H&E images of lymphovascular space invasion highlighted by blue arrows. Figure a shows LVI at 20x objective. Figure b shows a case of LVI at 40x objective.

4.1.11. P63 IMMUNOHISTOCHEMISTRY

P63 immunohistochemical staining was performed on a total of 133 cases (16.9%). Of these cases, 60.9% (n=81) were benign (P63 staining was retained within basal cells). 38.3% (n=51) of cases had absent P63 staining within the basal cell layer. Thirty-six (70.6%) of these were diagnosed as PCa. Fifteen cases (29.4%) were diagnosed as ASAP.

In cases with retained/positive P63 basal cell staining, a significant proportion were diagnosed as acute prostatitis (n=45, 54.9%) and HGPIN (n=17, 20.7%), $p<0.005$. No cases of PCa showed P63 positive staining. The breakdown of positive P63 staining is shown in Table 8.

Table 8: Breakdown of the cases with positive/retained P63 immunohistochemical staining across the different diagnostic categories

Diagnostic categories	Absolute Number	Percentage (%)
Atrophy	4	4.9
Acute prostatitis	45	54.9
Basal cell hyperplasia	1	1.2
Atypical small acinar proliferation	1	1.2
High grade prostatic intraepithelial neoplasia	17	20.7
Chronic inflammation	9	10.9
Normal	5	6.1

In the cases with negative P63 basal cell staining, 36 (70.6%) cases were diagnosed as PCa and 15 (29.4%) cases were diagnosed as ASAP. This is shown in Table 9.

Table 9: Cases with negative P63 immunohistochemical staining

	Absolute number	Percentage (%)
Prostate carcinoma	36	70.6
Atypical small acinar proliferation	15	29.4

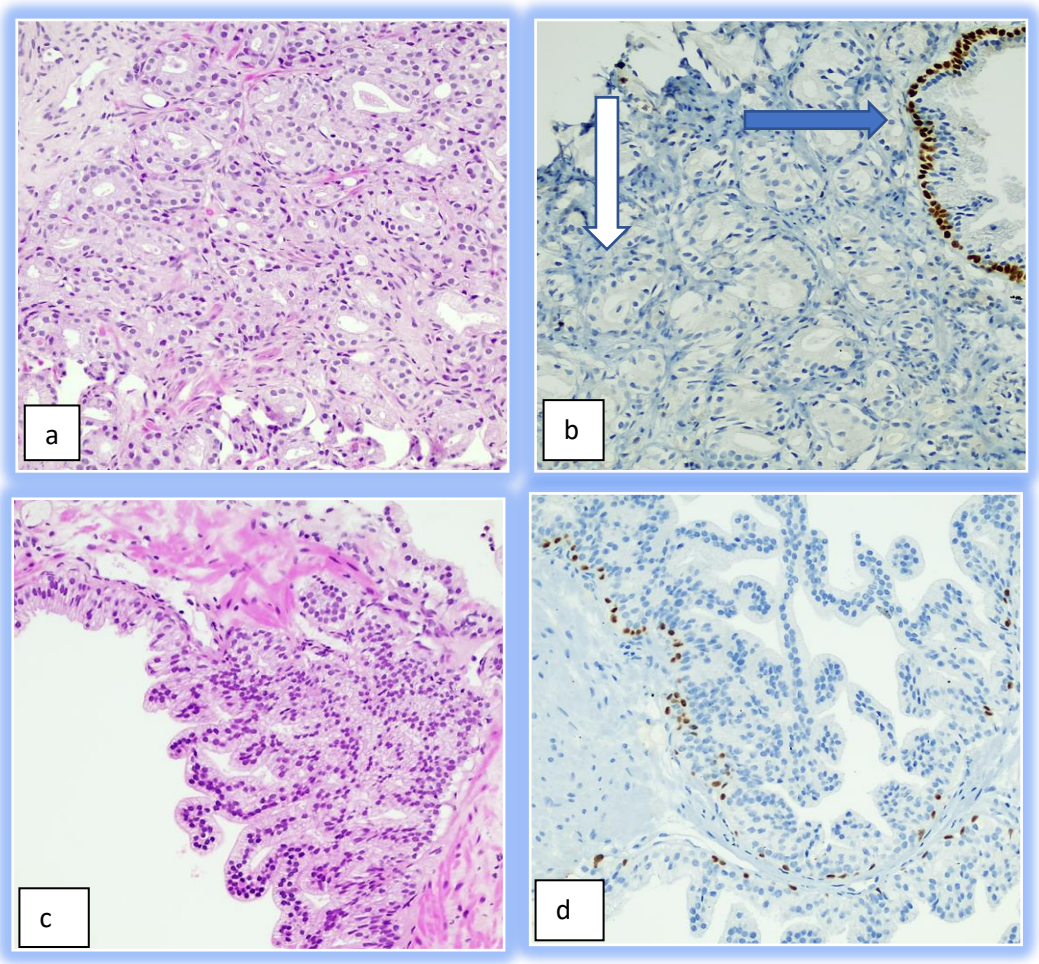

Figure 16: Figure a is an H&E image of prostate adenocarcinoma (20x objective) and Figure b is the corresponding P63 immunohistochemical stain, negative in the carcinoma (white arrow) (20x objective). A benign prostate gland (blue arrow) is also seen and is useful as an internal control. H&E image of HGPIN (Figure c 20x objective) with the corresponding P63 immunohistochemical stain showing retained basal cells (Figure d 20x objective).

