EXPRESSION OF ERG AND SPINK1 IN PROSTATE CARCINOMA AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS

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This thesis is dedicated to my parents

For their endless love, support and encouragement

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ABSTRACT

Prostate cancer is the second most common adult male malignancy worldwide and rank 6th in Pakistan. Altered genes play a driving role in cancer development and can serve as specific diagnostic and prognostic biomarkers. TMPRSS2:ERG fusion is the commonest molecular alteration present in more than 50% of prostate cancers this leads to overexpression of ERG which can be observed on immunohistochemistry. The serine peptidase inhibitor kazal type1 (SPINK1) is suggested to be an aggressive molecular subtype of ERG fusion negative Prostate cancer and is associated with poor prognosis. The aim of this study was to evaluate the expression of ERG and SPINK1 in prostate cancer and BPH samples and to determine their co-relation with various clinic-pathological parameters. A cross-sectional study was conducted in PNS Shifa Hospital, Karachi, over a period of one year. 33 cases of prostate cancer and 7 cases of BPH were retrieved and examined for immunohistochemical expression of ERG and SPINK1. The results of immunohistochemistry were correlated with various clinicopathological parameters namely age, clinical presentation, Gleason score, Gleason grade group, lymphovascular invasion, perineural invasion and intraductal carcinoma. Out of 33 cases of prostate carcinoma 20 (60.6%) showed ERG expression and none of BPH sample expressed ERG. Among 13 ERG negative prostate carcinoma cases SPINK1 expression was observed in only three cases, thus undermining its significance as a diagnostic marker or a marker of advance lesions. ERG expression was seen in both high and low grade prostate carcinoma, suggesting TMPRSS2-ERG fusion as an early event in carcinogenesis of these tumors and its persistence throughout the disease. One case expressed both ERG and SPINK1 thus questioning the mutual exclusivity of the expression of these markers. The current study is expected to pave way for further researches regarding the effectiveness of these two markers as diagnostic/prognostic markers and as therapeutic targets for prostate carcinomas.

Keywords: Prostate cancinoma, ERG, SPINK1, immunohistochemistry, clinicopathological parameters

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LIST OF ABBREVIATIONS

S No	Abbreviations	Stand For
1	DALYs	Disability-Adjusted Life Years
2	WHO	World Health Organization
3	ACS	American Cancer Society
4	GLOBOCAN	WHO Global Cancer Observatory
5	PSA	prostate-specific antigen
6	BRCA1	Breast Cancer gene 1
7	BRCA2	Breast Cancer gene 2
8	HPC1	Hereditary prostate cancer gene 1
9	BPH	Benign prostatic hyperplasia
10	IPSS	International Prostate Symptom Score
11	QOL	Quality of life index
12	PIN	Prostatic intraepithelial neoplasia
13	HG-PIN	High-grade prostatic intraepithelial neoplasia
14	ETS	Erythroblast transformation-specific
15	TMPRSS2-ERG	Transmembrane protease, serine 2 gene - erythroblast
16	NDRG1-ERG	N-myc downstream-regulated gene 1 - erythroblast
17	PCR	Polymerase chain reaction
18	IHC	Immunohistochemistry
19	Akt	Protein kinase B
20	STAT3	Signal transducer and activator of transcription 3
21	ERK	Extracellular Signal-Regulated Kinase

CHAPTER 1

INTRODUCTION

1.1.1 Global Epidemiology

Cancer is the most significant factor to produce clinical, public and economic challenges for Disability-Adjusted Life Years (DALYs) in all human-related diseases. The specialized agencies accountable for international public health, World Health Organization (WHO) and American Cancer Society (ACS), have estimated the incidence, mortality and life expectancy of top 15 cancers globally. They found 20.2% risk to develop cancer between the age of 0 to 74 years. Their estimates revealed 18 million newly diagnosed cases, in which lung (2.09 million), breast (2.09 million), and prostate (1.28 million) cancers were the most frequent. Besides, cancer was the second most common cause of mortality (8.97 million), followed by ischemic heart disease worldwide. However, the possibility of it becoming the topmost cause in the year 2060 is high, with projected estimate of approximately 18.63 million deaths (Mattiuzzi & Lippi, 2019). Prostate carcinoma is one of the major cancers prevalent in males globally. According to the Global Cancer Observatory (GLOBOCAN) report of 2020, 1414259 (14.1%) cases of newly diagnosed prostate cancer were found globally. The number of deaths reported in 2020 from prostate carcinoma were 375,304 (Figures 1 and 2). One in seven men in the US and one in 25 men worldwide is diagnosed with prostate cancer in his lifetime. According to American Cancer Society, 192,000 new cases and 33,330 prostate cancer related deaths were recorded in 2020 in US, accounting for 10.6% of all new cancer diagnoses (Barsouk et al., 2020). National cancer institute Surveillance, Epidemiology and End Results (SEER) Program have estimated 248,530 new cases and 34,130 deaths due to prostate cancer in year 2021.

1.1.2 Local Epidemiology

The GLOBOCAN 2020 revealed significant differences in prostate cancer incidence and mortality rates between Western and Asian countries. Low incidence estimate (ASR 13.6 per 100 000) is reported in Asia with a relatively high mortality rate (ASR 4.4 per 100 000). In recent decades, prostate cancer incidence has been growing rapidly in Asia particularly in developed countries. According to the GLOBOCAN 2020 report, prostate cancer was the fifth most commonly diagnosed cancer and the seventh leading cause of cancer mortality among Asian men (GLOBOCAN 2020). High-income Asian nations such as Japan, Singapore, and South Korea recorded lower mortality rates but higher incidence rates compared to other low-to-middle income Asian countries (Lim et al., 2021). In Pakistan, 4550 new cases and 2188 deaths due to prostate cancer were documented in all ages of men in 2020 (GLOBOCAN). According to Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH&RC) Annual cancer registry report (2019), prostate cancer is 2nd most common adult male malignancy with 11.4 per 100,000 population men diagnosed with this cancer. Age-specific incidence rates for prostate cancer were also observed to increase at the age of 55 years and reaches a peak at 75 years (Badar et al. 2020). According to Karachi Cancer Registry, which is a part of 'National Cancer Registry' collected data from eight major hospitals in Karachi (2017-2019) and found prostate cancer to be 6th most common cancer of adult male in Pakistan (Pervez et. al., 2020). In Lahore incidence of cancer were recorded from 2010 to 2019, highest ASIRs recorded among male adults were of prostate cancer (10.7) (Badar et al., 2021). India, neighboring country of Pakistan also follows the same pattern of incidence. According to Indian National Cancer Registry Program (2020) 41,532 cases were reported and analysis was made that 1 in 125 men were diagnosed by prostate cancer (Mathur et al., 2020).

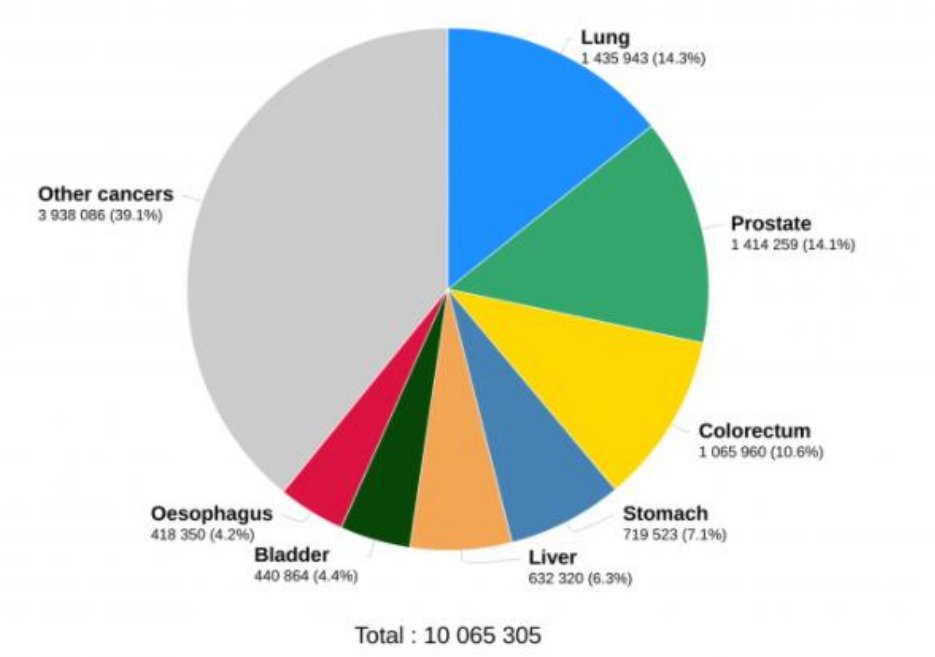


Figure 1: Incidence of cancer in males in 2020

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.

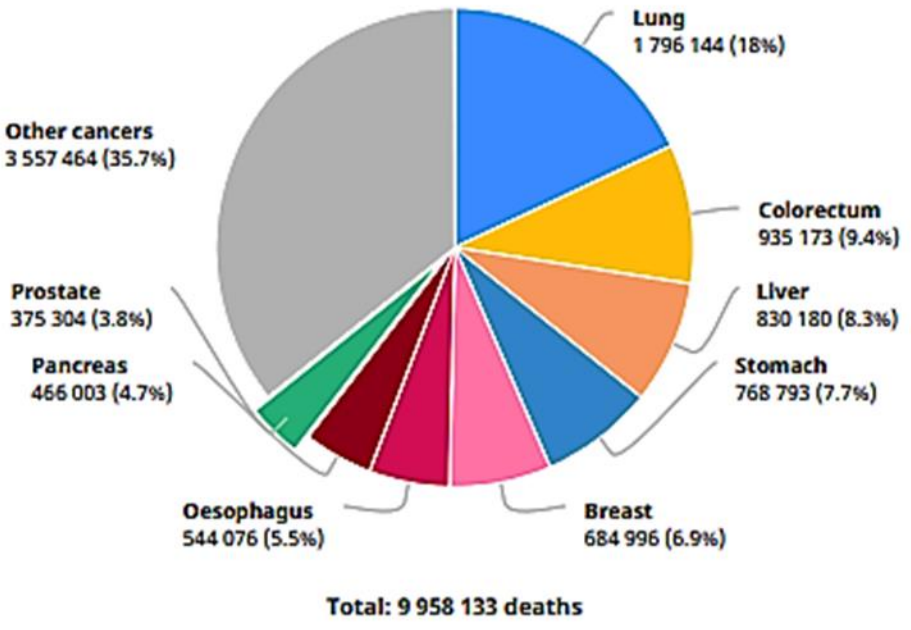


Figure 2: Incidence of cancer-associated deaths in 2020

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249

1.1.3 Benign prostatic hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is defined as noncancerous enlargement of prostate gland (Jin et al., 2018). Onset of BPH is usually seen in men after age of 40 years (Lim et al., 2017). Most of the males are affected at or over 50 years of age, this frequency drastically increases with increasing age and around 90% of males are affected after 80 years (Ng & Baradhi, 2020).

1.1.4 Risk factors

Number of factors responsible for the progression of BPH are still unclear. Some of the risk factors include erectile dysfunction, type 2 diabetes, obesity, genetics and a sedentary lifestyle (Calogero et al., 2019). The use of certain medications such as anticholinergics, pseudoephedrine and calcium channel blockers possibly worsen these symptoms (Zaman Huri et al., 2014). Numerous studies reported that androgens (e.g. testosterone) and other related hormones play a crucial part in BPH development (Rastrelli et al., 2019). Administration of exogenous testosterone does not increase the risk of BPH development. Dihydrotestosterone (DHT) plays a role in development and maintenance of the normal prostate as well as in the pathogenesis of BPH (Asiedu et al., 2017). DHT is synthesized in the prostate and mediate prostatic growth. The enzyme 5α-reductase acts on testosterone to produce dihydrotestosterone. The mitogenic signal transduction pathways of DHT are regulated by autocrine or paracrine fashion in which it binds to nuclear androgen receptors in epithelial and stromal cells, respectively (Swerdloff et al., 2017). The evidences from clinical observations of 5α-reductase inhibitor (finasteride) showed that DHT causes nodular hyperplasia (Madersbacher et al., 2019). The use of DHT inhibitor markedly lowers the content of DHT in prostate gland, which subsequently declines volume of prostate and symptoms of BPH.

Estrogen is thought to promotes the development of BPH (Ajayi & Abraham, 2018). The underlying mechanism is initiated by converting androgen to estrogen in the prostate gland compared to a direct molecular action of estrogen (Nicholson & Ricke, 2011). An in-vivo study conducted on the canine castration model showed a significant reduction of androgen levels, but the estrogen levels remained the same, which subsequently caused prostate atrophy (Sun et al.,

2017). The previously reported studies have shown no correlation between prostatic hyperplasia and serum estrogen levels in humans (Gangkak et al., 2017).

Failure of spermatic venous drainage system is another factor for BPH development (Goren & Gat, 2018). As it increases hydrostatic pressure and level of local testosterone around 100 fold as compared to serum levels of testosterone (Gat et al., 2008). These mechanisms explain the reason behind the lack of correlation between serum androgen levels and BPH. It also defines the underlying cause of no difference in progression of BPH with administration of exogenous testosterone.

1.1.5 Clinical manifestations

BPH presents most commonly with symptoms including bladder outlet obstruction, polyuria, nocturia, dysuria, urinary hesitancy, urinary retention or urinary incontinence (Vasanwala et al., 2017). BPH may lead to urinary tract infections (UTI), renal calculi and chronic renal complications (Vuichoud & Loughlin, 2015).

The treatment possibilities of BPH include lifestyle modification, medications and surgery (Lokeshwar et al., 2019). The patients who suffers from mild symptoms are recommended weight reduction, caffeine intake and regular exercise (Bradley et al., 2017; Yee et al., 2015). The patients having severe symptoms may be treated with alpha-blockers (e.g., terazosin) or 5α-reductase inhibitors (e.g., finasteride) (Jiwrajka et al., 2018; Rompay et al., 2019). Surgical removal of part of the prostate is done in patients who are not cured with medication (Foster et al., 2018).

1.1.6 Pathophysiology

BPH degenerate the prostate gland myofibers (Wang et al., 2015). “Misrepair-accumulation aging theory” suggests fibrosis and prostate muscle weakening as a major cause

in the development of BPH (Rashan et al., 2020). In BPH, the collagen fibers in muscular tissues are used for replacing broken myofibers, which are injured during ejaculations. Muscular fibrosis

results in accumulation of prostatic fluid and expansion of the prostate. Therefore, increasing accumulation of prostate fluid in glands elevates muscular tissue resistance during contractions and dilations. All of these events eventually break myofibers and replace them with collagen fibers (Wang et al. 2015).

With aging, aromatase and 5-alpha reductase activity increases, converting androgen into estrogen and DHT, respectively (Sánchez et al., 2018). BPH occurs due to age related changes in prostate androgen metabolism that favors the accumulation of DHT and responsible for cell growth in prostate gland and thus results in rapid prostate enlargement (BPH) (Dhingra, 2021). Many of the studies suggests that the extent of stromal hyperplasia is predominant as compared to glandular epithelial hyperplasia.

1.1.7 Classification of Benign Prostatic Hyperplasia

Classification of benign prostatic hyperplasia is based on significant obstruction and presences or absences of symptoms. Obstruction is comparatively more commonly found than any other symptom. It accelerates and accounts for organ dysfunctions if left untreated. Identifying BPH symptoms is usually done with the International Prostate Symptom Score (IPSS) and the quality of life index (QOL). The studies have been shown a positive corelation between IPSS and benign prostatic obstruction but no association is observed with quality of life index (QOL). An old aged person with nocturia (maximum of 4 times) is probably not considered serious as compared to nocturia (maximum of 2 times) at a younger age (Foo, 2017).

Benign prostatic hyperplasia executes different extent of obstruction. The urinary bladder serves two essential functions; storage and emptying. During impairment in emptying function, the consistently high residual urine is reported. Whereas impairment in storage function, the maximum voided urinary volume level is less and easily identified. Therefore, obstruction is characterized by increased post void residual urine (PVRU) > 100 ml and low maximum voided volume (MVV) <100 ml. According to the cut-off values, BPH are classified into 4 stages (figure 4) (Foo., 2017).

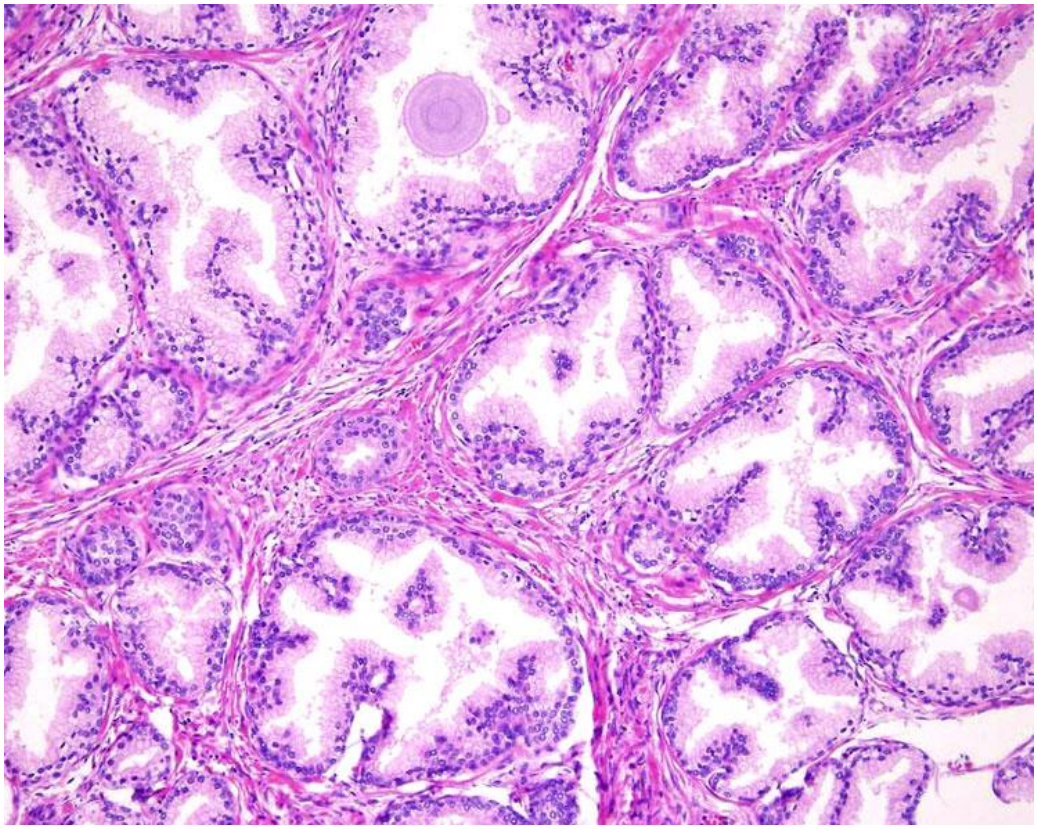


Figure 3: Histology of benign prostatic hyperplasia

https://www.auanet.org/education/auauniversity/education-products-and-resources/pathology-for-urologists/prostate/non-neoplastic-lesions/benign-prostatic-hyperplasia

Stage	Significant obstruction*	Bothersome symptoms†	Suggested treatment
I	Absent	Absent	Watch and counsel
II	Absent	Present	Medical treatment
III	Present	Irrespective	Surgical options
IV	Complications of clinical BPH	Complications of clinical BPH	Surgery

*Defined as persistent post-void residual urine volume > 100 mL or maximum voided volume < 100 mL. †Quality of life score ≥ 3.

Figure 4: Clinical stages of Benign prostatic hyperplasia

Foo, K. T. (2017). Pathophysiology of clinical benign prostatic hyperplasia. *Asian journal of urology*, *4*(3), 152-157.

1.1.8 Prostatic intraepithelial neoplasia (PIN)

Prostatic intraepithelial neoplasia (PIN) is a pathological state that arises within pre-existing benign prostatic acini or ducts by increasing the neoplastic growth of epithelial cells (Zhou, 2018). HGPIN refers to proliferation of prostate glandular epithelial cells that display significant cytological atypia within the confines of prostatic ducts and acini, and is considered as a precursor lesion for prostate carcinoma (Zhou, 2018). While many other prostate impairments are possible to be associate with increased cancer rates but PIN is the most important precursor for maximum number of prostatic carcinomas and is considered as an ideal candidate for chemoprevention programs (Cui et al., 2017).