4.1.12. PRESENCE OF METASTATIC DISEASE

No cases within the cohort had evidence of metastatic disease indicated in the reports.

4.2. DISCUSSION

PCa although accounting for large proportions of cancers diagnosed in males remains a public health concern due to lack of screening and education. Unlike breast carcinoma, PCa is not widely discussed in media. Results from this study will hopefully enhance what is known about the disease locally. A discussion of these findings and how they compare to the literature follows.

4.2.1. Sample size

Prostate samples accounted for approximately 5% of the cases received and reviewed in the CHBAH Histopathology Laboratory during the study period. This serves as a crude indication of the relative burden of prostate pathology seen at CHBAH during the study period.

4.2.2. Benign versus malignant

Of all the prostate needle core biopsies reviewed, more (59.6%) were diagnosed with carcinoma. The finding that majority of prostate biopsies were malignant in this study aligns to studies done previously showing that PCa is the commonest cancer in the male population (Aref-Eshghi *et al* 2018). Adeloye *et al* (2016) described that Sub Saharan Africa has one of the highest incidence rates of PCa. PCa is also one of the four most common cancers diagnosed in South Africa (Alleyne *et al* 2013). The predominance of malignancy diagnosed on cores in this study may also be due to this hospital serving as a large referral centre, therefore distilling out cases from the general population at large.

HGPIN was diagnosed in 11.7% of biopsies in the current study. This is higher than the median incidence of 5-6% reported by Epstein & Potter (2001). The higher proportion of cases seen with HGPIN may be due to its presence in cases with concomitant invasive carcinoma. Conversely, it should also be noted that the presence of HGPIN in cases with carcinoma may not reported. HGPIN is an important diagnostic entity and if diagnosed in the absence of carcinoma, warrants follow up with repeat biopsies as Antonelli *et al* (2011) showed that 32% of patients will have carcinoma diagnosed in subsequent biopsies.

ASAP was diagnosed in 3.8% of biopsies. This is in keeping with than the incidence cited in the literature of between 2 and 5% (Iczkowski & Bostwick 2014).

Acute prostatitis accounted for 31.1% of biopsies and chronic inflammation accounted for 7.5% of cases. This is important as there is a recognised link between prostatitis and PCa in some studies (Jiang *et al* 2013, Hughes *et al* 2005). Perletti *et al* (2017) and Davidsson *et al* (2011) both report an increased incidence of PCa in patients with prostatitis. Cai *et al* (2019) have also shown a correlation between a history of prostatitis and PCa development. Prostatitis, however, was not found to be associated with an increased risk of PCa by Rybicki *et al* 2016. This is further supported by Langston *et al* (2019) who reported that a link between prostatitis and PCa in some studies are likely to be due to detection-based bias. Given this conflicting evidence it remains prudent that the presence of inflammation be well documented in histopathology reports.

Atrophy accounted for 12.6% of cases. This is significantly lower than the incidence on atrophy cited in literature. Postma *et al* (2005) reported an incidence of 91% whilst Billis *et al* (2010) and Kryvenko *et al* (2012) cited incidence rates of 83.7% and 88-100% respectively. It should be noted that there is interobserver variability in the diagnosis of atrophy (Giunchi *et al* 2017).

Basal cell hyperplasia was diagnosed in 6.9% of cases, lower than the incidence rate of 10.2% reported in literature (Thorson *et al* 2003). Like atrophy, basal cell hyperplasia is important to recognise as it may be misdiagnosed as malignancy (Hameed *et al* 2010).

4.2.3. Age

Patients diagnosed with malignancy were older (mean are 66.65 years) than those with benign diagnoses (65.19 years) on prostate core biopsy and this was shown to be statistically significant (p=0.014). The findings of the current study are comparable to Kgatle *et al* (2018) who described the increasing incidence of prostate cancer in men over the age

of 50 years old. Others have also documented the high prevalence of prostatic cancer in men older than 65 years (Cai *et al* 2019, Hall *et al* 2005, Rawla 2019).

Like Janbaziroudsari *et al* (2016) and Stangelberger *et al* (2008), we did not find a correlation between patients' ages and tumour grades. Hall *et al* (2005) described conflicting evidence regarding the patients age and the aggressiveness of the tumour with some studies showing an older age is associated with more aggressive disease (Carter *et al* 1999, Morgans *et al* 2017) and other studies describing the converse (Bratt *et al* 1998, Cook *et al* 1968 and Johnson *et al* 1972).

Hayes *et al* (2018) describes that men in South Africa present later when compared to the American male population who usually present on average 5 years earlier. The mean age of presentation of American men was reported as 68 years old by Gupta *et al* (2017). Lindh *et al* (2018) reported a mean age of presentation of 62.7 years within a New Zealand population. These findings are similar to our study where the mean age of presentation was 66.65 years old.

4.2.4. Race

A large proportion of our cases did not have a race category assigned to the patient. Of the cases that did have an assigned race, most (36.2%) PCa cases were found in African men followed by 0.2% in Coloured men. Where race was recorded, there were no tumour cases in White men or Indian/Asian men. This may be related to the location of CHBAH which is in Soweto and services a predominantly Black African community. We were unable to show any significant correlation between race and prevalence of tumour or a significant association between race and tumour grade. This is due to the large proportion of missing data regarding race (race groups were not documented in 63.6% of malignant cases) and the lack of representation of all race groups in the cohort. Accurate documentation of race is important in retrospective studies such as these and clinicians should be encouraged to accurately complete patient information to ensure complete data. It is therefore a major shortfall of this study that conclusions could not be drawn in relation to race. It has been

well published that African descent is associated with a more aggressive disease as well as a higher incidence of PCa (Dewar *et al* 2018). African men have been noted to present with tumours of a higher grade characterized by higher Gleason scores and Grade Groups (Cai *et al* 2019, Peterson *et al* 2013, Schuster *et al* 2010, Stefflova *et al* 2011). Our study did not find a statistically significant correlation. Studies also show a greater risk for metastases, faster PSA doubling times, higher tumour volume as well as a younger age at diagnosis in Black males (Berger *et al* 2006, Bigler *et al* 2011, Heath *et al* 2008, Moul *et al* 1995, Powell *et al* 2010, Sundi *et al* 2014, Tewari *et al* 2005).

4.2.5. Prostate specific antigen level

The median PSA was 15.2ug/L (IQR 14.60ug/L). It was also noted that the median PSA in patients with PCa was significantly higher than the median PSA in patients with benign diagnoses (23.35ug/L vs 9.60ug/L respectively, $p<0.0005$).

One third (33.3%) of patients with normal PSA levels had tumours – although it should be noted that this amounted to a very small number of cases (n=2). For those with raised PSA, 60% were diagnosed with tumour with the remainder having benign diagnoses. This highlights that fact that other causes of a raised PSA should be considered and that a raised PSA is not synonymous with carcinoma. Race and ethnicity have also been shown to be a factor for varying PSA levels with higher levels being found in men of African descent (Laguna *et al* 2000). Unfortunately, due to the lack of data regarding race and the lack of adequate representation of all race groups, meaningful conclusions regarding race could not be drawn.

Janbaziroudsari *et al* (2016) failed to show a correlation between PSA levels and histologic grade of tumour on biopsy specimens. Our study showed a significant difference in PSA levels across Grade Groups with for example PSA for Grade Groups 2-5 being significantly different than the PSA levels for Grade Group 1. Overall, results show that there is a

moderate increasing relationship between PSA and Grade Group. A moderate positive correlation exists between PSA and the major and minor Gleason scores. This is relevant as a pathologist may use the PSA level as a rough guide to the grade of the tumour. Perhaps, pathologists should be extra vigilant when assessing biopsies with lower PSA levels as it may indicate a lower grade and a better differentiated tumour that may be overlooked.

This study found that PSA levels in patients with PNI (mean PSA 60.10ug/L) were significantly higher than for those without PNI (mean PSA 14.00ug/L). Similarly, significantly higher PSA levels were noted in patients with LVI (mean PSA 113.50ug/L) compared to patients without LVI (mean PSA 20.60ug/L). Translated into everyday practice, one is more likely to see PNI and LVI with higher PSA levels.

A comparison between PSA levels in PCa and PSA levels in acute prostatitis was conducted in this study. This showed that PSA levels were significantly higher in patients with PCa as compared to patients with acute prostatitis.

The recommended threshold for PSA has been 4ng/ml as the upper limit of normal however studies have shown that patients with PCa may present with significantly lower PSA values (Punglia *et al* 2006). This threshold has been questioned and it is important to consider the age of the patient amongst other factors. Also, determination of local reference ranges are crucial (Maphayi *et al* 2020). Whilst PSA levels can be used as a guide to indicate the likelihood of malignancy, a normal PSA value should not lull a pathologist into complacency. Every biopsy should be scrutinised no matter the PSA level.

4.2.6. Number of cores

This study showed that the median number of cores submitted in those patients with benign diagnoses was 12 and in the carcinoma category was 10. This is in keeping with recommendations that at least 10 appropriately directed cores should be submitted as this optimises the detection of carcinoma (Pretsi 2003). In addition, the accuracy of the Gleason

score assigned on the biopsy is improved with the submission of 10 cores compared to 8 or 6 (Coogan 2006).