HGPIN and prostate carcinoma have many similarities, such as the increase frequency with age and elevated rates of incidence in the peripheral zone of the prostate (Chen et al., 2018). HGPIN is found predominantly in the peripheral zone of the prostate, rarely in the transition and central zone (Pradhan & Sharan, 2016). And also have similar genetic and molecular biomarkers; telomere shortening (Graham & Meeker, 2017), loss of alleles from chromosome 8p12-21 (Jung et al., 2016) and gain of chromosomes 7, 8, 10, and 12 (Jung et al., 2016). More than 400 abnormally expressed genes are reported in HGPIN and invasive prostatic carcinoma by using cDNA microarray analysis (Köseoğlu, 2018).

Pathologically and genotypically, HGPIN represents an intermediate stage between benign epithelium and invasive carcinoma (Barakzai, 2019). PIN and cancer cells are distinguished by expression of various tumor markers (Barakzai, 2019; Rycaj & Tang, 2015).

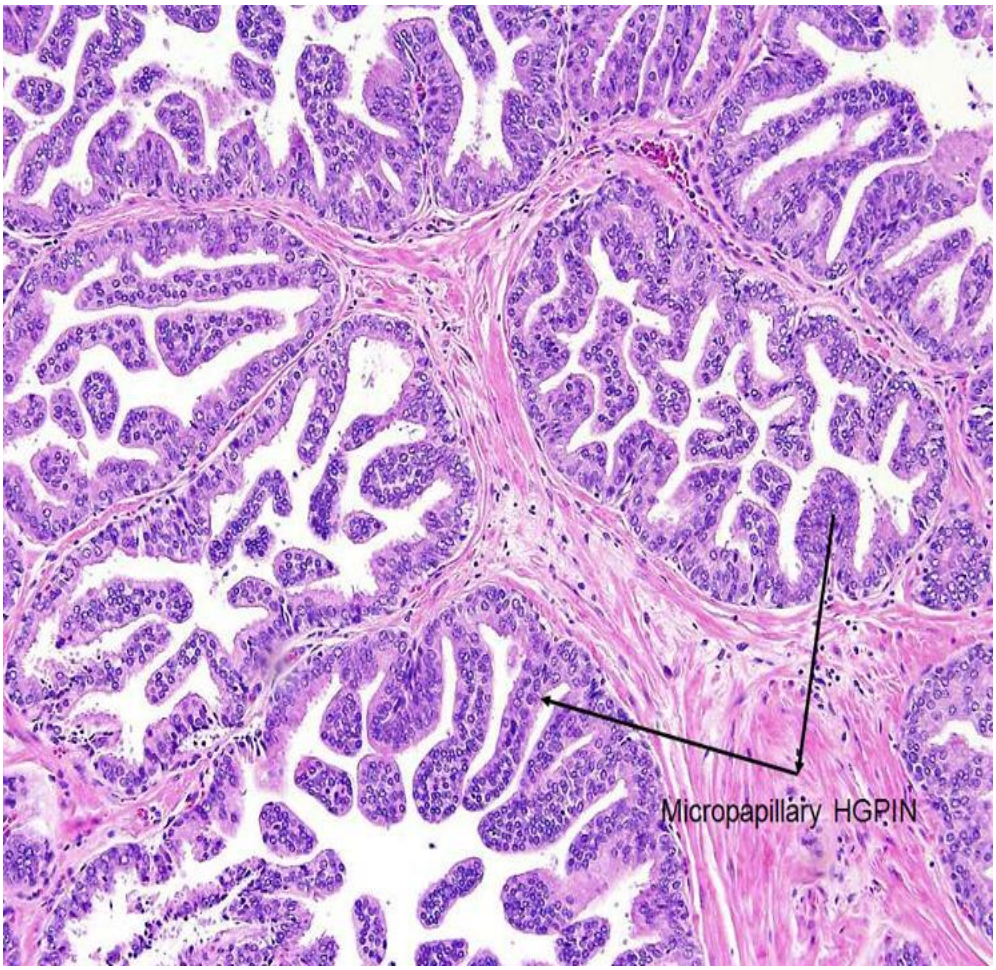


Figure 5: Histology of high-grade prostatic intraepithelial neoplasia (HG-PIN)

https://www.auanet.org/education/auauniversity/education-products-and-resources/pathology-forurologists/prostate/putative-precursor-lesions/prostatic-intraepithelial-neoplasia-(pin)

1.1.9 Prostate carcinoma

Histologically, prostatic carcinoma is divided into two different groups of variants; acinar adenocarcinoma and non-acinar. Acinar adenocarcinoma includes foamy, signet ring, lymphoepithelioma, pseudohyperplastic, atrophic, colloid, and oncocytic-like carcinomas. In contrast, non-acinar carcinoma that accounts for around 5 to 10% of carcinomas includes ductal adenocarcinoma, sarcomatoid carcinoma, basal cell carcinoma, urothelial carcinoma, adenosquamous carcinoma, and neuroendocrine tumors (specifically small-cell). While, other variants such as pleomorphic giant cell carcinoma, large-cell neuroendocrine carcinoma, microcystic adenocarcinoma and prostatic intraepithelial neoplasia-like adenocarcinoma are not present in the classification of WHO-2004 (Humphrey, 2012).

1.1.10 Clinical manifestations

In early disease, prostate carcinoma is non-symptomatic, while later in the disease, dysuria, hematuria, nocturia, and pelvic pain or back pain may be the presenting features. Prostate cancer may also cause erectile dysfunction and painful ejaculation (Mustafa et al., 2016). Prostate cancer cells may metastasize to distinct body parts, mainly in the lymph nodes and bones (Manna et al., 2018). Metastatic prostate cancer may produce bone pain involving the vertebral column, ribs and pelvis (Trent et al., 2020). Prostate cancer can also cause spinal compression resulting in leg weakness, paresthesia, urinary incontinence, and fecal incontinence (Miyoshi et al., 2020).

1.1.11 Risk factors

Several factors are associated with increased susceptibility to Prostate carcinoma including; obesity, age, genetics, sedentary lifestyle, hypertension, certain consumable products, medication and levels of hormones. Among all of these, obesity has been found to be associated with high mortality in prostate cancer (Bandini et al., 2017; Vidal et al., 2017). Incidence of Prostate cancer increases with age, it is rarely seen in younger males (<50 years) whereas its incidence rate increases upto 60% in men over the age of 65 years (Rawal., 2019).

Human genetics is a decisive risk factor. As, previous studies have suggested that men with one or two-first-degree relative (father or brother) with prostate cancer are two to five-times more susceptible to develop cancer. It is more common in brothers of affected individuals as compared to those with affected father (Albright et al., 2015). In the United States, the incidence and mortality rate of prostate cancer is higher in black men than in white or Hispanic males. Moreover frequency and death rates in Hispanic males are one-third of the rates in non-Hispanic whites (Rawla, 2019; Siegel et al., 2020; Taitt, 2018). Furthermore, inherited factors are the prominent cause of prostate cancer development. These include mutation in *BRCA1* and *BRCA2*, hereditary prostate cancer gene 1 (*HPC1*), the androgen receptor gene and the vitamin D receptor genes (Mehrgou & Akouchekian, 2016; Nunes et al., 2016; K. Wang et al., 2016). TMPRSS2 fusion, particularly TMPRSS2-ERG or TMPRSS2-ETV1/4, accelerate the growth of cancerous cell in prostate (Mustafa et al., 2016).

Evidence supports that diet rich in fruits, vegetables and low fat diet has slight preventive role (Lin et al., 2015). However, some studies have also reported low vitamin D levels and higher meat consumption rendering an increased risk of prostate cancer (Kim et al., 2018). A growing body of literature has established to link between prostate cancer and medicines, medical procedures, and pathological states. Statins, a lipid-lowering medication, are reported to decrease the risk of prostate cancer (Rompay et al., 2019).

Infections, such as prostatitis, human papillomavirus and sexually transmitted infections (*Chlamydia* gonorrhea or syphilis) seems to increase the risk of prostate carcinoma (Gandomani et al., 2017).

Previously reported studies for identifying hormone levels for prostate cancer indicated an insignificant association for serum testosterone (Klap et al., 2015). In contrast, numerous studies have linked increased serum levels of IGF-1 with prostate cancer development. A molecular study identified that IGF-1 might works as a mitogenic agent for prostate cancer cells, decrease sex hormone-binding globulin (SHBG) and increase androgen synthesis (Cao et al., 2015). These findings are inadequate, and the quantification of serum IGF-1 levels is not suggested.

1.1.12 Staging of prostate carcinoma

Prostate cancer is categorized into clinical and pathological stages. Most widely used staging system for prostate cancer is the AJCC (American Joint Committee on Cancer) TNM system to describe the extension of tumor to lymph nodes, bones or other organs. Table 1 shows AJCC TNM staging of prostate carcinoma. N0 represents no lymph nodes involvement, whereas N1 shows tumor spread into nearby lymph nodes. Besides, M0 shows that cancer has not metastasized to distant sites whereas M1 means spreading of cancer to distant body parts, such as lymph nodes (M1a), bones (M1b), other site with or without bone involvement (M1c).

1.1.13 Gleason grading system

Gleason scoring system is the most widely accepted grading system and was established in 1966 by Donald Floyd Gleason. It is based on the microscopic glandular pattern, ranging from grade 1, which is the most well-differentiated, to grade 5, which is the most undifferentiated pattern (Figure 6). It is obtained by adding primary and secondary patterns (McNeal & Gleason, 1991). This grading system was upgraded in 1974 and again in 2005 by the International Society of Urological Pathology (ISUP) (Epstein, 2005). According to new grading system, Gleason score ≤ 6 is Grade Group 1, Gleason score $3 + 4 = 7$ is Grade Group 2, Gleason score $4 + 3 = 7$ is Grade Group 3, Gleason score $4 + 4 = 8$, $3 + 5 = 8$, $5 + 3 = 8$ is Grade Group 4 and Gleason score 9 – 10 is Grade Group 5 (Barakzai, 2019). Together with other parameters, Gleason score or grade group predicts prognosis of prostate cancer. Cancers with a higher Gleason score are more aggressive and have a worse prognosis.

TUMOR	
Tx	Tumor cannot be evaluated (due to lack of information)
T0	No evidence of a primary tumor
T1*	Tumor was not detected during a digital rectal exam (DRE) and cannot be seen on imaging studies (tumor may be discoverd during surgery for a reason other cancer)*
T2 T2a T2b T2c	Tumor can be detected during a DRE but is present in the prostate only Tumor is in half or less than one side (lobe) of the prostate Tumor is in more than half of one prostate lobe, but has not yet invaded the other lobe Tumor is in both prostate lobes
T3 T3a T3b	Tumor extends outside of the prostate Tumor extends outside the prostate on one or both sides Tumor has spread to the seminal vesicles (the glands on each side of the bladder)
T4	Tumor has spread to tissues near the prostate other than the seminal vesicles, such as the bladder or wall of the plevis
Nearby (regional) lymph nodes (N)	
Nx	Nearby lymph nodes are not evaluated
N0	No cancer cells are found in nearby lymph nodes
N1	Cancer cells are found in nearby lymph nodes
Distant Metastasis (M)	
M0 M1 M1a M1b M1c	Cancer has not spread beyond the prostate Cancer has spread beyond the prostate Cancer has spread to distant lymph nodes Cancer has spread to bone Cancer has spread to another organ or site, with or without bone disease

Table 1: AJCC TNM staging of prostate cancer

The AJCC TNM staging system:

https://www.prostateconditions.org/about-prostate-conditions/prostate-cancer/newly-diagnosed/staging

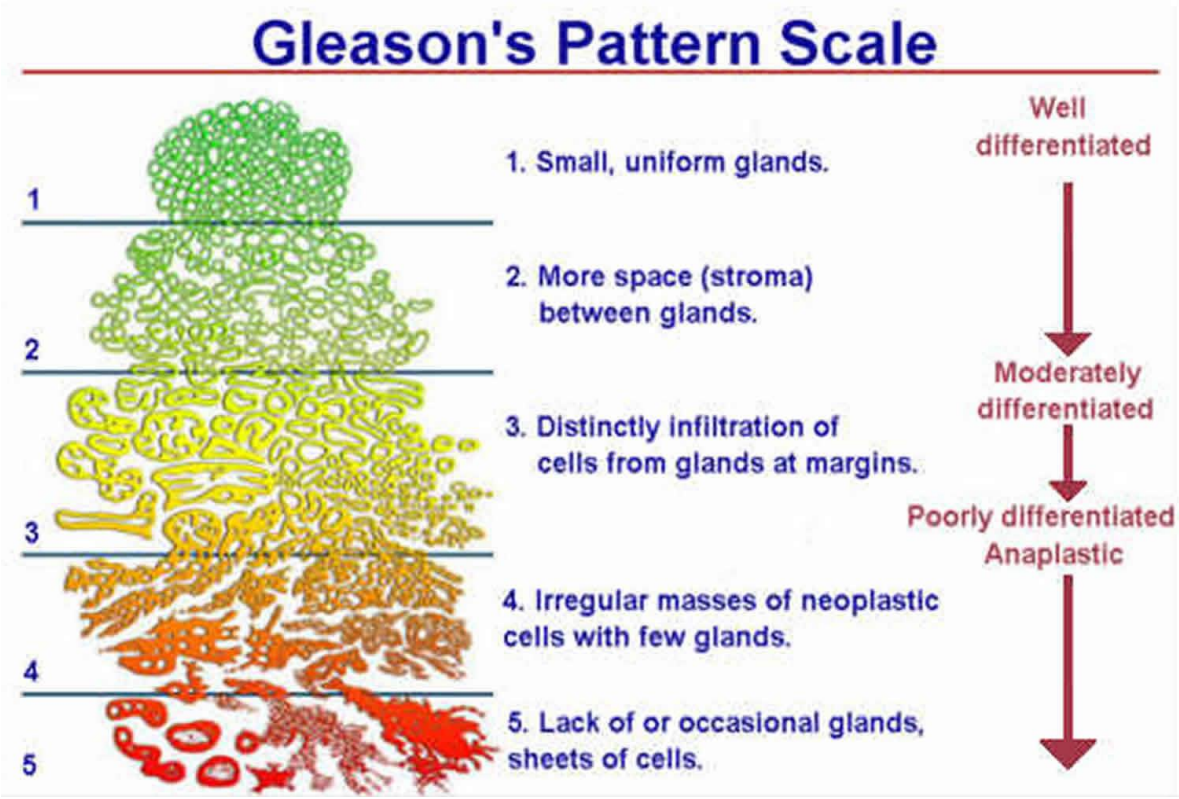


Figure 6: Gleason grading system for prostate carcinoma

Harnden, P., Shelley, M. D., Coles, B., Staffurth, J., & Mason, M. D. (2007). Should the Gleason grading system for prostate cancer be modified to account for high-grade tertiary components? A systematic review and meta-analysis. *The lancet oncology*, *8*(5), 411-419.

1.1.14 TMPRSS2-ERG gene fusion in prostate cancer

ERG belongs to an erythroblast transformation-specific (ETS) family of transcription factors and is categorized as a proto-oncogene (Adamo & Ladomery, 2016). All members of this family are key regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation and apoptosis. The protein encoded by this gene is mainly expressed in the nucleus (Adamo & Ladomery, 2016). Transmembrane serine protease 2 (TMPRSS2) is a cell surface protein primarily expressed by endothelial cells. It is expressed in normal prostate epithelial cells and is needed for normal prostate function (Shabir 2021). The promoter region of TMPRSS2 becomes fused to the coding region of ERG in prostate carcinoma to form a fusion gene. Both genes are present on chromosome 21 and fusion is caused by chromosomal translocation between TMPRSS2 and ERG. The promoter of TMPRSS2 is under control of androgen, subsequently this fusion drives the overexpression of ERG in the presence of androgens (Adamo & Ladomery, 2016). High-grade PIN that are adjacent to aggressive fusion positive cancer may also occasionally contain the fusion and thus can express ERG, fusions are also been detected at low frequency in benign prostatic hyperplasia (Robert., 2013). This indicates that fusion is an early event and that their presence in BPH can increase the risk of developing carcinoma.

From past few years, TMPRSS2-ERG fusion in prostate carcinoma has been the focus of attention for investigators. This fusion is more often seen in Caucasians then in African Americans and Asians (Galluzzi et al., 2011). The fused TMPRSS2-ERG gene product is a potential biomarker detected in various biological samples via several biological methods, such as, fluorescence in situ hybridization (FISH) (Sung et al., 2016), Polymerase chain reaction (PCR) (Lee et al., 2017) and immunohistochemistry (IHC) (Font-Tello et al., 2015). A noninvasive diagnostic procedure was also introduced to identify the TMPRSS2-ERG fusion transcripts in urine samples of patients (Sanguedolce et al., 2016). Studies that suggested TMPRSS2-ERG gene fusion as the prognostic biomarker for prostate carcinoma are still controversial. The present study was performed to analyze the diagnostic significance of this fusion by assessing ERG expression on immunohistochemistry in prostate carcinoma patients.

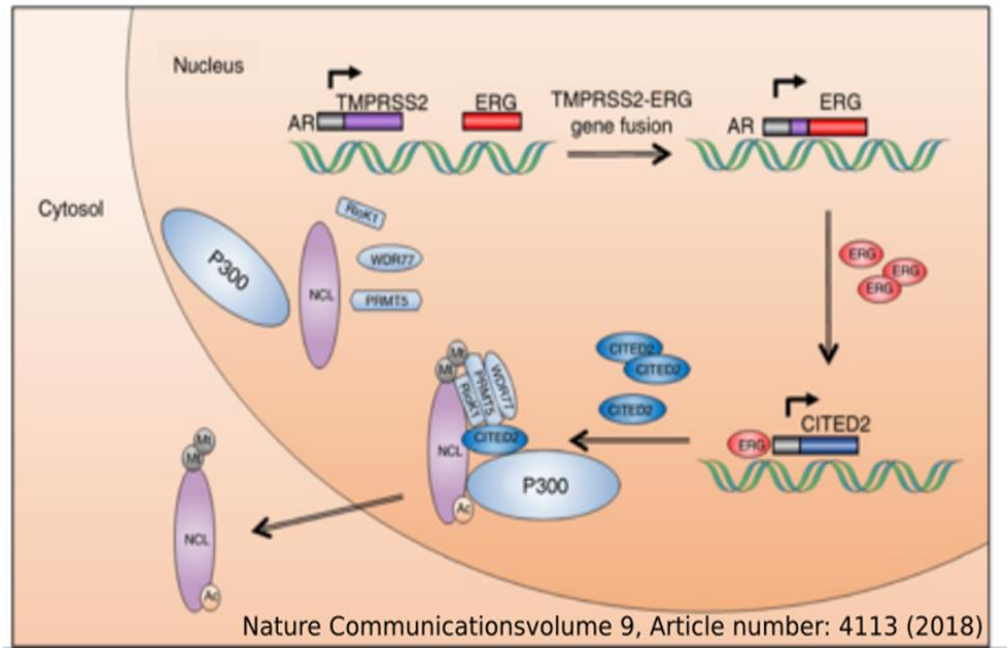


Figure 7: TMPRSS2-ERG gene fusion in prostate cancer

Shin, S. H., Lee, G. Y., Lee, M., Kang, J., Shin, H. W., Chun, Y. S., & Park, J. W. (2018). Aberrant expression of CITED2 promotes prostate cancer metastasis by activating the nucleolin-AKT pathway. *Nature communications*, *9*(1), 1-14.

1.1.15 Serine Peptidase Inhibitor Kazal Type 1 (SPINK1) overexpression in prostate carcinoma

Serine Peptidase Inhibitor Kazal Type 1 (SPINK1; also called pancreatic secretory trypsin inhibitor or tumor-associated trypsin inhibitor) was initially identified in the pancreas (Ohmuraya & Yamamura, 2011). It inhibits the function of serine protease, including trypsin of pancreas (Lin et al., 2021). Normally SPINK1 is expressed in colon, liver, pancreas, and other gastrointestinal organs. Numerous variant of SPINK1 are found to be associated with the development of chronic pancreatitis (Bagul et al., 2009; Derikx et al., 2009). The role of pancreatic SPINK1 in autophagy of exocrine pancreatic cells shows its importance in cell survival. In hepatocytes Hepatitis B virus elevates SPINK1 expression and thus prevent hepatocytes from initiating serine proteinase-mediated apoptosis (Wang & Xu, 2010).