4.2.7. Tumour volume

The median tumour volume for the study cohort was 40%. Tumour volume is an important prognostic indicator for biochemical recurrence (Hong *et al* 2011, Tanaka *et al* 2012). An increased tumour volume as assessed on histopathology has been shown to be associated with extraprostatic extension and lymph node involvement, all of which are poor prognostic indicators (Knoedler *et al* 2014). Using evidence from these previous studies, it can be deduced that a higher tumour volume is seen in patients with more aggressive disease. Our study showed a significant correlation between PSA level and tumour volume, with a higher PSA level seen in tumours with a higher tumour volume. There was also a significant difference in tumour volume between the five Grade Group categories for example a higher tumour volume was seen in tumours belonging to Grade Groups 2, 3, 4 and 5 compared to Grade Group 1. This translates to Grade Group 1 tumours having a lower volume. As they are well differentiated and more likely to be of smaller volume, they may be easily overlooked on core biopsy. It should also be noted that estimation of tumour volume may be subjective. At CHBAH Histopathology, tumour volume is assessed as number of cores involved and the total percentage of tumour per case rather than per core. All prostate cores are submitted in one specimen container at the lab.

4.2.8. Grade Group

A significant number of carcinoma cases were assigned Grade Groups 2 (32.3%), 3 (22.2%) and 5 (27.8%). In 2014 the International Society of Urological Pathology (ISUP) reviewed and improved this scoring system with a Grade Group index (Epstein *et al* 2016) which is more well correlated with patient prognosis (Sehn *et al* 2018) and is used more widely as a strong predictor of cancer related mortality in patients with prostate cancer (Milonas *et al* 2021). The risk of mortality increases as the Grade Group increases (Milonas *et al* 2021). The South African Prostate Cancer Study showed 35.5% of patients had tumours with a Gleason score of 7 or more (Tindall *et al* 2014). This correlates with a high Grade Group category as the sum of the Gleason score is used to calculate the Grade Group. Our findings of higher Grade

Group tumours in our cohort of patients as well as a correlation between tumour volume and Grade Group are all in keeping with previously published literature as mentioned above.

4.2.9. Perineural invasion and Lymphovascular space invasion

PNI was present approximately 50% of cases. It is an important independent predictor of recurrence of PCa (Zhang *et al* 2018). It was also noted in our study that Grade Groups 1 and 2 were less likely to show PNI than Grade Group 5 tumours. This finding is in keeping with the findings of Saadat *et al* (2012). Some studies have shown that although PNI is associated with higher Gleason scores and a more aggressive tumour, these findings do not correlate with an increase in the risk of biochemical recurrence (Kraus *et al* 2019). Dell'Atti (2016) considers the significance of PNI in prostate needle core biopsies as controversial.

Most (88%) of the PCa cases did not show LVI. This is not surprising considering the comments made by Freeman (2009) who stated that LVI may be under-reported and difficult to distinguish from retraction artefact. LVI is a marker of poor prognosis and is a risk factor for metastases. Jiang *et al* (2018) found that LVI could be used as an indicator of biochemical recurrence. LVI is usually seen in higher grade tumours and in patients with more advanced disease (Jiang *et al* 2018). In our cohort, only a small percentage (11.7%) of cases had LVI. This finding could be explained by lack of recognition by pathologists or lack of representation of lymphovascular space invasion within the sampled prostate gland (Baydar *et al* 2008).

4.2.10. P63 immunohistochemistry

A total of 133 cases (16.96%) required P63 immunohistochemical staining. P63 immunohistochemical stains were used less often than in other studies (Watson *et al* 2013, p63 was used in 40% of cases and Bonert *et al* 2020 where it was used in 60% of cases). The frequency of p63 use may be related to a pathologist's experience, training and individual preferences and the availability of immunohistochemistry. Also, when working in a

government facility, one must exercise prudence in ordering expensive tests that drive up cost.

Of these cases where p63 staining was done, 60.9% (n=81) were benign with most subsequently being diagnosed with acute prostatitis (n=45) and HGPIN (n=17). As noted by Hameed *et al* (2010) reactive atypia that may accompany acute prostatitis may mimic PCa necessitating the use of p63 to assist in the differentiation. Atrophy, another mimicker of carcinoma (Hameed *et al* 2010, Beltran *et al* 2019) was diagnosed in 4 (4.88%) of the cases that required p63 staining. It is not surprising that cases prompting p63 staining were diagnosed with HGPIN, as the cytological characteristics of HGPIN are identical to invasive carcinoma (Brawer 2005) making this distinction challenging. No carcinoma cases were P63 positive therefore our cohort did not have the rare P63 positive tumours described by Tan *et al* (2015). Bonert *et al* (2020) and Srinivasen *et al* (2011) describes the usefulness of P63 immunohistochemistry in differentiating malignant cases from benign mimics such as atrophy, particularly partial atrophy and adenosis. Post atrophic hyperplasia is another important mimic of prostate adenocarcinoma and the use of P63 immunohistochemistry is crucial in these biopsies. Of the 51 cases that were negative for P63 immunohistochemistry, the majority (n=36) were diagnosed as invasive carcinoma with a smaller proportion (n=15) being diagnosed as ASAP most likely due to the limited representation of atypical glands within the submitted biopsy.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSION

This study contributes information to the field of prostate pathology especially PCa histologically diagnosed in a major hospital in Johannesburg, South Africa.

Our findings are largely in keeping with findings in the literature with the following key points noted in our study:

1. Most cases received were diagnosed as PCa (59.60%).
2. The mean age for PCa within our population during the study period was 66.65 years.
3. The median PSA for benign pathologies (9.60ug/L) was lower than the median PSA in patients with carcinoma (23.35ug/L).
4. PSA levels increase as the Grade Group increases.
5. PSA levels in patients with PNI and LVI are higher than in patients without.
6. Due to lack of adequate data on race, a meaningful conclusion could not be made.

It is imperative that studies focussing on Black African men are conducted to elucidate the reasons behind their well-documented late presentations and more aggressive disease at presentation. Awareness regarding PCa and the importance of screening should be a primary health priority just as breast carcinoma has become. Lower volumes of tumour seen in lower tumour grade groups serve as reminders to histopathologists to always remain vigilant when assessing prostate biopsies.

5.2. LIMITATIONS

Race was not available in a significant proportion of cases. These have been recorded as "unknown" or left blank in the data collection sheet. The lack of this demographic data impacts the study objective of examining the relationship between race and other tumour variables such as PSA level, tumour volume, Gleason score and Grade group.

The study is based in Gauteng at CHBAH. This limits the extent of the population surveyed to the south of Johannesburg and resulted in sample population bias. Also, this hospital is a public based hospital servicing the needs of the lower income population with an anecdotally predominantly Black African patient base.

This study did not assess outcomes which would have added value. It would have been interesting to follow-up patients with raised PSA levels and no tumour on initial biopsy or those diagnosed with the categories PIN or ASAP.

5.3. RECOMMENDATIONS

A strong correlation between Black African race and a higher grade of tumour has been observed in other studies. To date, limited studies have been done within Africa and South Africa to explore the reasons behind the increased risk of PCa and more aggressive disease in Black African patients. Further studies are therefore required and will hopefully raise awareness regarding PCa in South Africa.

PCa is a relatively indolent tumour if diagnosed early, therefore morbidity and mortality can be reduced through effective primary health care interventions. With the projected increase in life expectancy and subsequently larger elderly population, the focus on such cancers is critical. Better early screening and prevention strategies are needed in the male population. Hopefully, this and other similar studies will cast a spotlight on prostate cancer and encourage allocation of resources to this important disease.

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APPENDICES

Appendix 1: Data collection sheet

Patient study number	Age	Race (if available)	PSA level	Number of cores	Diagnostic category[1]	P63 IHC	Tumour type	Tumour volume (%)	No of cores involved	Gleason major	Gleason minor	Grade group	Perineural invasion	Lymphovascular invasion	Repeat biopsy	Histologically confirmed metastasis

Appendix 2: Ethics approval

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Karishma Chandraser

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190710

NAME: (Principal Investigator)	Dr Karishma Chandraser
DEPARTMENT:	Anatomical Pathology
PROJECT TITLE:	DEMOGRAPHIC,BIOCHEMICAL AND HISTOPATHOLOGICAL FEATURES OF PROSTATE ADENOCARCINOMA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOUTH AFRICA
DATE CONSIDERED:	26/07/2019
DECISION:	Approved Uncondtionally
CONDITIONS:	
SUPERVISOR:	DR REENA MOHANLAL
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/08/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

02/08/2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Professor Martin Hale

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150884

NAME: (Principal Investigator)	Professor Martin Hale
DEPARTMENT:	Division of Anatomical Pathology NHLS
PROJECT TITLE:	Use of Pathology Specimens Archived in the Department of Anatomical Pathology (Previously M00/08/29/Prof A Paterson)(M10744)
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Renewal (2015 - 2020)
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	09/09/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