SPINK1 overexpression has been found in various cancers, such as those of breast, ovary, cervix, urinary bladder, renal, GI, prostate, lung, colon and liver (Räsänen et al., 2016; Stenman, 2011). Elevated expression of SPINK1 has been reported to be associated with liver metastasis (Marshall et al., 2013; Xu et al., 2018). It is an independent biomarker for diagnosing colon and breast cancer (Chen et al., 2016). Furthermore, its overexpression is responsible for increasing cell growth and metastasis of tumors and has been suggested to include SPINK1 along with serum α-fetoprotein and osteopontin as a combined prognostic biomarker for hepatocellular carcinoma (Lee et al., 2007; Xu et al., 2018).

The expression of SPINK1 is associated with the expression of tumor-associated trypsin, which ultimately stimulates numerous matrix metalloproteinases. SPINK1 expression is associated with poor prognosis in many carcinomas (Paju & Stenman, 2006). SPINK1 and epidermal growth factor receptor (EGFR) interaction plays an important role as SPINK1, epidermal growth factor (EGF) and EGF receptor (EGFR) ligand have 50% amino acid homology and many other structural similarities. Like EGF, SPINK1 phosphorylates EGFR and downstream signaling through mitogen activated protein kinase (MAPK), Janus kinase (JAK) or phophoinosite 3-kinase (PI3K) pathways

(Figure 8), and act as an autocrine growth factor (Ohmuraya & Yamamura, 2011).

1.1.2 Prostate specific antigen (PSA)

Clinical screening for cancer is recommended for diagnosing the disease at initial levels and limiting morbidity and mortality rate. Screening of prostate carcinoma aids in improving the quality of life and declines disease-specific and overall death rate due to carcinoma (Ilic et al., 2013). The screening test for prostate carcinoma is the detection of increase prostate-specific antigen (PSA) levels in the blood. PSA is found between the range of 1.0-1.5 ng/mL in the serum of healthy prostates males. The serum PSA level between 4-10 ng/mL are supposed to be doubtful and is recommended to repeat test for its confirmation (Shtricker et al., 2009). A study was conducted to compare incidence and mortality rate of prostate carcinoma in USA, where PSA screening is a standard screening test, and UK, where it is not done. Even though dramatically increased number of diagnosed people with cancer were found in USA, but the mortality rate remained the same (Feletto et al., 2015; Jatoi & Sah, 2019).

PSA is highly controversial test because it is elevated in prostate cancer as well as in other prostate lesions. A study has reported an increased serum PSA levels in PIN (Banerjee et al., 2016) and in patients undergoing simple prostatectomy. PSA is an unspecific prognostic marker of prostate carcinoma as PSA level is also elevated in inflammation of normal prostate and BPH. It is low sensitive and specific test, around 75% shows false-positive PSA results (Bates, 2017, Markin et al., 2020). False-positive test results cause confusion and anxiety and can lead to unnecessary medication and procedure that may cause harmful consequences, such as incontinence, erectile dysfunction and psychological distress. While, false-negative results increase a false sense of security in a person with carcinoma (Bernal-Soriano et al., 2019). Researchers are trying to resolve the issues regarding prostate cancer screening by developing more accurate screening tools and is the focus of attention for investigators. In health and the broader community, prostate cancer screening is considered a controversial topic because varying recommendations exist that are made by healthcare organizations and governed by national policies (Ilic et al., 2013). Therefore, it is of utmost importance to identify promising and effective biomarkers for the early detection of prostate cancer in order to facilitate management and improve

the health policy decisions.

1.1.16 Rationale of the study

Prostate cancer is among top 10 malignancies of adult males in Pakistan. Current clinical practice guidelines for prostate cancer screening are debatable, to some extent, because ambiguity in findings ultimately results in increased risk of overdiagnosis and overtreatment. PSA has been approved for annual screening of prostate carcinoma in males of age above or equal to 50 years by the Food and Drug Administration (FDA) but is still controversial screening modality because of varying recommendations by healthcare organizations. Researchers are working hard to answer the challenges of prostate cancer screening by introducing more precise diagnostic markers. There is a great need to identify promising biomarkers for the early detection of prostate cancer and targeted treatment.

Limited data is available regarding immunohistochemical (IHC) expression of ERG and SPINK1 in prostate tumors in Pakistan. This study aims to evaluate the immunohistochemical expression of ERG and SPINK1 in prostate carcinoma cases in our population and asses its correlation with clinicopathological parameters.

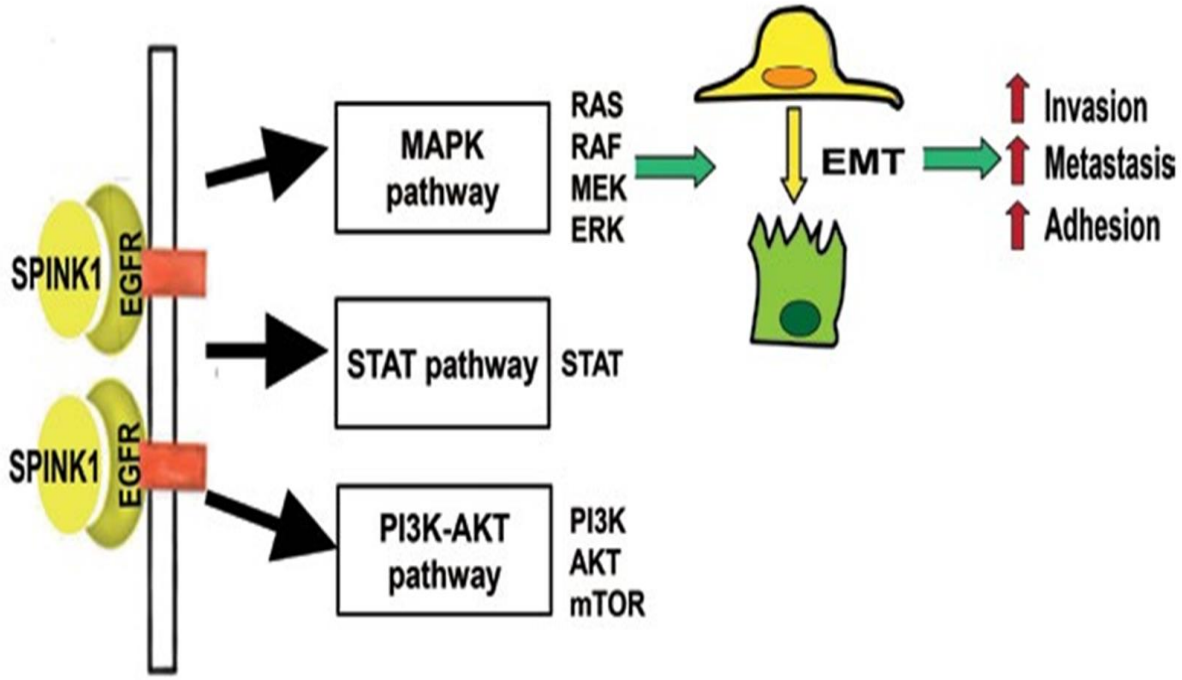


Figure 8: Intracellular serine Peptidase Inhibitor Kazal Type 1 (SPINK1) mechanism to initiate prostate carcinoma

Ohmuraya, M., & Yamamura, K. I. (2011). The roles of serine protease inhibitor Kazal type 1 (SPINK1) in pancreatic diseases. *Experimental animals*, *60*(5), 433-444.

1.2 Hypothesis

A) Null hypothesis

There is no association between the expression of ERG and SPINK1 neither in selected prostate tumour cases nor with clinico-pathological parameters.

1.3 Objectives of the study

i. To evaluate the expression of ERG and SPINK1 in Prostate carcinoma cases using Immunohistochemistry.

ii. To assess the relationship of ERG and SPINK1 expression in prostate carcinoma.

iii. To determine the relation of ERG and SPINK1 expression with the clinico-pathological parameters.

1.4 Statement of the problem

Prostate cancer is among top 6^{th} most common neoplasm responsible for morbidity and mortality of adult males in Pakistan. Majority of prostate tumors are curable at the time of diagnosis, hence there is a need to detect the expression of different proteins using immunohistochemistry for early diagnosis and timely effective therapeutic intervention.

1.5 Significance of study

Prostate tumor is second most commonly diagnosed malignancy of adult men globally and is the fifth leading cause of death worldwide. Both genetic and environmental factors are implicated in the pathogenesis. Various markers are being utilized for diagnosing and prognosticating the disease, and many studies are underway to identify novel and more effective markers specially as targets for precision therapy.

This study was conducted with aim to evaluate the expression of ERG and SPINK1 in prostate carcinoma and to assess its effectiveness as diagnostic markers. This was achieved via immunohistochemistry on paraffin-embedded tissues and correlation of the results with clinicopathological parameters. Identification of ERG in prostate cancer signifies the importance of ERG fusion as a driving molecular event in our male population. The present study and similar future studies can prove to be of great value in identifying suitable patients for Anti-ERG therapy and for evaluating SPINK1 as a marker for aggressive disease.

1.6 Operational definitions

i. ERG expression

The staining intensity in the nuclei of Prostate cancer cells were scored as negative (no staining = 0), weakly positive (only evident at high magnification [x10 objective magnification] = 1+), moderately positive (evident at low magnification [x4 objective magnification] = 2+), and strongly positive (striking at low magnification = 3+). Nuclear staining of endothelial cells served as a positive control for the staining procedure.

A patient was labelled as ERG positive if all foci demonstrate ERG expression and a patient with exclusively negative foci was labelled ERG negative.

Reference: Berg, K. D. (2016). The prognostic and predictive value of TMPRSS2-ERG gene fusion and ERG protein expression in prostate cancer biopsies. *Dan Med J*, *63*(12), B5319.

ii. SPINK1 expression

SPINK1 the cytoplasmic staining was scored as 0 = no staining, 1 = less than 50% of cells staining in scattered individual cells, 2 =less than 50% of cells staining in complete glands, 3 =50–80% of cells staining, and 4 =greater than 80% of cells staining. SPINK1 cytoplasmic staining was recorded for each core.

Reference: Brooks, J. D., Wei, W., Hawley, S., Auman, H., Newcomb, L., Boyer, H., ... & Carroll, P. R. (2015). Evaluation of ERG and SPINK1 by immunohistochemical staining and clinicopathological outcomes in a multi-institutional radical prostatectomy cohort of 1067 patients. *PloS one*, *10*(7), e0132343.

iii. Gleason grading system

According to new grading system:

- Gleason score ≤ 6 → Grade Group 1
- Gleason score 3 + 4 = 7 → Grade Group 2
- Gleason score 4 + 3 = 7 → Grade Group 3
- Gleason score 4 + 4 = 8, 3 + 5 = 8, 5 + 3 = 8 → Grade Group 4
- Gleason score 9 – 10 → Grade Group 5

Reference:Barakzai, M. A. (2019). Prostatic adenocarcinoma: A grading from Gleason to the new grade-group system: a historical and critical review. *Asian Pacific journal of cancer prevention: APJCP*, *20*(3), 661.

iv. Clinico-pathological parameters

This includes age, tumour grade, lymph node metastasis, lymphovascular invasion, perineural invasion and intraductal carcinomas.

CHAPTER 2

LITERATURE REVIEW

Carcinoma of Prostate is the most commonly diagnosed non-cutaneous carcinoma in men in over half of the nations of the world, it is the main cause of cancer death in 46 countries (Globocan, 2020). According to GLOBOCAN 2020, 1,414,259 new cases and 375,304 deaths due to prostate carcinoma were reported worldwide in 2020, with higher prevalence in the developed nations. 2,293,818 new cases and 379,005 deaths due to Prostate cancer worldwide are estimated until 2040, with highest mortality rate in Africa, then in Asia, while the least frequency will be found in Europe (Globocan, 2020). According to annual cancer report 2019 of The Shaukat khanum memorial cancer hospital and research center Pakistan, carcinoma of prostate is the second most common neoplasm of adult male. Strong association with advance age is observed with the highest incidence in men more than 65 years of age. The frequency of prostate carcinoma varies over the regions and populations, with African American men having the highest frequency and more aggressive type of prostate tumor as compared to non-Africans (Globocan, 2020). This incongruity is due to social, environmental and genetic differences. The known risk factors are advancing age, family history, ethnicity and genetic factors. Other factors include lifestyle; such as diet with high saturated fat, less intake of fruits and vegetables, physical inactivity, and exposure to chemicals or radiation. Several studies stated that about 5% of disease risks is associated with genetics (Chung et al.,2019). Prostate tumor is a leading public health challenge for men around the globe and early diagnoses of disease by effective diagnosis is critical for the treatment.

Prostate carcinoma patients present with various clinical manifestations such as lower urinary tract symptoms in early stages of disease, while more advanced stage may present with hematuria, haematospermia, bone pain. Hamilton et al. (2016) and Masood et al. (2018) studied

significant association between clinical presentation and prostate cancer. The identification of these associations need attention as this will help in early diagnosis and treatment and will also be beneficial for the mental and physical well-being of patients.

For the last decade, scientists have been making noteworthy advancements in the development of therapeutic strategies and studying progression of carcinoma with regards to genetic alterations. But all of these developments still need more investigation, for example; identification of promising diagnostic biomarkers, controlling of metastatic impairments and evaluating prognostic biomarkers that gives an advantage to medical practitioners in early diagnosis and eventually effective treatment (Mohler et al., 2016). Several biomarkers have been identified in prostate carcinoma, but lot of conflicting theories also exists against their use as an approved diagnostic and prognostic biomarker.

Prostate-specific antigen (PSA) was used as a detection tool for prostate carcinoma, but has shown various weaknesses, as it is not able to differentiate between (i) prostate cancer and benign prostatic hyperplasia (BPH), (ii) between indolent and aggressive types of cancers. Furthermore, PSA's screening level fail to identify patient`s response to a treatment. Many studies have been found that lower free PSA (fPSA) to total PSA (tPSA) ratio and elevated serum PSA level represents cancer as these changes are not seen in BPH. Many biomarkers, for instance prostate cancer antigen (PCA) 3, PSA (pro-PSA, benign PSA, and intact PSA), kallikreins and many others helps to reach better diagnosis(Adhyam & Gupta, 2012; McNally & Ruddock, 2020; Romero Otero et al., 2014).

Several diagnostic markers in urine, serum, tissue, and semen have been identified as an effective target approach for prostate carcinoma and BPH. But none of these candidates prove to be useful in clinical practice and have not progressed more than the discovery stage. Multiple studies are directed towards finding targets for precision therapy like monoclonal antibodies. The use of monoclonal antibodies (mAbs) for cancer therapy has achieved considerable success in recent years, identification of therapeutic antibodies needs i) well-documented research of cancer serology, ii) understanding of protein techniques, iii) mode of action and resistance, iv) immune system-cancer cells mechanism and v) its associated adverse or side effects (Scott et al., 2012).

In the initial stages of Prostate carcinoma tumorigenesis, gene fusions are known to be important (Breg K. D, 2016). A retrospective study was carried out to **e**valuate the ERG via immunohistochemistry in various benign and malignant tissues. It was found to be expressed in 44% of low to intermediate grade prostatic adenocarcinomas; 22% of high grade prostatic adenocarcinomas; and in 22% of High grade PIN. No ERG expression was detected in benign lesions and non-prostate carcinomas. This study demonstrated that ERG is a highly specific marker which may help in interpretation of prostate biopsies, while working on a tumor of uncertain origin (Haiyan, 2013).

In another study 95% concordance was found between ERG expression and ERG gene rearrangement by immunohistochemistry and FISH. ERG expression was detected in 52.4% of Prostate cancer. Clinical outcome and tumor phenotype was unrelated to ERG expression. High AR expression was noted in ERG-positive cancers. The distinction in AR levels between ERG-positive and negative supports a potential response to hormonal therapy (Minner, 2011).

ERG expression varies with race, a study conducted on East Asians (China) and Black Americans, reported 15% and 27% of ERG expression, respectively by using immunohistochemistry (Dong et al. 2014; Khani et al. 2014). Similarly, Abdelsalam et al. (2020) performed immunohistochemical study and reported higher incidence of ERG in Western population ranging between 50 to 70%. Caucasian-Americans showing more ERG expression than Black-Americans (41.9% vs. 23.9%). In Chinese patients, the frequency of ERG was found at approximately 14%. A similar frequency of ERG was also noted in the population of Asian descent from Japan, Korea, and India (Abdelsalam et al. 2020).

Aldaoud et al. identified ERG expression in Jordanian-Arab population via immunohistochemistry and found the lower incidence (33.2%) in Arabs as compared to North Americans and Europeans. No significant association was found with Gleason grade group (Aldaoud et al., 2017). ERG expression is well documented in the peripheral zone tumors as compared to transition zone tumors (Liu et al., 2011). This can be the reason of inconsistency between the results of various studies, as Aldaoud et al. (2017) reported incidence of ERG

expression in mixed cases from peripheral zone on needle biopsy and transition zone on TURP, and concluded that both zones showed comparatively lower expression of ERG.

Serine protease inhibitor Kazal-type1 is a trypsin inhibitor that prevents trypsinogen activation in pancreatic tissues. Huhtala et al. (1983) first identified the levels of SPINK1 in urine samples of patients with gynecological carcinoma and suggested it as a tumor marker. Expression of SPINK1 was also observed in solid tumors, such as prostate carcinoma (Paju et al., 2007). The expression of SPINK1 was found in about 9% of Caucasian prostate cancer patients and 23% in Black Americans on immunohistochemistry (Khani et al., 2014). The study conducted in the middle east noted only 4% SPINK1 expression via immunohistochemistry, which was comparatively less than Western population. No significant association was found for SPINK1 to be a prognostic biomarker. The molecular landscape of prostate carcinoma among regions differs from one race to another and yielding conflicting results (Abdelsalam et al., 2020). This might be due to difference in genetics, regional environment, lifestyle and dietary habits among different population (Ornish et al., 2008). Molecular alterations in different ethnic groups are not well characterized, and for this reason conflicting results are observed on several published reports (Abou-Ouf et al., 2016; Lee et al., 2015).

Many researchers are interested to identify the association of SPINK1 and ERG as a diagnostic and prognostic marker for prostate carcinoma. Tomlins et al. carried out a study to determine clinicopathological association of microarray-based molecular subtyping of prostate carcinoma and found positive association of ERG with lower PSA and Gleason scores, while SPINK1 positive tumors were associated with higher Gleason scores and were more frequently expressed in African Americans. Clinical outcomes were not significantly different between subtypes (Tomlins et al., 2015).

Several studies found that a subset of ETS-rearrangement negative prostate tumors shows over-expression of SPINK1 at the RNA and protein levels, suggesting a molecularly distinct subset with worse outcomes (Faisal et al., 2019). US Physicians Health Study was conducted on 879 prostate carcinoma patients that were treated by radical prostatectomy. SPINK1 expression was analyzed *via* immunohistochemistry in 8% of the cases. Higher PTEN and stathmin expression

and lower AR expression were seen in SPINK1 positive tumors. SPINK1 over-expression was seen in 4% ERG - positive and 11% ERG- negative samples. No association was found between SPINK1 and Gleason grade. This study also suggested that SPINK1 was not a predictor of recurrence or lethal prostate cancer (Flavin et al., 2014).

A study was conducted to evaluate expression of different markers in young patients and to observe its relation to racial background and genetics by using dual immunohistochemistry and dual RNA in situ hybridization. Out of 151 men 56% expressed ERG, 40% expressed SPINK1, 6% ETV1 and 3% ETV4. 17% of cases demonstrated both ERG and SPINK1 in either similar or various foci. ERG overexpression was seen more in Caucasian patients ≤45 years old and with Gleason groups1 and 2, whereas SPINK1 was observed more in African Americans (Lu et al., 2020).