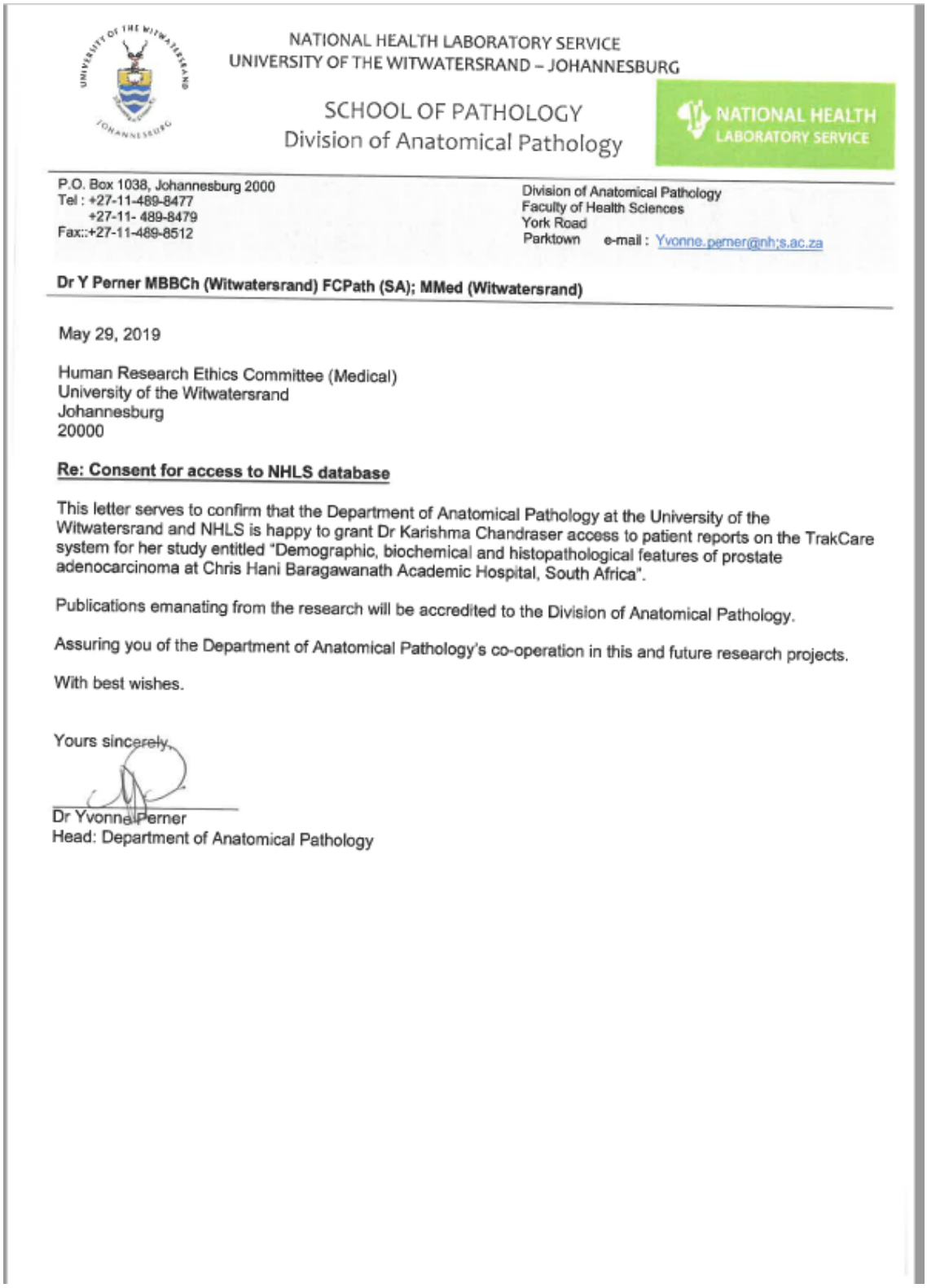
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 01/07/2019

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3: Permission to access the NHLS database



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+27-11- 489-8479
Fax::+27-11-489-8512

Division of Anatomical Pathology
Faculty of Health Sciences
York Road
Parktown e-mail : Yvonne.perner@nhls.ac.za

Dr Y Perner MBBCh (Witwatersrand) FCPath (SA); MMed (Witwatersrand)

May 29, 2019

Human Research Ethics Committee (Medical)
University of the Witwatersrand
Johannesburg
20000

Re: Consent for access to NHLS database

This letter serves to confirm that the Department of Anatomical Pathology at the University of the Witwatersrand and NHLS is happy to grant Dr Karishma Chandraser access to patient reports on the TrakCare system for her study entitled "Demographic, biochemical and histopathological features of prostate adenocarcinoma at Chris Hani Baragawanath Academic Hospital, South Africa".

Publications emanating from the research will be accredited to the Division of Anatomical Pathology.

Assuring you of the Department of Anatomical Pathology's co-operation in this and future research projects.

With best wishes.