Another study was carried out to examine the association of ERG and SPINK1 expression with hormone responsiveness. 178 biopsies were selected, out of which 34% were hormonally treated patients. By using FISH and immunohistochemistry, ERG expression was observed in association with age, tumor area, Gleason score, PSA and progression-free survival. No association was seen between TMPRSS2:ERG fusion and prognosis that suggesting absence of hormonal dependency . 11% of biopsies expressed SPINK1 and was associated with aggressive disease suggesting it to act as a biomarker in endocrine-treated Prostate carcinoma (Leinonen et al., 2010).

A study was conducted to see clinical relation of SPINK1 expression with other genomic alterations of Prostate carcinoma by immunohistochemistry. 5.9% of prostate cancer cases expressed SPINK, whereas none of benign prostate lesions showed its expression. SPINK1 expression was seen more in ERG negative than in ERG positive cancers, and its expression was not related to phenotype and recurrence. It was concluded that SPINK1 is a relevant biomarker for malignant prostate cancer and is firmly connected to 6q15-and 5q21-deleted ERG negative prostate tumor (Grupp et al., 2013)**.**

A new clinically feasible and cost-effective approach, two dual-color immunohistochemistry for evaluation of ERG– PTEN and ERG–SPINK1 in Prostate lesions. For ERG–PTEN, 35% ERG-positive and 33% PTEN-deleted cases were identified. For ERG–SPINK1, 39% of cases were positive for ERG, SPINK1 was expressed in 9% of the remaining 173 ERG-negative cases. SPINK1 expression was mutually exclusive with ERG expression, but two cases showed the corresponding expression of ERG and SPINK1 either in the same foci or different foci of the same tumor. Homogeneous staining was observed in ERG, whereas heterogeneous staining was observed in most SPINK1 cases (Bhalla et al., 2013).

An analysis was done to evaluate the clinical significance and expression of ERG, SPINK1, and EZH2 in pure and mixed ductal adenocarcinoma of the prostate. 22 pure and 39 mixed ductal/acinar cases were collected. None of the clinical parameters showed a significant difference between the two tumor types except for tumor growth patterns. 98% of tumors expressed EZH2, 20% of tumors expressed ERG, and 36% expressed SPINK1. Pure and mixed ductal adenocarcinomas showed no difference in protein expression, clinical behavior, and molecular alterations. Higher EZH2 and SPINK1 protein expression might account for more aggressive ductal adenocarcinoma (Patil et al., 2018).

A multi-institutional study was conducted to test the prognostic value of ERG and SPINK1 proteins via immunohistochemistry. 39% ERG positive were related with lower Gleason scores, young age and low PSA levels, and better recurrence-free survival (RFS), in univariate analysis. There was no correlation of ERG with disease-specific survival (DSS), recurrence-free survival (RFS), and overall survival (OS) on multivariate analysis. In both analyses, 3% of SPINK1 protein expression was associated with improved RFS. Over-expression of both ERG and SPINK1 was not associated with clinical outcome. Both expressions were inversely correlated but not mutually exclusive. None of these expressions seems to be helpful prognostic biomarkers for initial-stage prostate tumor (Brooks et al., 2015).

In primary Prostate carcinoma SPINK1 was expressed in 25% and ERG were expressed in 42.7% of cases and in 91.7% PTEN loss was observed via immunohistochemistry. Primary Prostate tumor showed SPINK1+/ERG+, SPINK1+/ERG, SPINK1-/ERG+ and SPINK1-/ERG-

phenotype. SPINK1+/ERG+ phenotype was found to be related to higher Gleason grade, more aggressive cancers and lymph node metastases (Huang et al., 2016).

Prognostic role of SPINK1 in prostate cancer is controversial. Zhang and colleagues performed meta-analysis to evaluate the association between SPINK1 and clinical outcomes in prostate cancer and concluded that SPINK1 was not a predictor of PCa-specific mortality or overall survival (OS) among patients who underwent radical prostatectomy but in metastatic prostate cancer, SPINK1 was significantly associated with PCa-free survival and OS (Zhang et al., 2017). Another study observed that knockdown of SPINK1 might reduce cell invasion but not affect its proliferation (Ateeq et al. 2011). This finding might explain why SPINK1 is associated with adverse prognosis in aggressive prostate tumors.

Some studies found significant associations between SPINK1 and clinical outcomes, while others demonstrated insignificant or even contrary correlations. Terry et al. enrolled 279 patients who underwent radical prostatectomy at Henri Mondor Hospital, analyzed ERG, TFF3 and SPINK1 expression by immunohistochemistry. Significant correlation was observed among ERG, TFF3, and SPINK1 expression with patient age, prostate-specific antigen (PSA), Gleason score, tumor stage, and biochemical recurrence. This study categorized ERG and TFF3 as two different subsets of prostate carcinoma, SPINK1 was expressed more in aggressive tumors (Terry et al., 2015).

Many studies have been conducted on metastatic diseases and suggests a vital role of SPINK1 in chemo resistance. There is an inadequate evidence for SPINK1 as an independent marker so combination with other prognostic factors is required.

Benign prostatic hyperplasia (BPH) is a commonly diagnosed disease in men older than 50 years of age. It refers to non-malignant growth of the prostate and is defined as prostate gland enlargement secondary to hyper-proliferation of stromal and granular cells with predominance of mesenchymal cells. Chokkalingam et al. (2015) reported that patients with BPH are 10% more likely to develop prostate cancer after 5-years of follow-up. The etiology and pathogenesis of BPH is still not completely understood. Many factors play a role in the development of BPH, including

inflammatory genes, inflammatory mediators, hormones, dietary factors, oxidative stress, positive family history and genetic polymorphisms (Winchester et al. 2015).

Winchester et al. investigated the association between single nucleotide polymorphisms (SNPs) in the promoter of Serine Protease Inhibitor Kazal Type 1 (SPINK1) and the increased risk of BPH and prostate cancer by applying multiple logistic regression models. An inverse association was found between SNP rs10035432 and BPH but no association was found between these SNPs and prostate cancer. However, association between SNP rs1432982 and lower-grade prostate cancer was seen. Hence SPINK1 promoter variants are likely to be associated with the risk of BPH (Winchester et al. 2015).

Many studies have found a correlation among ALDH2 and age-related disorders, such as Benign prostate hyperplasia (BPH). Association of a Missense ALDH2 Single Nucleotide Polymorphism with Benign Prostate Hyperplasia was found in a Korean Population (Seok et al., 2013). El Ezzi and colleagues conducted a study in Lebanese males to interpret BPH-single nucleotide polymorphisms (SNPs) correlation in the VDR gene. They found the correlation of VDR with the increased risk of BPH development and suggested it as a good diagnostic marker of BPH. (El Ezzi et al., 2014). Yoo et al. found a strong correlation of Nitric oxide synthase 2 SNP (rs10459953) with BPH in Korean men (Yoo et al., 2010).

TMPRSS2-ERG has also been detected in prostate intraepithelial neoplasia (PIN) lesions and rarely in benign prostatic hyperplasia (BPH). A study was carried out to see the possibility of TMPRSS2-ERG fusion in BPH samples in the absence of apparent prostate cancer. Out of 115 BPH samples, three were found positive on RT-PCR, the presence of the fusion gene was confirmed by FISH. These findings indicates that TMPRSS2-ERG fusion may or may not lead to prostate cancer development (Velaeti et al. 2014). Whereas expression of TMPRSS2-ERG was determined by real time PCR showed no expression in BPH and PIN but was detected in 40% of prostate cancer cases. Thus, suggesting the expression of TMPRSS2-ERG cannot be used for differential diagnosis of BPH and low and high grade PIN but is diagnostic for prostate cancer (Mikhaylenko et al. 2015). Similarly, Ibrahim et al. (2019) reported that expression of ERG was

restricted to malignant tissue (Prostatic carcinoma) and was negative in BPH and PIN specimens. ERG is highly specific but less sensitive marker (Ibrahim et al. 2019).

A study was conducted to quantify the expression level TMPRSS2-ERG in benign prostatic hyperplasia (BPH) and prostate carcinoma tissue samples. Out of 48 BPH and 48 prostate cancer cases, TMPRSS2:ERG gene fusion was found in 8.3% of the BPH and 50% of the prostate cancer samples (Robert et al. 2013).

Väänänen and colleagues conducted a study to identify mRNA expression of PCA3, TMPRSS2-ERG fusion gene and SPINK1 *via* RT-PCT on radical prostatectomy (RP) samples to observe that these biomarkers are specific to cancerous lesions alone and found all these expressions more frequently in cancerous specimens than in BPH tissues. They suggested that identification of this fusion gene can be used for histological interpretation and can potentially diagnose future susceptibility of cancer in males with negative biopsies (Väänänen et al., 2014).

The presence of TMPRSS2-ERG fusions has been widely considered to be a cancer-specific event, but TMPRSS2-ERG III and/or VI mRNAs are also detected in 51% of HBP (Histologically benign prostate) tissues of RP specimens with clinically localized prostate cancer. TMPRSS2-ERG positivity in benign-appearing prostate tissue has been reported (Watson et al., 2009). In previous studies, it has mainly been considered an anomaly. However, a conceivable explanation could be a carcinogenic field effect (Nurmi et al., 2000), which suggests that area adjacent to the tumor focus is also changed in neoplastic events because of a carcinogenic signal. This type of area can appear histologically normal but molecular changes might still be detectable (Mazzucchelli et al., 2011; Nonn et al., 2009).

In Pakistan, very scarce data is available regarding expression of ERG and SPINK1 in prostate cancer and/or association with any clinicopathological parameter. There is a need to conduct more research for investigating the role of above mentioned markers in prostate cancer. Clinical trials and ongoing researches have been, and are, currently being used for investigating SPINK1 in different tumors especially in prostate cancers. This is especially vital for selection and initiation of different treatment modalities, such as novel targeted therapies for cure of prostate

cancer. It is hoped that by the usage of targeted therapies, prostate cancer can be stopped or at least its progression can be halted. Hence detection of SPINK1 and ERG is extremely imperative.

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN:

This cross-sectional study was conducted in Bahria University Medical and Dental College (BUMDC). Initially, the ethical approval was obtained from Ethical Review Committee (ERC) of BUMDC.

3.2 SUBJECTS:

Paraffin embedded prostate carcinoma and benign prostatic hyperplasia tissue blocks (cases taken from 2018- 2020).

3.3 SETTING

Department of Histopathology, PNS Shifa Hospital, Karachi, Pakistan.

3.4 INCLUSION CRITERIA

(a) All prostate carcinoma specimens prior to therapy.
(b) Benign prostatic hyperplasia cases for comparison.
(c) Patient of any age group.

3.5 EXCLUSION CRITERIA

(a) Metastatic tumors.
(b) Poorly fixed tissues.
(c) Specimens received after chemo/radio therapy.

3.6 DURATION OF STUDY:

(a) Individual study period: 2-3 days
(b) Total period of study: 1 year

3.7 SAMPLE SIZE CALCULATION:

Sample size of 40 was calculated by using following formula

$$\text{Sample size } n = \frac{[DEFF * Np\,(1-p)]}{[(d^2/Z^2_{1-\alpha 2} * (N-1) + p*(1-p)]}$$

Calculated by OpenEpi, Version 3.

95% confidence interval and 5% confidence limit.

3.8 SAMPLING TECHNIQUE:

Non-probability convenient.

3.9 HUMAN SUBJECTS AND CONCENT

Paraffin embedded prostate carcinoma and benign prostatic hyperplasia tissue blocks.

3.10 MATERIALS USED

(a) Formalin fixed specimens.

(b) Paraffin embedded blocks.

(c) Surgical pathology/ clinical records.

(d) Hematoxlyin and Eosin stained slides of all cases.

(e) Poly-L-lysine coated slides for immunohistochemical markers.

(f) ERG (EP111) Rabbit monoclonal antibody. Product identification Z2280RL. Procured from Zeta corporation, USA.

(g) SPINK1 (abx302123) Rabbit polyclonal antibody. Procured from Abbexa, USA.

(h) HRP Detection system

(i) DAB chromogen

(j) Blocking agent

(k) Enhancer

3.11 PARAMETERS OF STUDY

(a) Expression of ERG and SPINK1, by immunohistochemistry, on selected paraffin fixed blocks of prostate cancer specimens and BPH specimens

(b) Clinical data/history reviewed for data regarding age, clinical presentation and grade of tumor.

(c) H&E slides of the diagnosed specimens revised by two histopathologists, for information regarding morphology, grade, lymphatic/vascular invasion, peri-neural invasion and necrosis

3.11.1 CLINICOPATHOLOGICAL PARAMETERS

a) Age
b) Type of biopsy
c) Clinical manifestation
d) Gleason score
e) Gleason grade group
f) Lymphovascular invasion
g) Perineural invasion
h) Intraductal carcinoma

3.12 PROTOCOL OF STUDY

(a) Relevant cases were selected along with the clinical records

(b) Sections were taken through rotary microtome and H&E stained

(c) H&E slides were reviewed by two histopathologists

(d) Immunohistochemical staining was performed on formalin fixed paraffin embedded (FFPE) tissue slides according to the company's specified protocol

(e) All slides were studied under Nikon light microscope (YS100) using the scanner lens (4x10), low power lens (10x10) and high power (40x10) lenses, then reviewed by the supervisor

(f) Various parameters were recorded as mentioned in proforma

(g) Results were statistically analyzed

3.12.1 Staining

3.12.1.1 Haemotoxylin and Eosin (H&E) staining

Sections were passed through various solutions as follows:

a) Xylene I- 10 minutes
b) Xylene II- 10 minutes
c) Absolute alcohol- 10 minutes
d) 95% alcohol- 5 minutes
e) 80% alcohol- 5 minutes
f) 70% alcohol- 5 minutes
g) Tap water rinse- 2 minutes
h) Harris hematoxylin- 5-10 minutes
i) Acid alcohol 1%, 3-5 dips then washed with tap water
j) Ammonia water, 3-5 dips then rinse with tap water for 10 minutes
k) Eosin- 2minutes
l) 70% alcohol- 5 quick dips
m) 80% alcohol- 5 quick dips
n) 95% alcohol- 5 quick dips
o) Absolute alcohol – 2 changes- 5 minutes each
p) Xylene- 5 minutes

q) Mounted in Dako toluene free mounting media

Results:

The nucleus appeared blue in color.

The cytoplasm was stained with different shades of pink.

3.12.1.2 Immunohistochemical staining

Primary Antibodies

(1) ERG (EP111) Rabbit monoclonal antibody

Product identification : Z2280RL

Purchased from: Zeta corporation, USA

Localization: Nucleus

Positive Control tissue: Endothelium

Dilution: 1:100

(2) SPINK1 (abx302123) Rabbit polyclonal antibody

Product identification: abx302123

Purchased from: Abbexa, USA

Localization: Cytoplasm

Positive Control tissue: Pancreas

Dilution: 1:100

Procedure

- Sections were taken from Formalin-Fixed Paraffin-Embedded (FFPE) prostate cancer block of 3-5 micrometer thickness and were picked on poly L-lysine coated slides
- Slides were fixed in oven at 80℃ for 25-30 minutes

Antigen Retrieval

- Deparaffinization, hydration and antigen retrieval was done automatically through Dako PT link pretreatment system in 40-45 minutes. Temperature started from 65℃ when slides were put in, raised to 98℃ for 45 minutes, then cooled back to 65℃ and then slides were taken out
- Slides were than washed with washing buffer 2 times for 5 minutes each

Blocking

- Endogenous peroxidase was blocked by hydrogen peroxide blocking solution
- On tissue sections one to two drops of blocking solution was applied to cover the section
- Slides were incubated for 10- 15 min in humidity chamber at room temperature
- Slides were washed with washing buffer 2 times for 5 minutes each

Primary Antibody

- Both ERG and SPINK1 were diluted in the ration 1:100 as specified in the company's protocol. Dilution was done by antibody diluent
- Primary antibody was applied to cover the section
- Sections were incubated for 35-45 minutes in humidity chamber at room temperature
- Slides were then washed with buffer 2 times for 5 minutes each
- 50-100 microliter enhancer was applied for 10-15 minutes
- Slides were washed with washing buffer 2 times for 5 minutes each

Secondary HRP Antibody

- Sufficient Horseradish Peroxidase (HRP) was applied to the specimen
- Slides were incubated for at least 35 - 40 minutes at 37 °C in humidified chamber
- Slides were then rinsed gently with washing buffer 2 times for 5 minutes each

DAB Substrate Chromogen

- Diaminobenzidine (DAB) substrate chromogen was prepared with 1 ml of DAB substrate and 1 drop of DAB chromogen
- Slides were wiped
- Sufficient DAB substrate chromogen solution was applied to cover the section
- Slides were incubated for 1-2 minutes at room temperature
- Sections were washed with distilled water

Haemotoxylin Counterstaining

- Slides were counterstained with heamotoxylin stain, 1-3 dips

- Slides were rinsed gently with washing buffer
- Sections were dehydrated in ascending series of ethanol 60%, 80% and 100% and then cleared with xylene
- Slides were mounted in Dako toluene free mounting media
- Slides were then observed under microscope

3.12.1.2 Interpretation

For ERG, the staining procedure which was observed was ascertained to be either no staining, weakly positive, moderately positive or strongly positive in tumour cells. Nuclear staining was taken into account.

No staining = 0

Weakly positive = +1 (only evident at high magnification ×10)

Moderately positive = +2 (evident at low magnification ×4)

Strongly positive = +3 (striking at low magnification)

For SPINK1, staining was observed to be either no staining, less than 50% of cells staining in scattered individual cells, less than 50% of cells staining in complete glands, 50-80% of cells staining or more than 80% of cells staining. Cytoplasmic staining was recorded.

No staining = 0

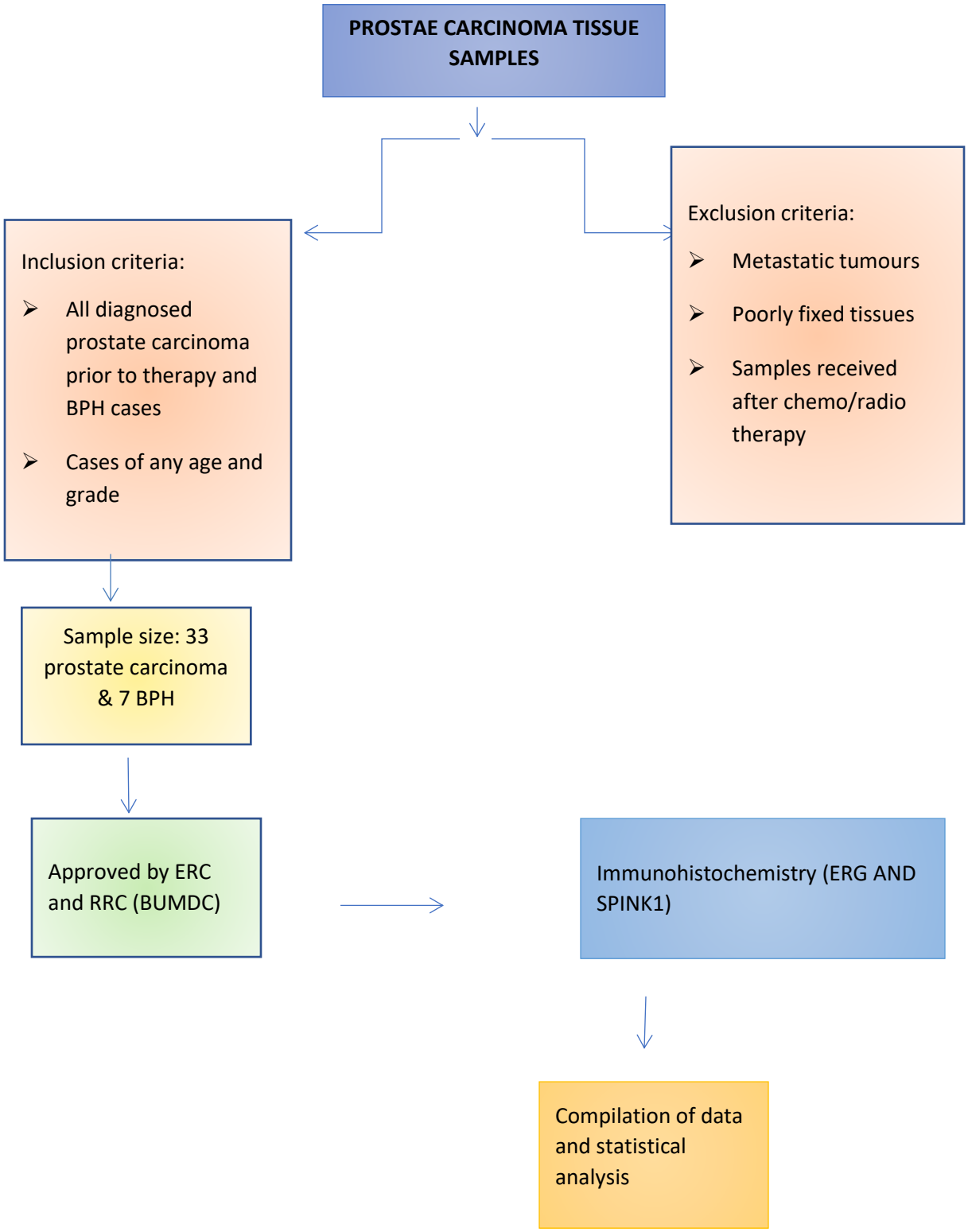
< 50% of cells staining in scattered individual cells = 1

< 50% of cells staining in complete glands = 2

50-80% of cells staining = 3

> 80% of cells staining = 4

3.13 FLOW CHART/ ALGORITHM OF STUDY



3.14 STATISTICAL ANALYSIS:

The analysis was done using IBM SPSS version 23. Continuous variables were shown as mean and standard deviation. Categorical data was recorded as frequencies and percentages. Appropriate test statistic such as Chi square test or Fisher exact test were used to see the relation between ERG and SPINK1 with different clinic- pathological features. p value < 0.05 was considered to be statistically significant.

CHAPTER 4

RESULTS

Over the period in which samples were received i.e. from 2018-2020, 33 cases of prostate carcinomas and 7 cases of BPH were extracted. Two categories of biopsy were recorded in this study; transurethral resection of prostate (TURP) and transrectal ultrasonography (TRUS). Majority of the prostate cancer specimens 28 out of 33 were obtained form TURP (84.8%), while 5 out of 33 (15.2%) prostate cancer specimens were obtained by TRUS.

Table 1 depicts the age-wise distribution of prostate carcinoma patients. The mean age of patients was 72 ± 7.4 years. The youngest patient was 58 years old while the oldest was 88 years old. The maximum number of carcinoma patients (14 out of 33) were observed in 61-70 age-group (42.4%). After that, 71-80 age group was slightly below than previous (n:12, 36.4%). Whereas, group 81-90 (n:4, 12.1%) and 51-60 (n:3, 9.1%) had least number of patients. BPH also followed the same age wise distribution pattern as prostate cancer.

Table 2 shows the clinical manifestations in prostate carcinoma patients. Among all 33 cases of prostate carcinoma, nocturia & dysuria (n:9; 27.3%), prostate enlargement (n:8; %: 24.2), frequent urination (n:4; 12.1%) and urinary retention (n:7; 21.2%) were most prevalent. Whereas, difficulty in starting urine (n:3; 9.1%) and blood in urine (n:2; 6.1%) were found with lesser prevalence. Whereas in BPH cases, frequent urination (57.1%) and loss of bladder control (42.9%) were the main symptoms.

Table 3 allots Gleason score of prostate carcinoma. Most of the prostate cancer cases (11 out of 33) (33.3%) were found to be with Gleason score 9, followed by Gleason score 10 (18.2%), 7 (18.2%), 8 (15.2%) and 6 (15.2%).

Table 4 shows Gleason grade group of prostate carcinoma cases. Majority of the cases (17 out of 33) (51.5%) were found in grade group 5, followed by grade group 4 (15.2%), 1 (15.2%), 3 (12.1%) and 2 (6.1 %).

Table 5 depicts the lymphovascular invasion in prostate carcinoma patients. Out of 33 cases, the status of 27 could be determined. 12 (36.4%) were identified to have lymphovascular invasion and 15 (45.5 %) of cases donot have lymphovascular invasion. Status of 6 cases was unknown.

Table 6 shows the perineural invasion in prostate cancer patients. Out of 33 cases, 15 (45.5%) showed perineural invasion and 12 (36.4%) did not show perineural invasion . While, in rest of 6 cases the status was unknown.

Table 7 represents intraductal carcinoma in prostate cancer patients. Out of 33 cases, 19 (57.6 %) were negative for intraductal carcinoma. While in 14 cases the status was unknown.

Table 8 depicts ERG expression in prostate carcinoma cases. The results show no nuclear staining in 13 (39.4%) patients, weakly and moderately positive staining in 3 (9.1%) and 3 (9.1%) patients respectively, and strongly positive ERG expression in 14 (42.4%) patients. No staining was observed in BPH cases.

Table 9 shows SPINK1 expression in prostate carcinoma cases. The results show no cytoplasmic staining in 30 cases (90.9%), 50-80% of cells staining in 1 case (3.0%), and > 80% of cells staining of SPINK1 expression in 2 cases (6.1%). While none of BPH cases showed expression for SPINK1.

Table 10 correlates ERG expression with SPINK1 expression in prostate cancer specimens. Majority of the cases (36.7%) showed no expression for both ERG and SPINK1. 43.3% of the cases had strongly positive expression for ERG but negative for SPINK1. 10.0% cases had weak to moderate expression of ERG but did not express SPINK1. 2 cases were those who expressed SPINK1 but were negative for ERG, and only 1 case was observed to express both ERG and SPINK1. No statistically significant association was seen between ERG expression and SPINK1 expression (p-value: 0.91).

Table 11 correlates ERG and SPINK1 expression with clinicopathological parameters namely age group, type of biopsy, clinical presentation, Gleason score, Gleason grade group, lymphovascular invasion, perinural invasion and intraductal carcinoma. The most frequent age group was 61-70 years with 14 out of 33 cases, followed by 71-80 years with 12 out of 33 cases. The majority of ERG strong positivity was seen in these age groups with 7 cases and 6 cases respectively. No statistically significant association was found between age group and ERG expression (p-value: 0.50). Regarding SPINK1 expression majority of the cases showed SPINK1 expression in 71-80 years age group. No statistically significant association was found between age group and SPINK1 expression (p-value: 0.44).

The most common type of biopsy was TURP with frequency of 28 cases (84.8%), out of these, the majority of cases (13) showed strong positivity for ERG expression. This was followed by those cases which were negative for ERG expression (11 cases). There was no statistically significant association found between ERG expression and type of biopsy (p-value: 0.52). SPINK1 expression was not found in majority of the TURP biopsy (25 cases) but 3 cases were found positive for its expression. No statistically significant association was found between SPINK1 expression and type of biopsy (p-value: 0.74).

The most common clinical presentation of prostate carcinoma patient was nocturia and dysuria with 27.3% frequency, followed by prostate enlargement (24.2%) and urinary retention (21.2%). Majority of strongly positive expression of ERG was also observed in these clinical manifestations. No statistically significant association was found between ERG expression and clinical presentation (p-value: 0.78). Majority of SPINK1 expression was also observed with

these clinical manifestations. No statistically significant association was found between SPINK1 and clinical presentation (p-value: 0.86).

Majority of the cases 11 out of 33 (33.3%) were found to be of Gleason score 9, out of these cases 6 cases showed strongly positive expression of ERG. No statistically significant association was found between ERG and Gleason score (p-value: 0.26). Expression of SPINK1 was seen in specimen with Gleason score 6,9 and 10. No statistically significant association was found between SPINK1 and Gleason score (p-value: 0.42).

Most of the cases 17 out of 33 (51.5%) were included in Gleason grade group 5, out of these 8 cases strongly expressed ERG, no statistically significant association was found between ERG and Gleason grade group (P-value: 0.13). SPINK1 was also expressed in >80% of cells with Gleason grade group 5 (p-value: 0.46).

Intraductal carcinoma showed no statistically significant association with ERG (p-value: 0.11) and SPINK1 (p-value: 0.67).

No statistically significant association was found between ERG expression and lymphovascular invasion and perinural invasion. Similarly, there was no statistically significant association between SPINK1 expression and these clinicopathological parameters.

Age Group	Frequency (n=33)	Percentage (%)
51-60	3	9.1
61-70	14	42.4
71-80	12	36.4
81-90	4	12.1
Total	33	100

Table 4.1: Age wise distribution of prostate carcinoma patients. Mean age:72 ± 7.4

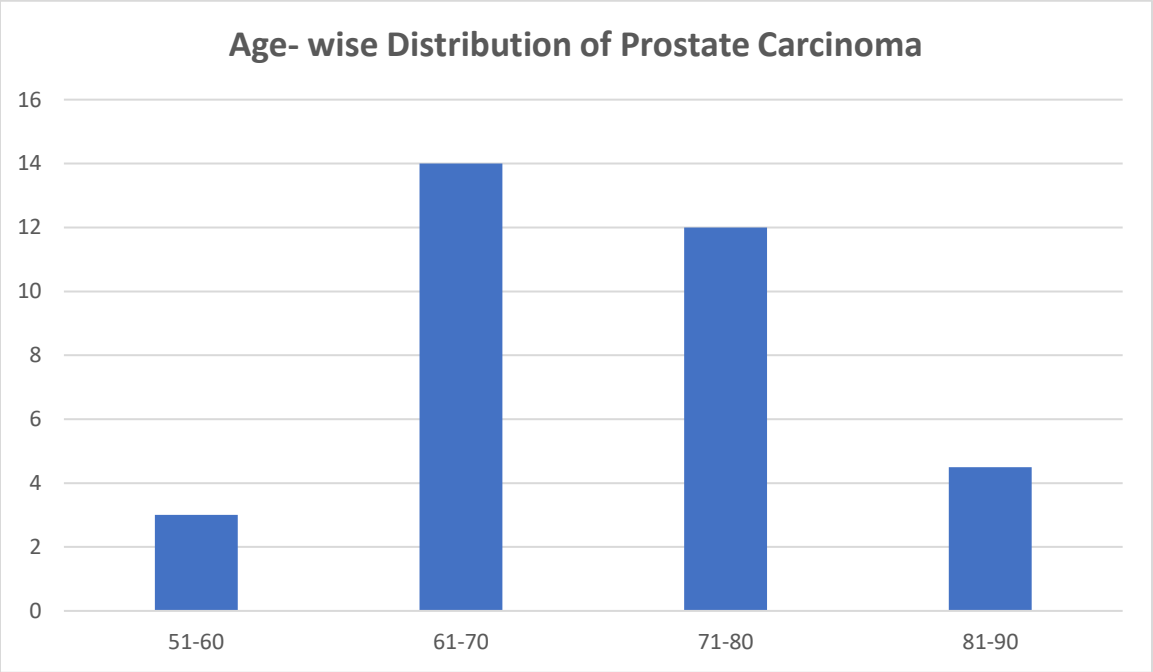


Figure 4.1: Age-wise distribution of prostate carcinoma.

Clinical manifestations	Frequency (n=33)	Percentage (%)
Urinary retention	7	21.2
Nocturia & dysuria	9	27.3
Frequent urination	4	12.1
Blood in urine	2	6.1
Prostatic enlargement	8	24.2
Difficulty in starting urine	3	9.1
Total	33	100

Table 4.2: Clinical manifestations in prostate cancer patients.

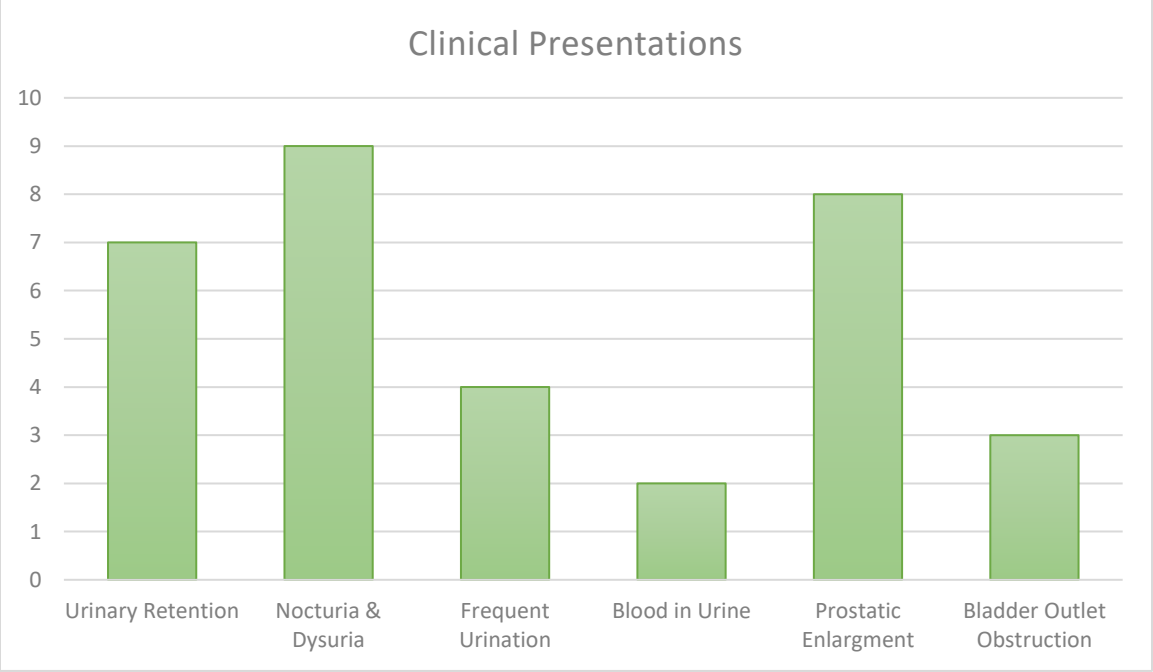


Figure 4.2: Clinical manifestations in affected patients.

Gleason score	Frequency (n=33)	Percentage (%)
6	5	15.2
7	6	18.2
8	5	15.2
9	11	33.3
10	6	18.2
Total	33	100

Table 4.3 : Gleason score of prostate carcinoma

Figure 4.3: Gleason score of prostate carcinoma

Gleason grade group	Frequency (n=33)	Percentage (%)
1	5	15.2
2	2	6.1
3	4	12.1
4	5	15.2
5	17	51.5
Total	33	100

Table 4.4: Gleason grade group of prostate carcinoma

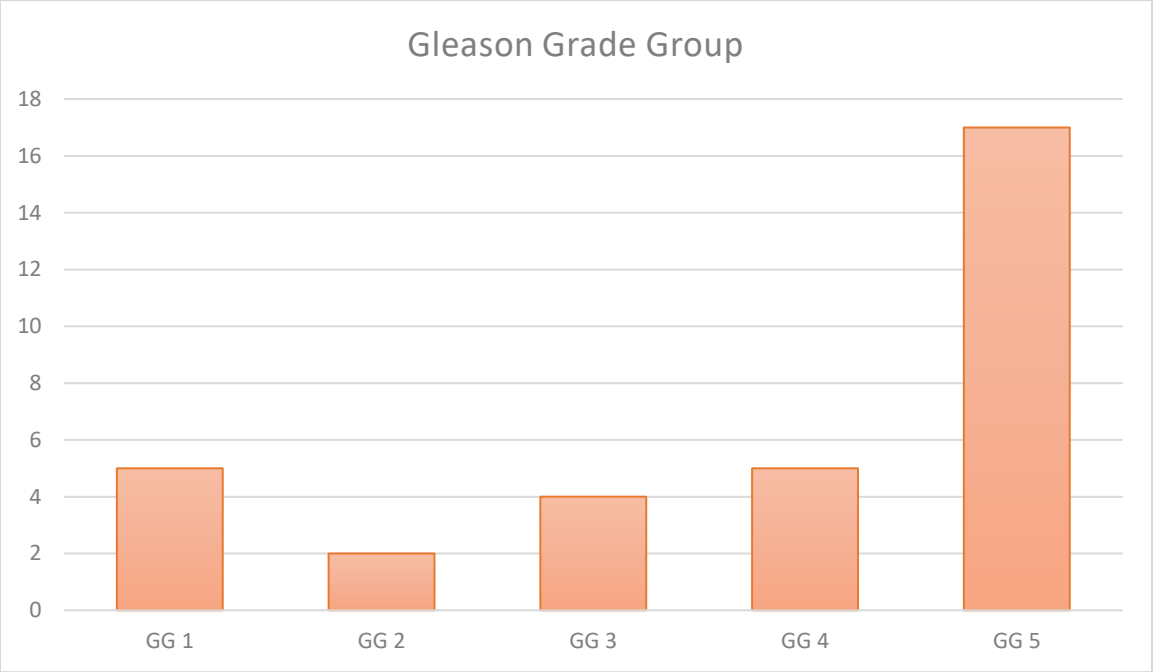


Figure 4.4: Gleason grade group of prostate carcinoma

Lymphovascular invasion	Frequency (n=33)	Percentage (%)
Identified	12	36.4
Not identified	15	45.5
Unknown	6	18.2
Total	33	100

Table 4.5: Lymphovascular invasion in prostate carcinoma cases.

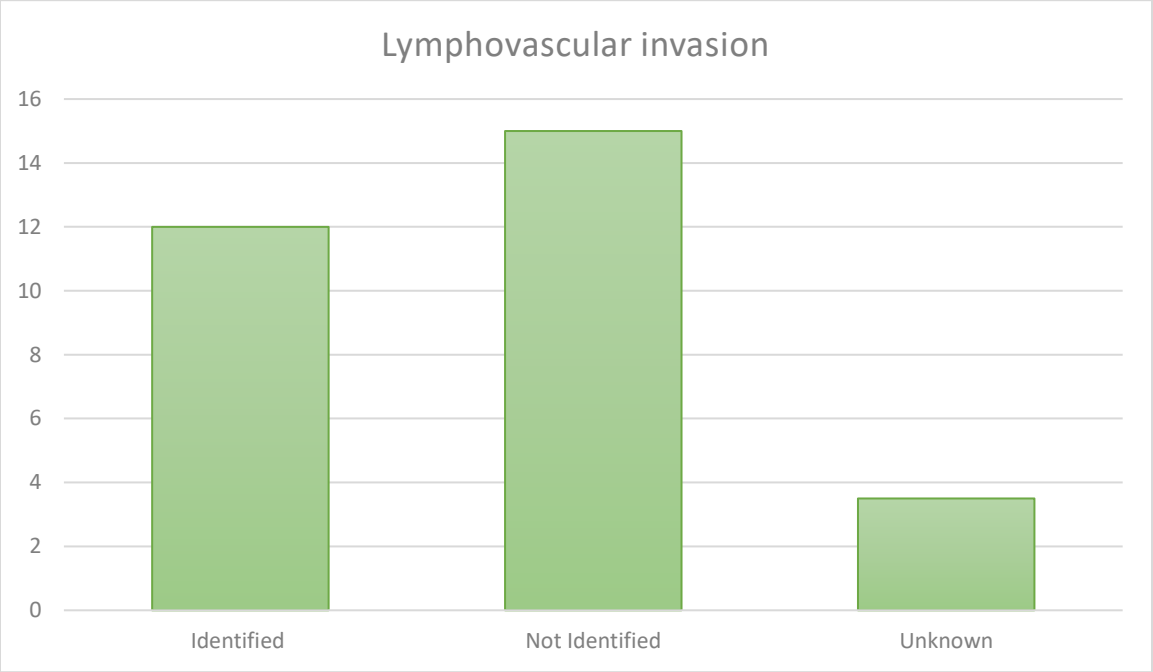


Figure 4.5: Lymphovascular invasion in cases.

Perineural invasion	Frequency (n=33)	Percentage (%)
Identified	15	45.5
Not identified	12	36.4
Unknown	6	18.2
Total	33	100

Table 6: Perineural invasion in prostate carcinoma cases.