Yours sincerely,

Dr Yvonne Perner
Head: Department of Anatomical Pathology

Appendix 4: Protocol application

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CANDIDATE'S SURNAME: CHANDRASER	FIRST NAME/S: KARISHMA	STUDENT NUMBER: 302859
CURRENT QUALIFICATIONS: MBCHB; Dip HIV Man SA		
TEL: 011 489 8468 CELL: 0836554500	E-MAIL: kari_chandraser@yahoo.com	FAX:
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: MMED ANAT PATH		
PART-TIME OR FULL-TIME: ~~PART-TIME~~ FULL TIME		
FIRST REGISTERED FOR THIS DEGREE:	TERM : 1	YEAR: 2018
DEPARTMENT: ANATOMICAL PATHOLOGY		
TITLE OF PROPOSED RESEARCH: Demographic, biochemical and histopathological features of prostate adenocarcinoma at Chris Hani Baragwanath Academic Hospital, South Africa.		
CANDIDATE'S SIGNATURE:		DATE:
SUPERVISOR 1 (NAME & SURNAME): DR R.D. MOHANLAL		100% Supervision
SUPERVISOR'S QUALIFICATIONS: FC PATH (Anat) MMed (Wits) (Anat Path) DMH		
SUPERVISOR'S DEPARTMENT: ANATOMICAL PATHOLOGY		
SUPERVISOR'S ADDRESS / TEL / E-MAIL: Chris Hani Baragwanath Laboratory, NHLS, Room 12, 4th Floor, NHLS building, Soweto, Johannesburg. 011 489 8715 reena.mohanlal@nhls.ac.za		
SUPERVISOR 2 (NAME & SURNAME): N/A		% Supervision
SUPERVISOR'S QUALIFICATIONS		
SUPERVISOR'S ADDRESS / TEL / E-MAIL:		
SUPERVISOR 3 (NAME & SURNAME): N/A		% Supervision
SUPERVISOR'S QUALIFICATIONS		
SUPERVISOR'S ADDRESS / TEL / E-MAIL:		
SYNOPSIS OF RESEARCH: *Please see next page*		

WITS ETHICS NOT REQUIRED: WITS ETHICS PENDING: WITS ETHICS APPROVED: (circle appropriate symbol)* ***Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research**	Yes (No) (Yes) No Yes (No)	IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.		
SIGNATURE OF SUPERVISOR/S:	...Kdlodiaulal.....	.....
SIGNATURE PG OFFICE STAFF	REGISTERED YES✓ NO.....	UNIVERSITY OF THE WITWATERSRAND AMP 07 JUN 2019 FACULTY OF HEALTH SCIENCE JOHANNESBURG

SYNOPSIS OF RESEARCH CONTINUED

Prostate cancer is one of the leading causes of cancer related mortality in males [1]. Prostate cancer is the most common cancer in men in South Africa [2]. In 2007, the incidence rate was 29.4 per 100 000. This increased to 67.9 in 2012 [2]. Prostate cancer is known to have a slower progression than other cancers however, it remains the leading cause of cancer related death in men [3]. There is a lack of statistics pertaining to prostate adenocarcinoma in Africa and more importantly in South Africa and therefore the aim of this study is to describe the demographic, biochemical and histopathological features of prostate carcinoma at Chris Hani Baragwanath Academic Hospital. The study will be a retrospective descriptive study. The prostate core biopsies of patients with histologically confirmed prostate adenocarcinoma will be reviewed over a one-year period at Chris Hani Baragwanath Hospital.

11 March 2019/MP

Appendix 5: Appointment of supervisor form

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

RECOMMENDATION FOR APPOINTMENT OF SUPERVISOR(S) OF RESEARCH REPORT, DISSERTATION OR THESIS

Motivation / Reason for Appointment:

Recommendation of Division / Department / School:
This project will provide valuable info mation regarding the epidemiology of prostate cancer in a particular population groups that has/were so far not been established. I give this study my full support.

Student Surname and Full name(s)	KARISHMA CHANDRASER
Student number	302859
Degree	MMED ANAT PATH
Div / Dept / School	DEPARTMENT OF ANATOMICAL PATHOLOGY
Title	DEMOGRAPHIC, BIOCHEMICAL AND HISTOPATHOLOGICAL FEATURES OF PROSTATE ADENOCARCINOMA AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOUTH AFRICA

(Supervisor 1): DR REENA MOHANLAL
(Name &Surname)

Supervision %: 100%

Supervisor Qualifications: HISTOPATHOLOGIST FCPATH (ANAT) MMED(WITS)(ANAT PATH) DMH

Supervisor Department: ANATOMICAL PATHOLOGY

Supervisor Telephone: 011 489 8715 E-mail: reena.mohanlal@nhls.ac.za

Supervisor 2:
(Name &Surname)

Supervision %:

Supervisor Qualifications:

Supervisor Department:

Supervisor Telephone: E-mail:

Student Signature:

Supervisor 3:
(Name &Surname)

Supervision %:

Supervisor Qualifications:

1

Supervisor Department: _____

Supervisor Telephone: _____ E-mail: _____

Student Signature: _____

Supervisor 1 Signature: _____

Supervisor 2 Signature: _____

Supervisor 3 Signature: _____

RECOMMENDATION BY HEAD OF DIVISION / DEPARTMENT / SCHOOL:

Yvonne Perner. _____ 31.05.2019.

(Full name(s) and Surname) (Sign) (Date)

APPROVAL BY CHAIR OF ASSESSOR GROUP:
(On behalf of the FGSC)

Appendix 6: Head of department approval letter

HREC 2019

8.9 Signatures:

I understand that no data may be collected until I have received the full ethics clearance certificate.