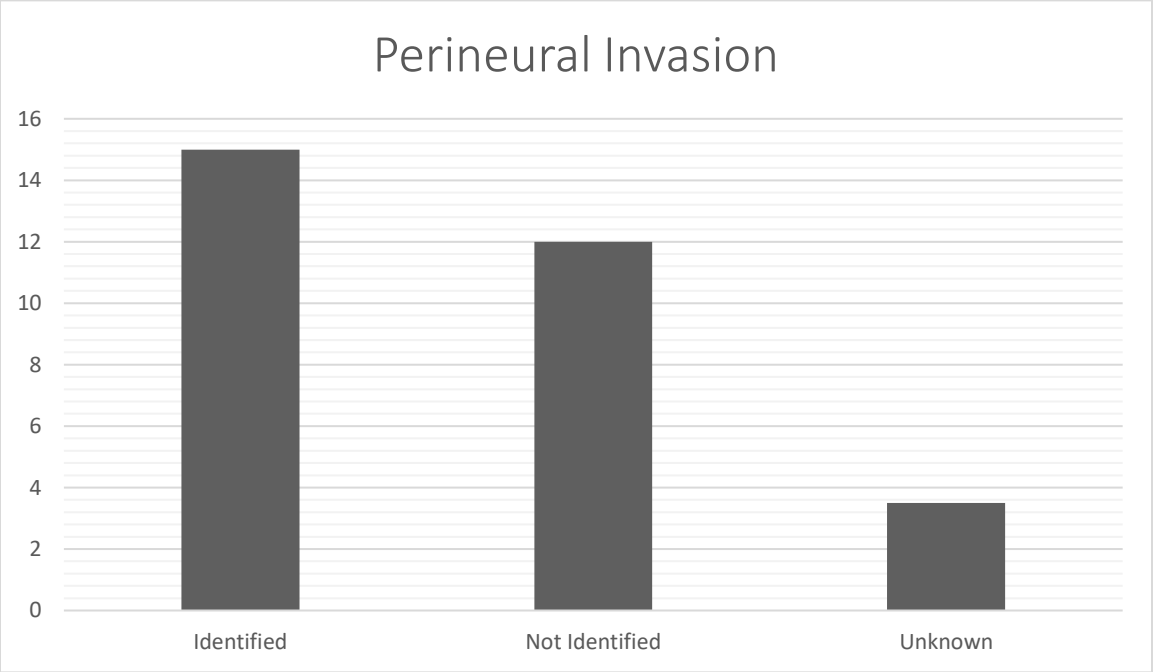


Figure 4.6: Perineural invasion in cases.

Intraductal carcinoma	Frequency (n=33)	Percentage (%)
Not identified	19	57.6
Unknown	14	42.4
Total	33	100

Table 7: Intraductal carcinoma in prostate cancer.

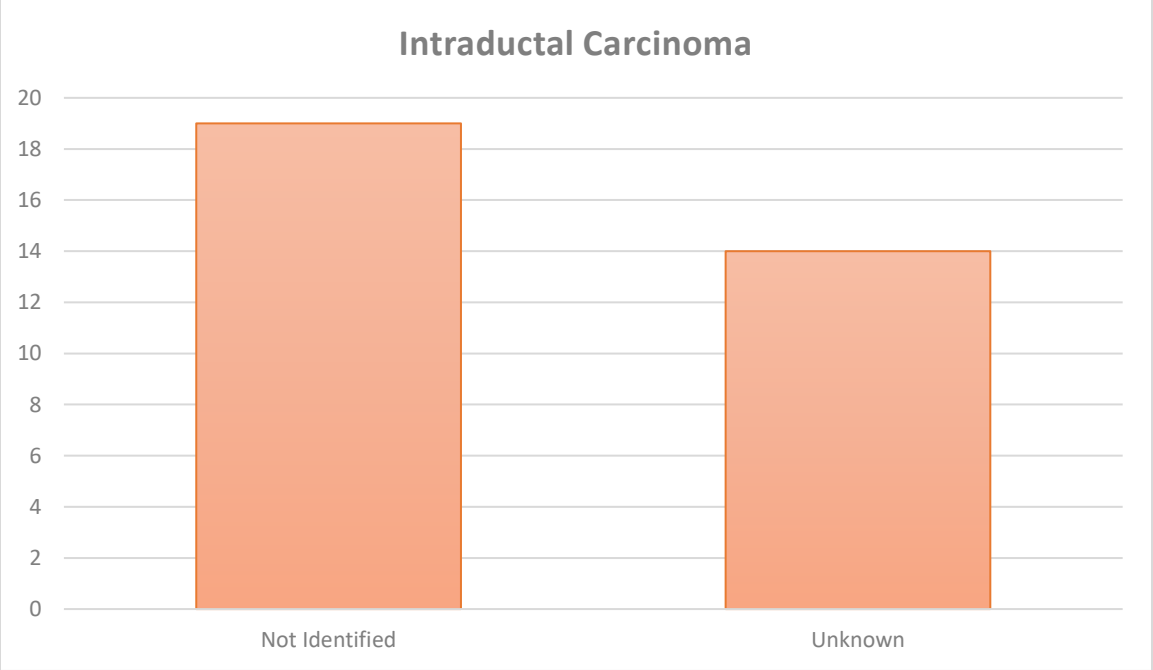


Figure 4.7: Intraductal carcinoma

ERG expression	Frequency (n=33)	Percentage (%)
No staining	13	39.4
Weakly positive	3	9.1
Moderately positive	3	9.1
Strongly positive	14	42.4
Total	33	100

Table 8: ERG expression in prostate carcinoma

(Nuclear staining) No staining = 0, Weakly positive = +1 (only evident at high magnification ×10), Moderately positive = +2 (evident at low magnification ×4), Strongly positive = +3 (striking at low magnification)

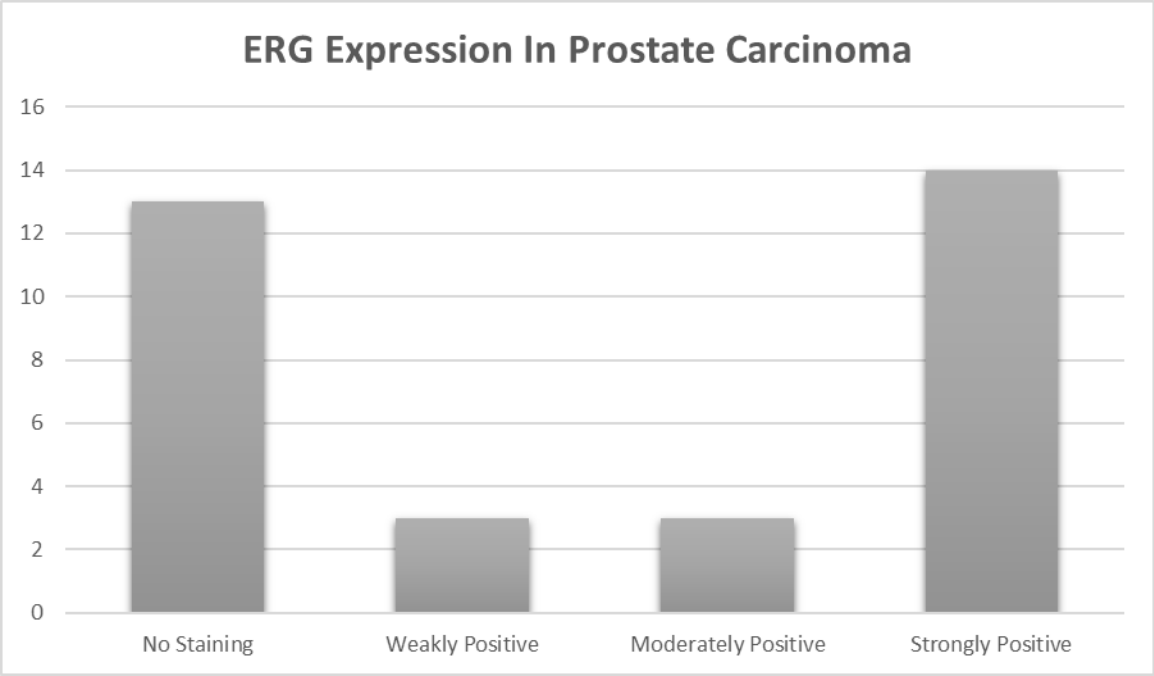


Figure 4.8: ERG expression in prostate carcinoma

SPINK1 expression	Frequency (n=33)	Percentage (%)
No staining	30	90.9
<50% staining in individual cells	0	0
<50% staining in complete glands	0	0
50-80% staining	1	3.0
>80% staining	2	6.1
Total	33	100

Table 9: SPINK1 expression in prostate carcinoma

(Cytoplasmic staining) No staining = 0, < 50% of cells staining in scattered individual cells = 1, < 50% of cells staining in complete glands = 2, 50-80% of cells staining = 3, > 80% of cells staining = 4

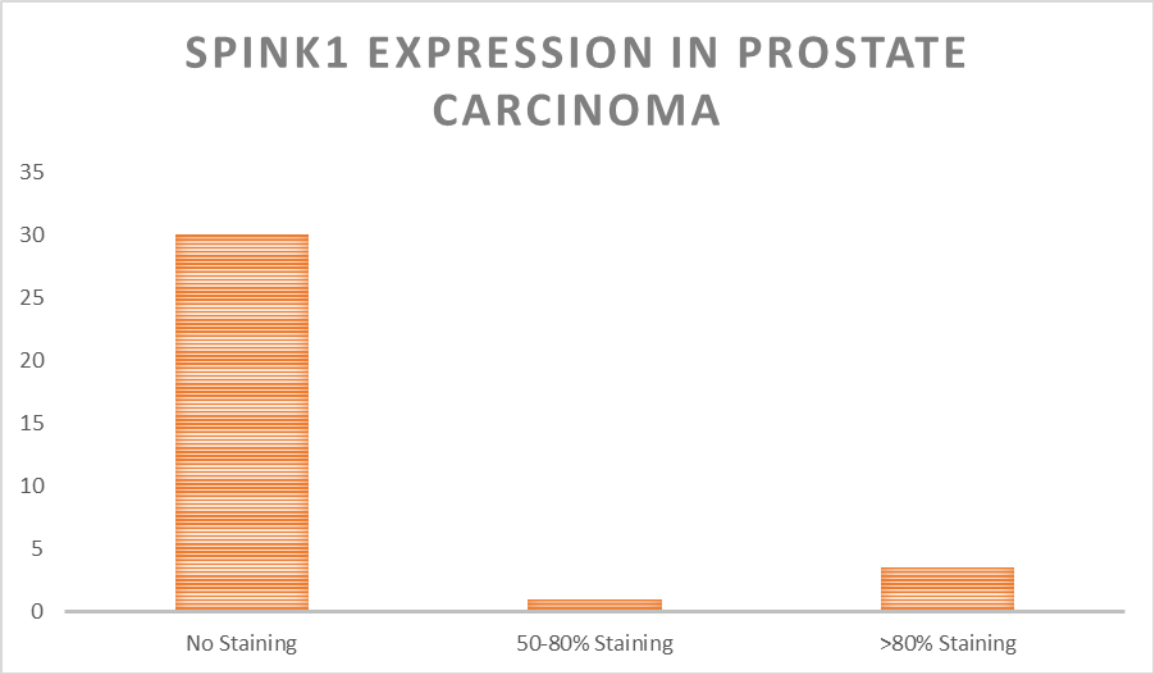


Figure 4.9: SPINK1 expression in prostate carcinoma.

ERG expression (n)	SPINK1 expression			Total	P-Value
	No staining (n)	50-80% staining (n)	>80% staining (n)		
No staining (13)	11	1	1	13	0.91
Weakly positive (3)	3	0	0	3	
Moderately positive (3)	3	0	0	3	
Strongly positive (14)	13	0	1	14	
	30	1	2	33	

Table 10: Co-relation between ERG and SPINK1 expression

Clinicopathological parameter	Frequency, n (percentage,%)	ERG expression 0 +1 +2 +3	p- value	SPINK1 expression 0 50-80% >80%	p-value
Age group	**n=33**	**13 3 3 14**		**30 1 2**	
• 51-60	3 (9.1)	2 0 1 0		3 0 0	
• 61-70	14 (42.4)	4 2 1 7	0.50	14 0 0	0.44
• 71-80	12 (36.4)	5 0 1 6		9 1 2	
• 81-90	4 (12.1)	2 1 0 1		4 0 0	
Type of biopsy	**n=33**	**13 3 3 14**		**30 1 2**	
• TURP	28 (84.8)	11 2 2 13	0.52	25 1 2	0.74
• TRUS	5(15.2)	2 1 1 1		5 0 0	
Clinical presentation	**n=33**	**13 3 3 14**		**30 1 2**	
Urinary retention	7 (21.2)	3 1 0 3		7 0 0	
Nocturia & dysuria	9 (27.3)	5 1 1 2		8 0 1	
Frequent urination	4 (12.1)	2 1 0 1		4 0 0	
Blood in urine	2 (6.1)	1 0 0 1	0.78	2 0 0	0.86
Prostatic enlargement	8 (24.2)	2 0 1 5		6 1 1	
Difficulty in starting urine	3 (9.1)	0 0 1 2		3 0 0	
Gleason score	**n=33**	**13 3 3 14**		**30 1 2**	
• 6	5 (15.2)	5 0 0 0		4 1 0	
• 7	6 (18.2)	3 0 0 3		6 0 0	
• 8	5 (15.2)	1 0 1 3	0.26	5 0 0	0.42
• 9	11 (33.3)	2 2 1 6		10 0 1	
• 10	6 (18.2)	2 1 1 2		5 0 1	
Gleason grade group	**n=33**	**13 3 3 14**		**30 1 2**	
• 1	5 (15.2)	5 0 0 0		4 1 0	
• 2	2 (6.1)	0 0 0 2		2 0 0	
• 3	4 (12.1)	3 0 0 1	0.13	4 0 0	0.46
• 4	5 (15.2)	1 0 1 3		5 0 0	
• 5	17 (51.5)	4 3 2 8		15 0 2	
Lymphovascular invasion	**n=33**	**13 3 3 14**		**30 1 2**	
▪ Identified	12 (36.4)	2 2 2 6		11 0 1	
▪ Not identified	15 (45.5)	7 0 1 7		13 1 1	0.78
▪ Not mentioned	6 (18.2)	4 1 0 1	0.24	6 0 0	
Perineural invasion	**n=33**	**13 3 3 14**		**30 1 2**	
▪ Identified	15 (45.5)	4 2 1 8		12 1 2	
▪ Not identified	12 (36.4)	5 0 2 5	0.39	12 0 0	0.41
Not mentioned	6 (18.2)	4 1 0 1		6 0 0	
Intraductal carcinoma	**n=33**	**13 3 3 14**		**30 1 2**	
▪ Not identified	19 (57.6)	5 1 3 10	0.11	17 1 1	0.67
▪ Not mentioned	14 (42.4)	8 2 0 4		13 0 1	

Table 11: Co-relations of ERG and SPINK1 with various clinicopathological parameters

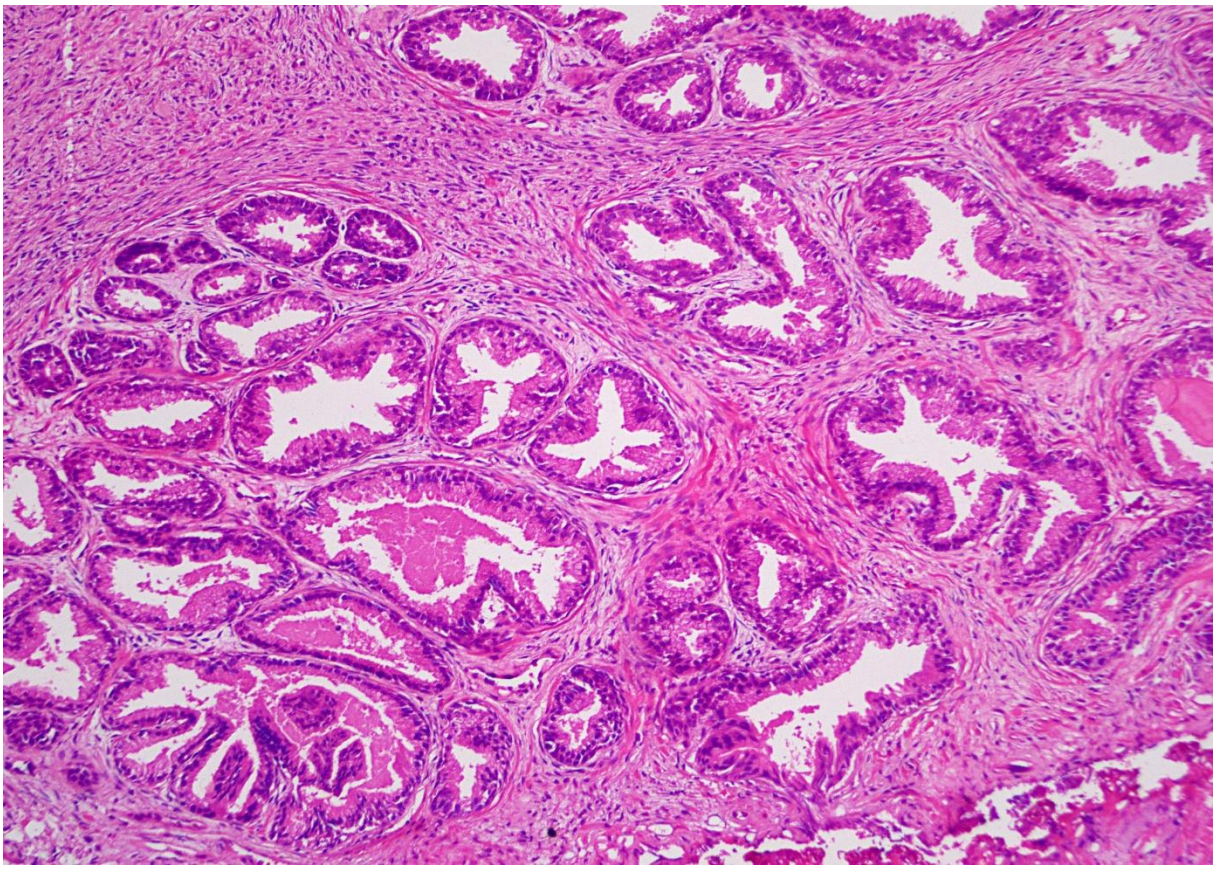


Figure 4.10: (Case no.# 1234) H&E slide of BPH (10×)

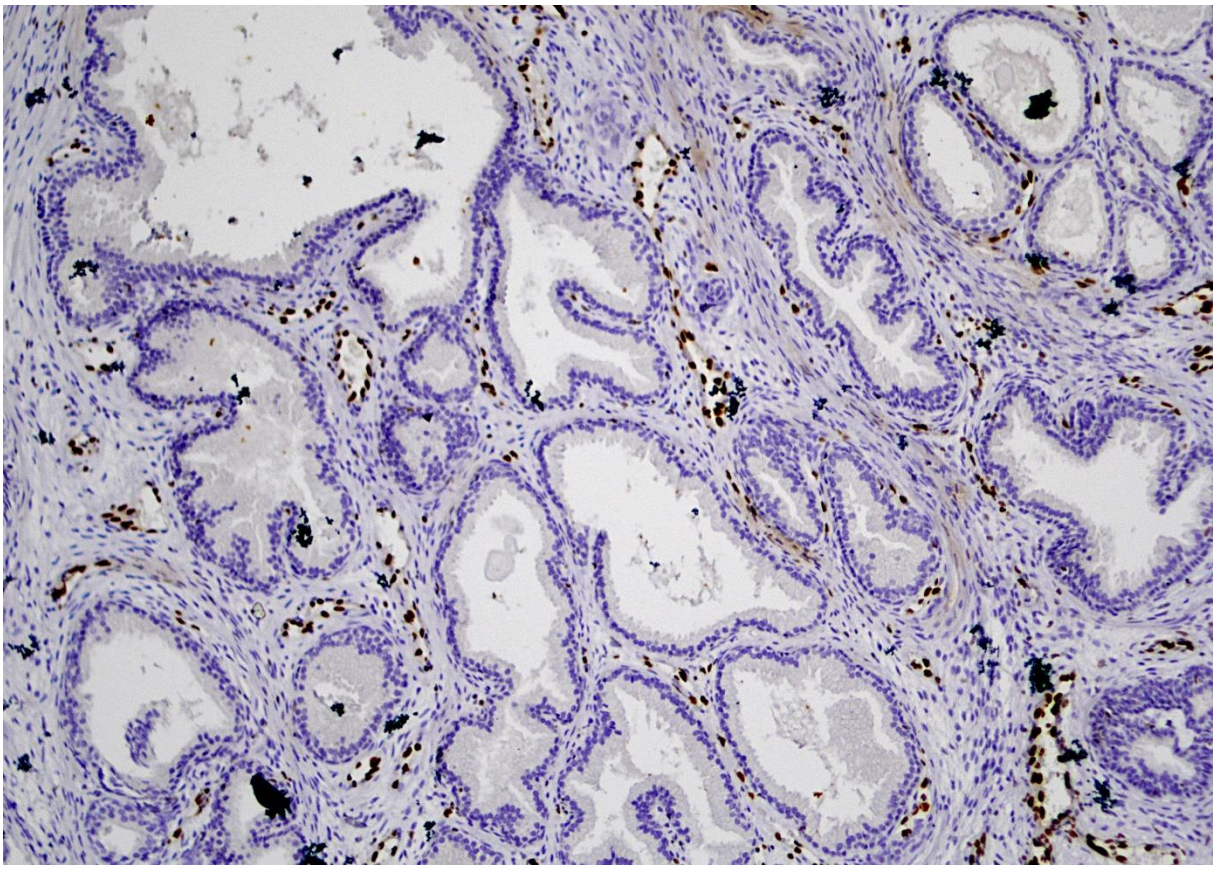


Figure 4.11: (Case no.#1234) BPH showing no ERG expression (10×)