Title of the Research Project:

Demographic, biochemical and histopathological features of prostate adenocarcinoma at Chris Hani Baragwanath Academic Hospital, South Africa.

Date: 04/07/2019 **Applicant's Signature:**

WHO WILL SUPERVISE THE PROJECT? (Where applicable)

Name Dr Reena Mohanlal **Department: Division of Anatomical Pathology, School of Pathology**

Telephone No: 011 489 8715 **Email: reena.mohanlal@nhls.ac.za**

Signature: RMohanlal **Date:** 4/07/19

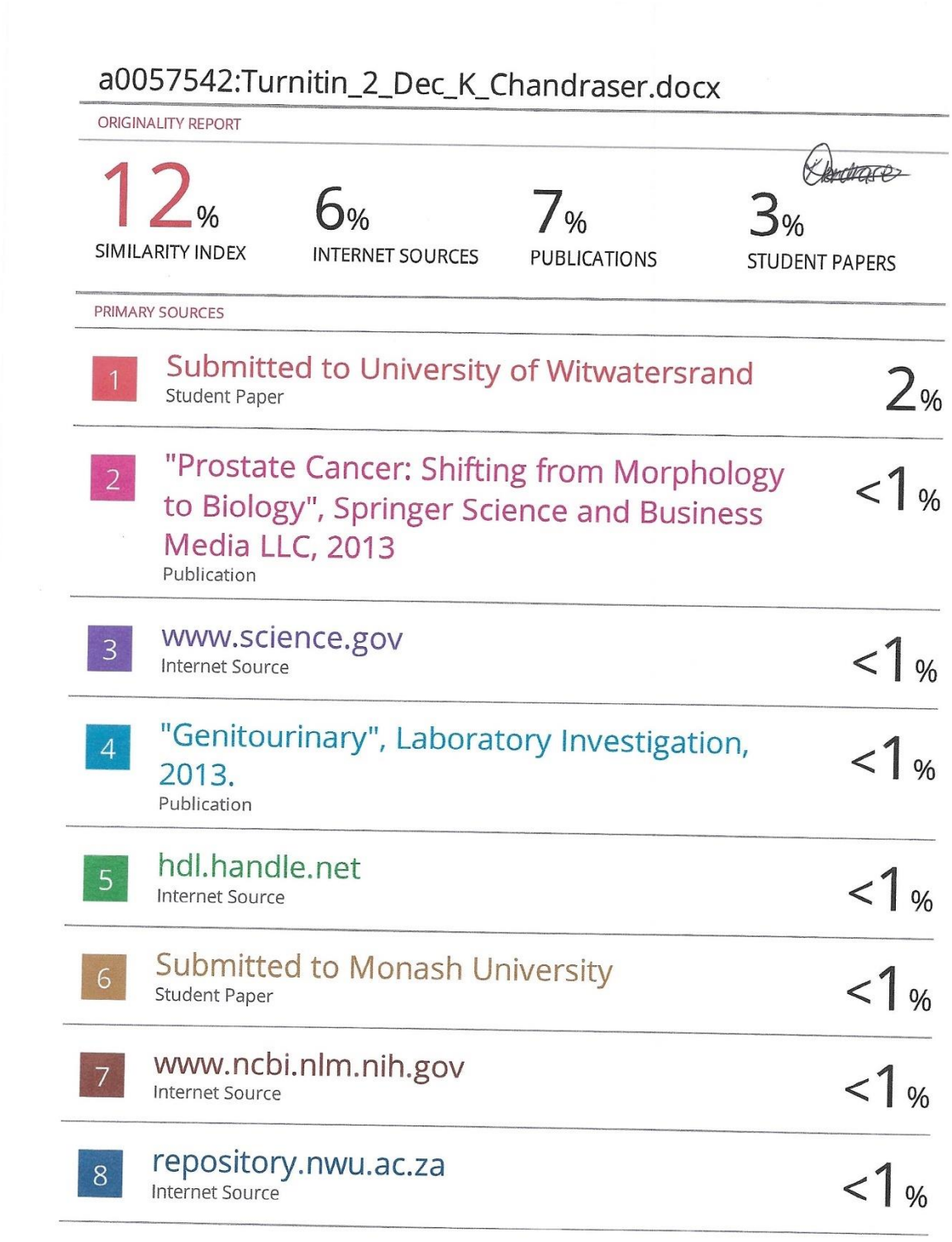
HEAD / RESEARCH COORDINATOR OF DEPARTMENT / ENTITY IN WHICH STUDY WILL BE CONDUCTED (Where applicable) (Wits Students Academic HOD must sign)

Name: Dr Peter Swart **Department / Entity:** Acting head of Department of Anatomical Pathology

Tel No: 011 489 8535 **Email:** peter.swart@nhls.ac.za

Signature: **Date:** 04/07/19

Appendix 7: Turnitin report



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