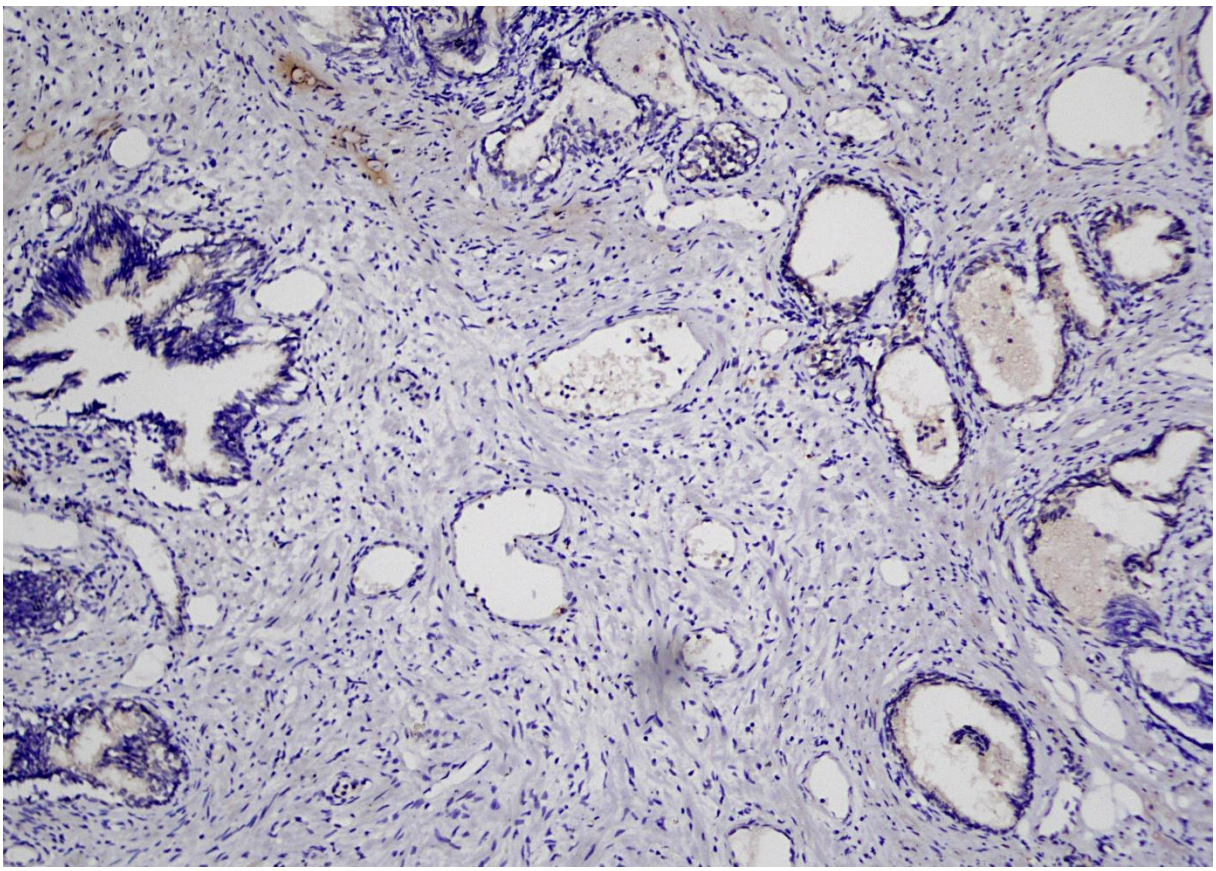


Figure 4.12: (Case no.# 1234) BPH showing no SPINK1 expression (10×)

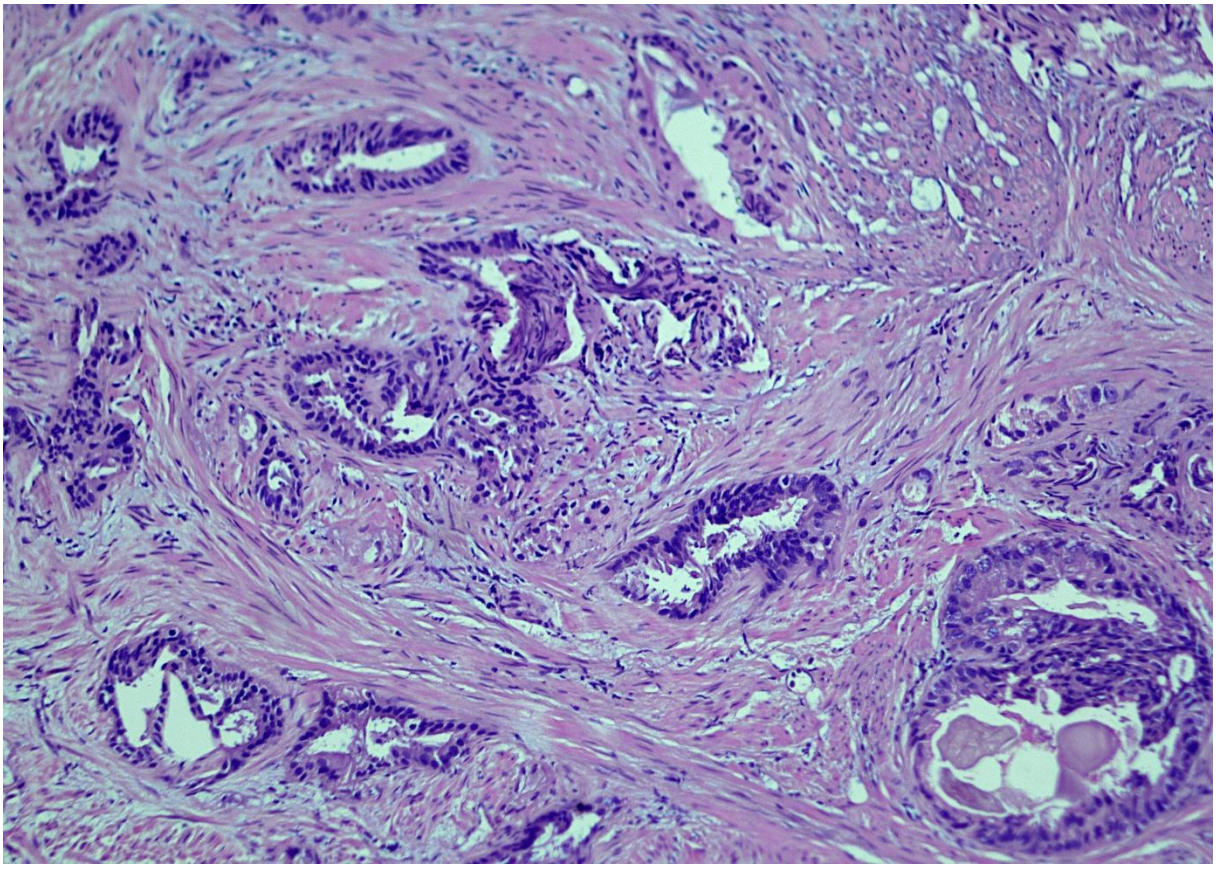


Figure 4.13: (Case no.# 2208) H&E slide of Gleason Grade Group 4 prostate carcinoma (10×)

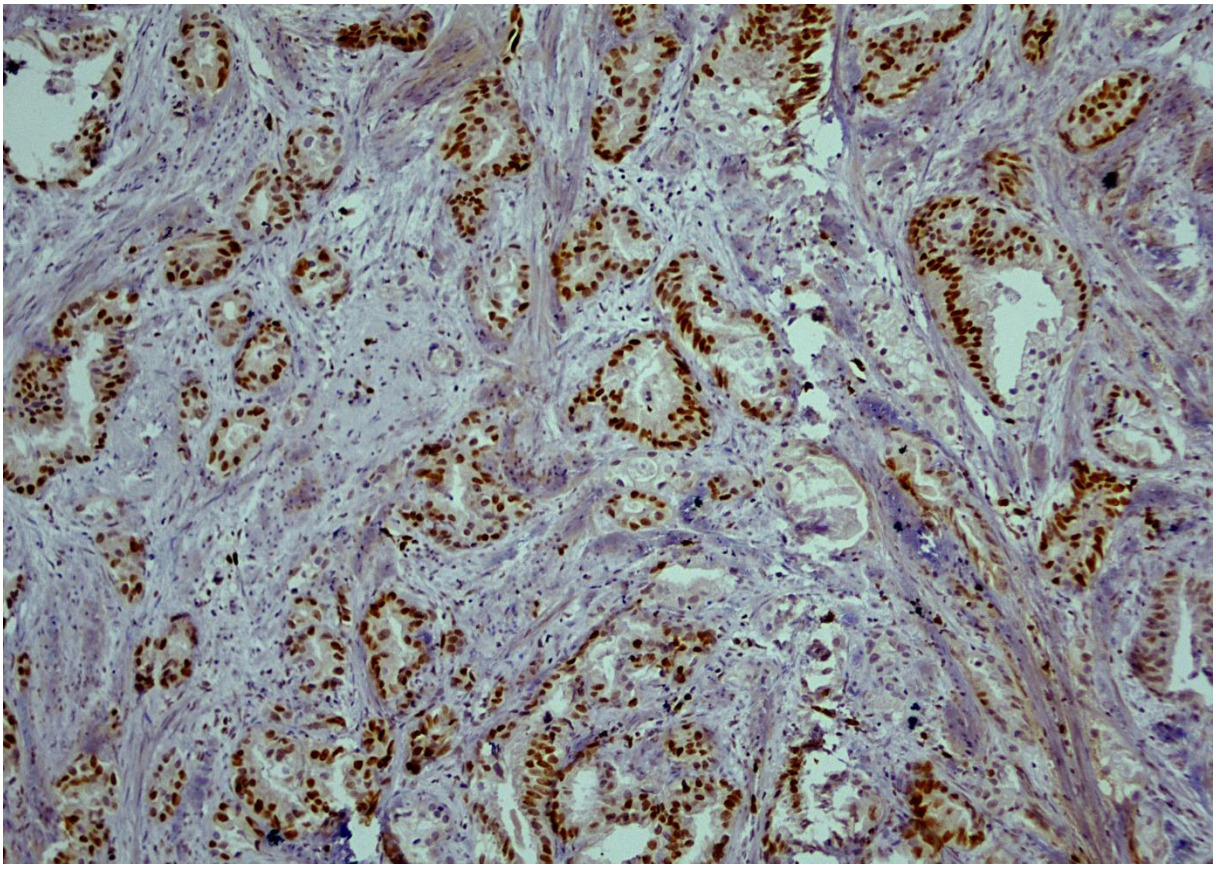


Figure 4.14: (Case no.# 2208) Gleason Grade Group 4 prostate carcinoma showing strong ERG expression (10×)

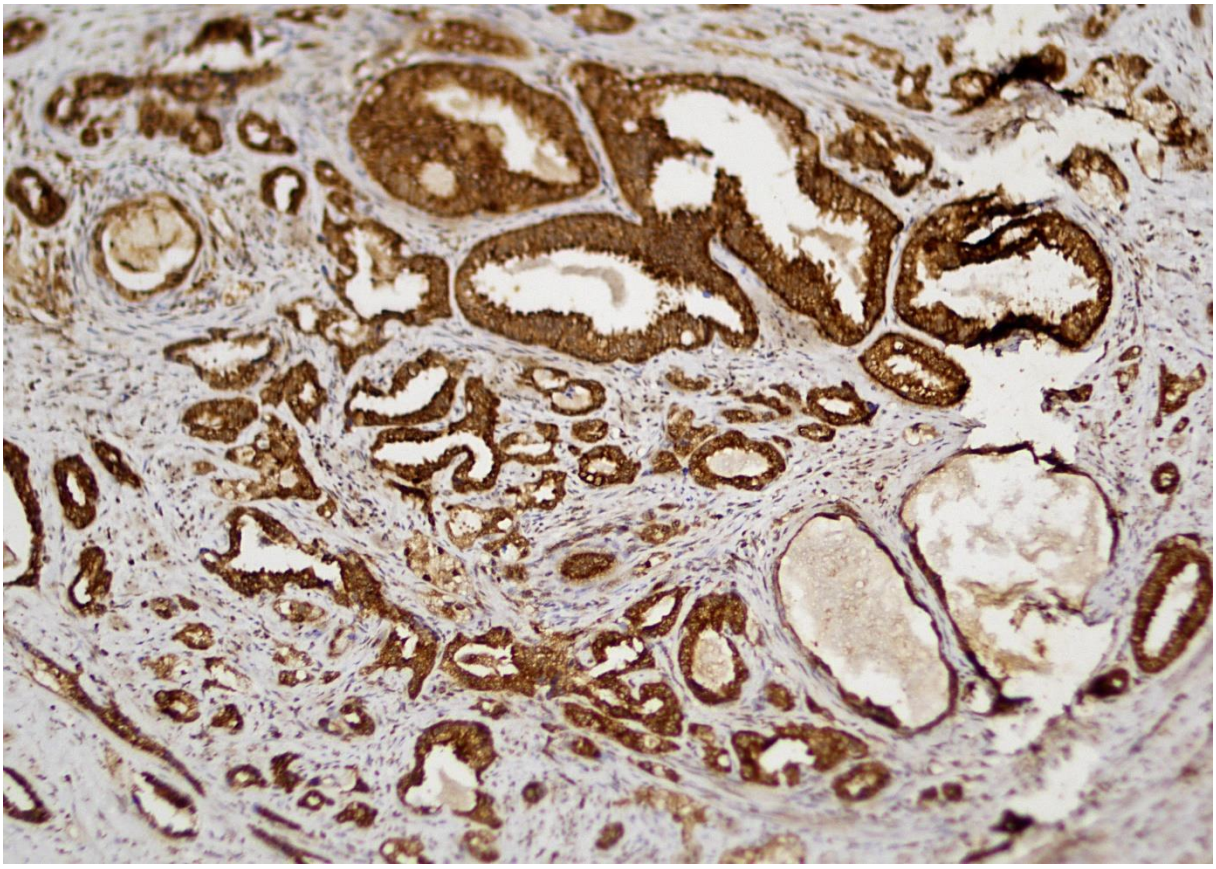


Figure 4.15: (Case no.# 2208) Gleason Grade Group 4 prostate carcinoma showing SPINK1 positivity (10×)

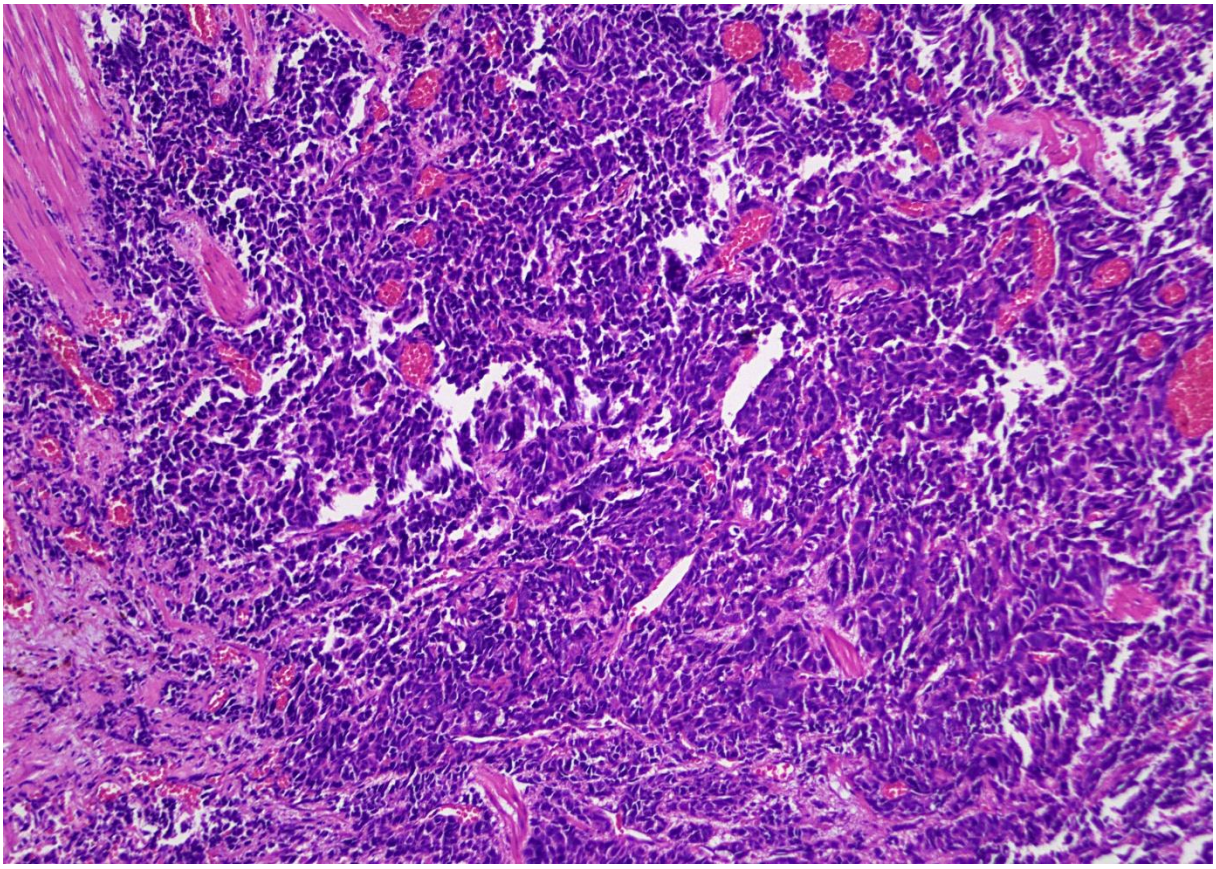


Figure 4.16: (Case no.# 2709) H&E slide of Gleason Grade Group 5 prostate carcinoma (10×)

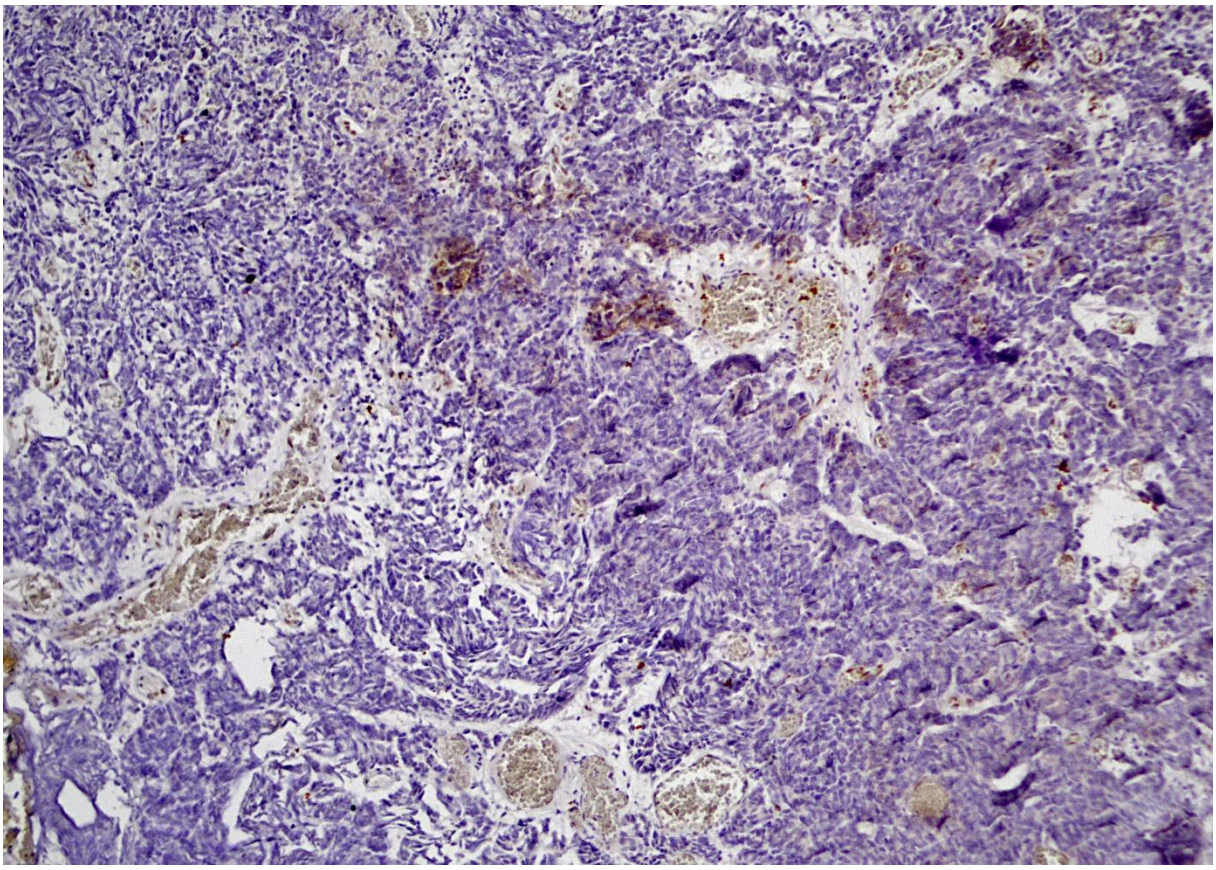


Figure 4.17: (Case no.#2709) Gleason Grade Group 5 prostate carcinoma showing negative ERG expression (10×)

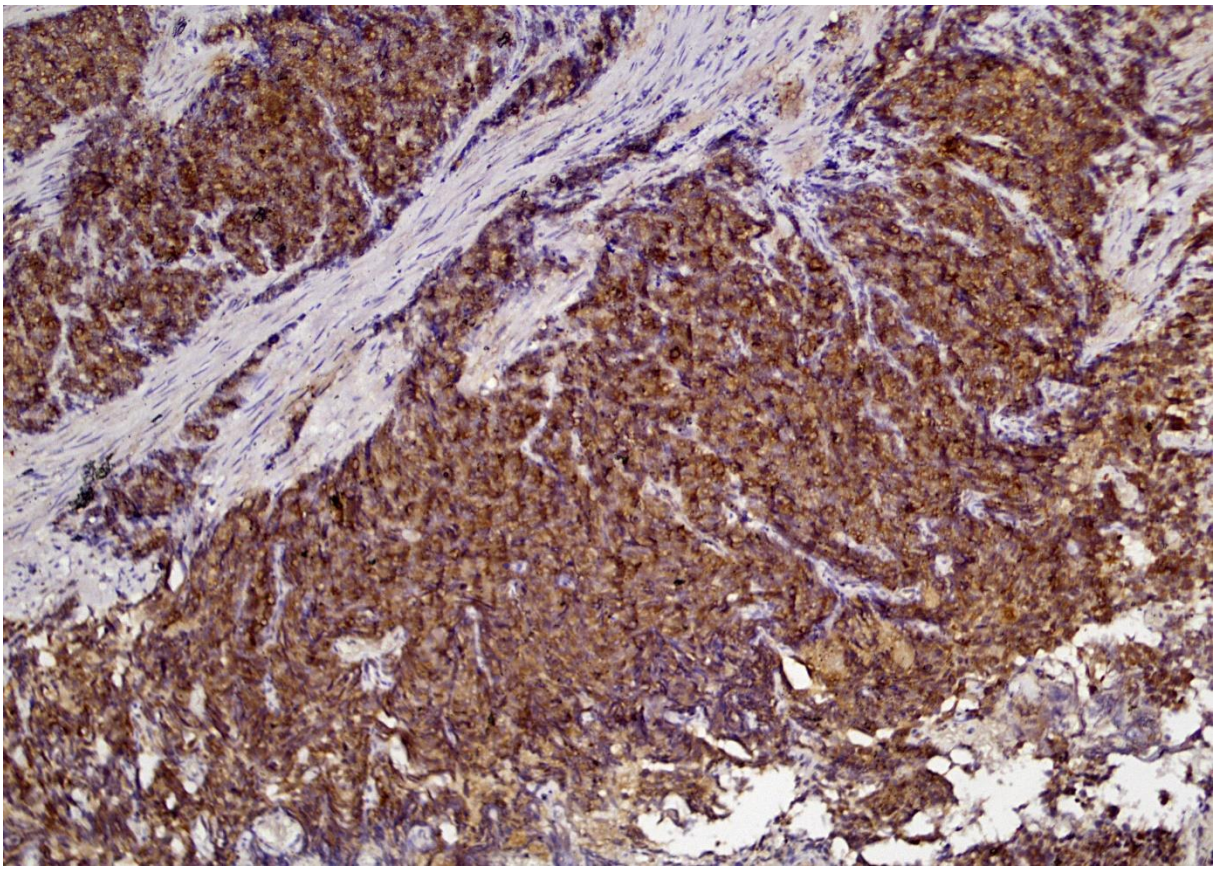


Figure 4.18: (Case no.#2709) Gleason Grade Group 5 prostate carcinoma showing strong SPINK1 expression (10×)

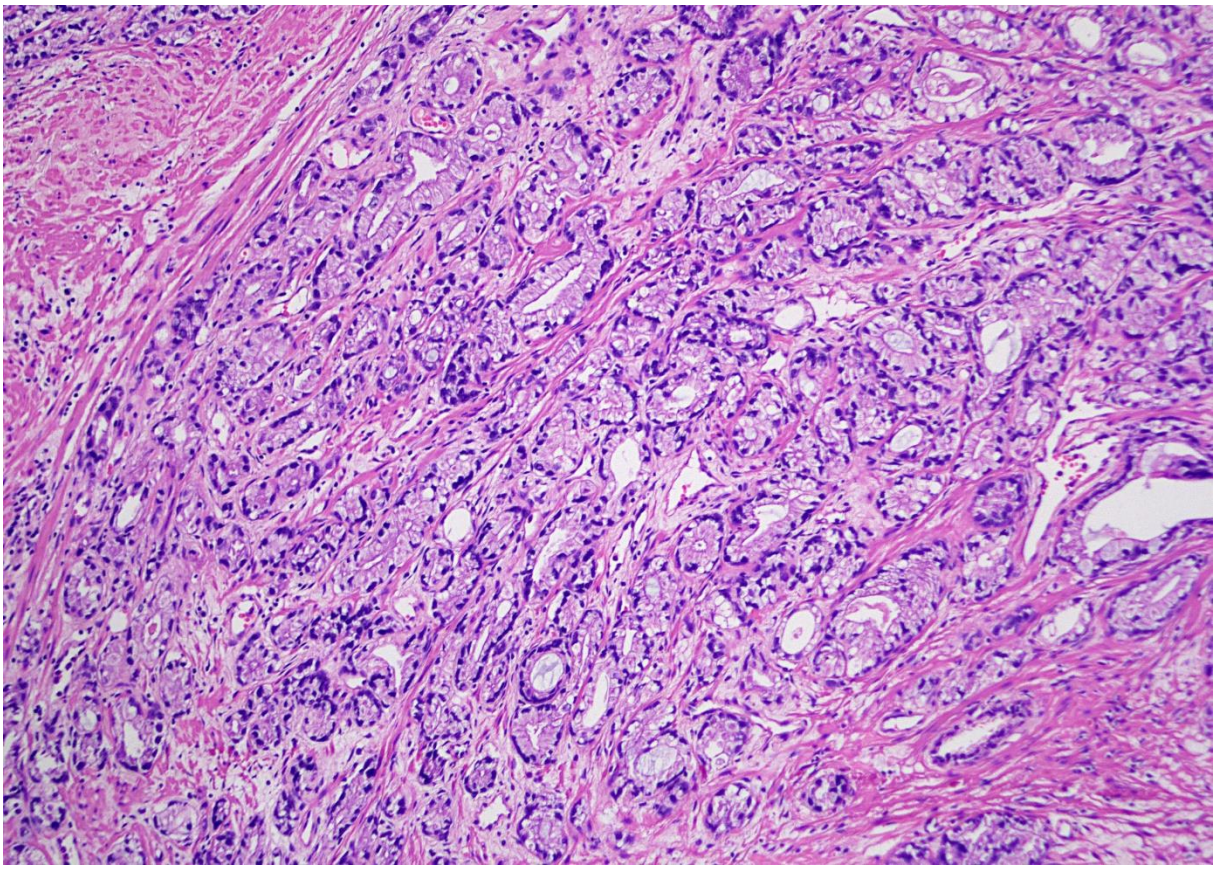


Figure 4.19: (Case no.#6221) H&E slide of Gleason Grade Group 4 prostate carcinoma (10×)

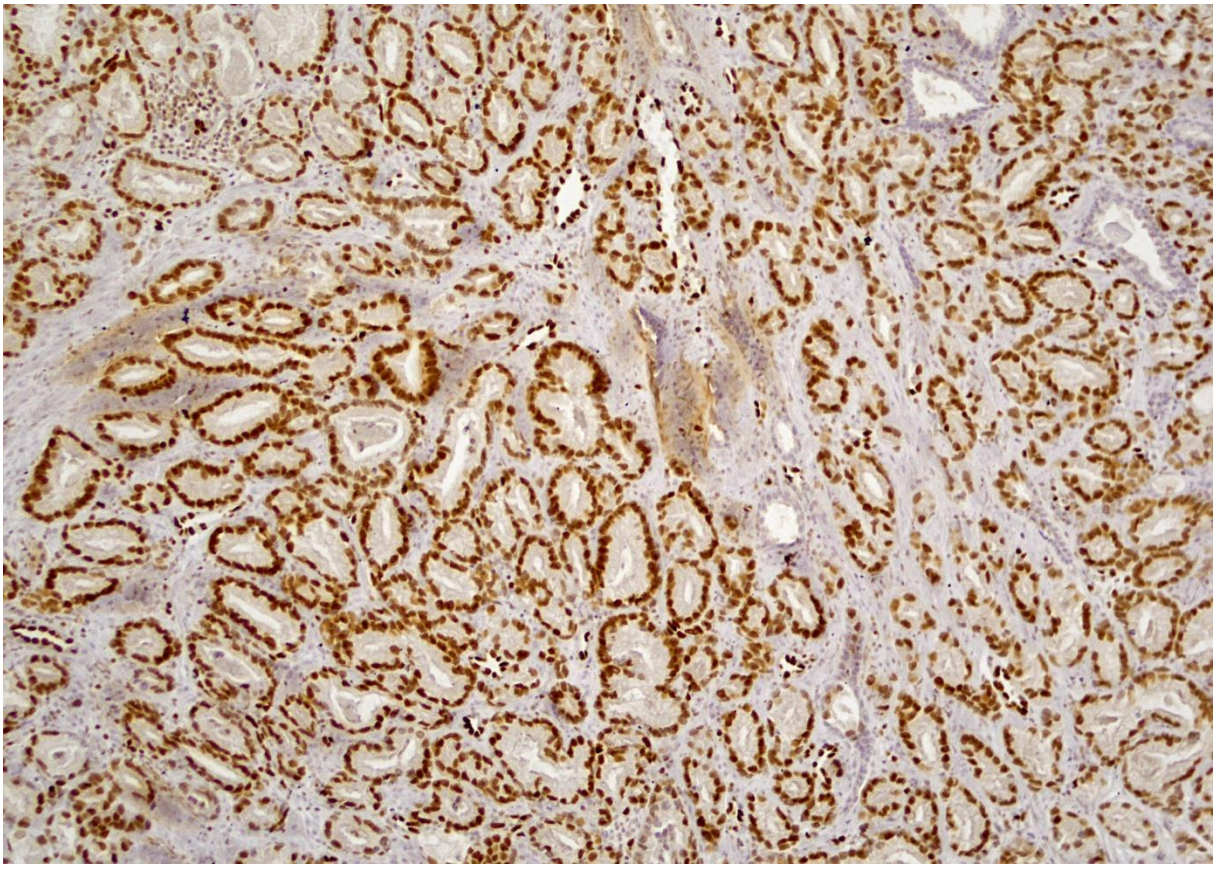


Figure 4.20: (Case no.# 6221) Gleason Grade Group 4 prostate carcinoma showing strong ERG positivity (10×)

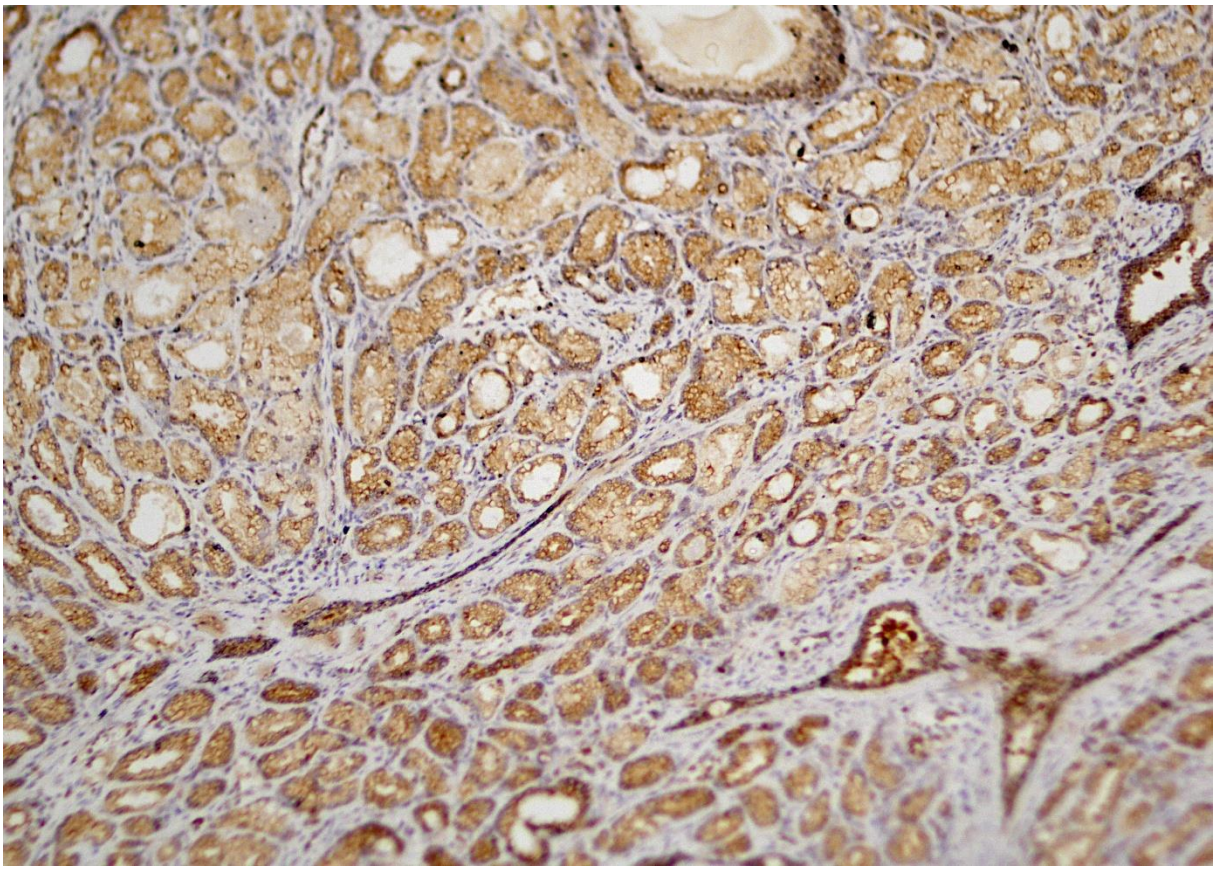


Figure 4.21: (Case no.# 6221) Gleason Grade Group 4 prostate carcinoma showing weak SPINK1 expression (10×)

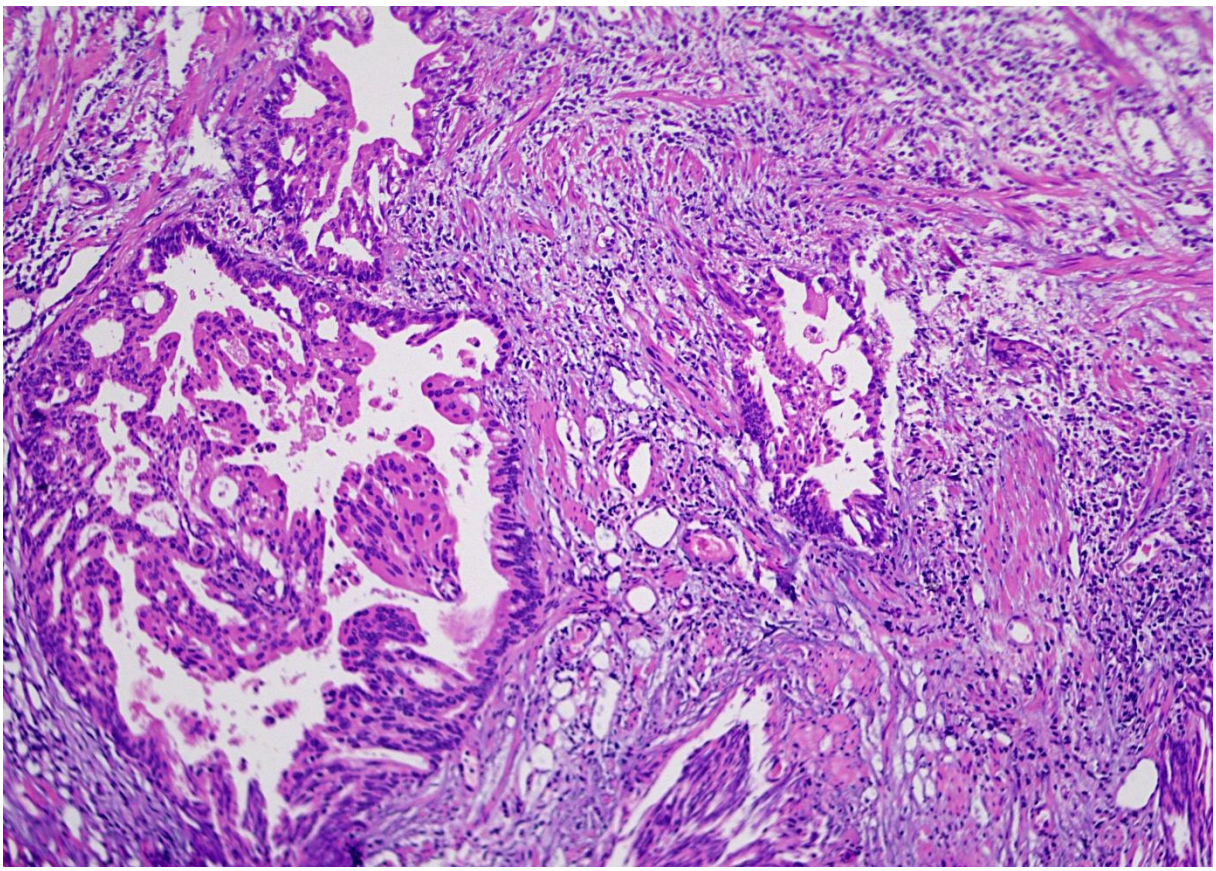


Figure 4.22: (Case no.# 1302) H&E slide of Gleason Grade Group 3 prostate carcinoma (10×)

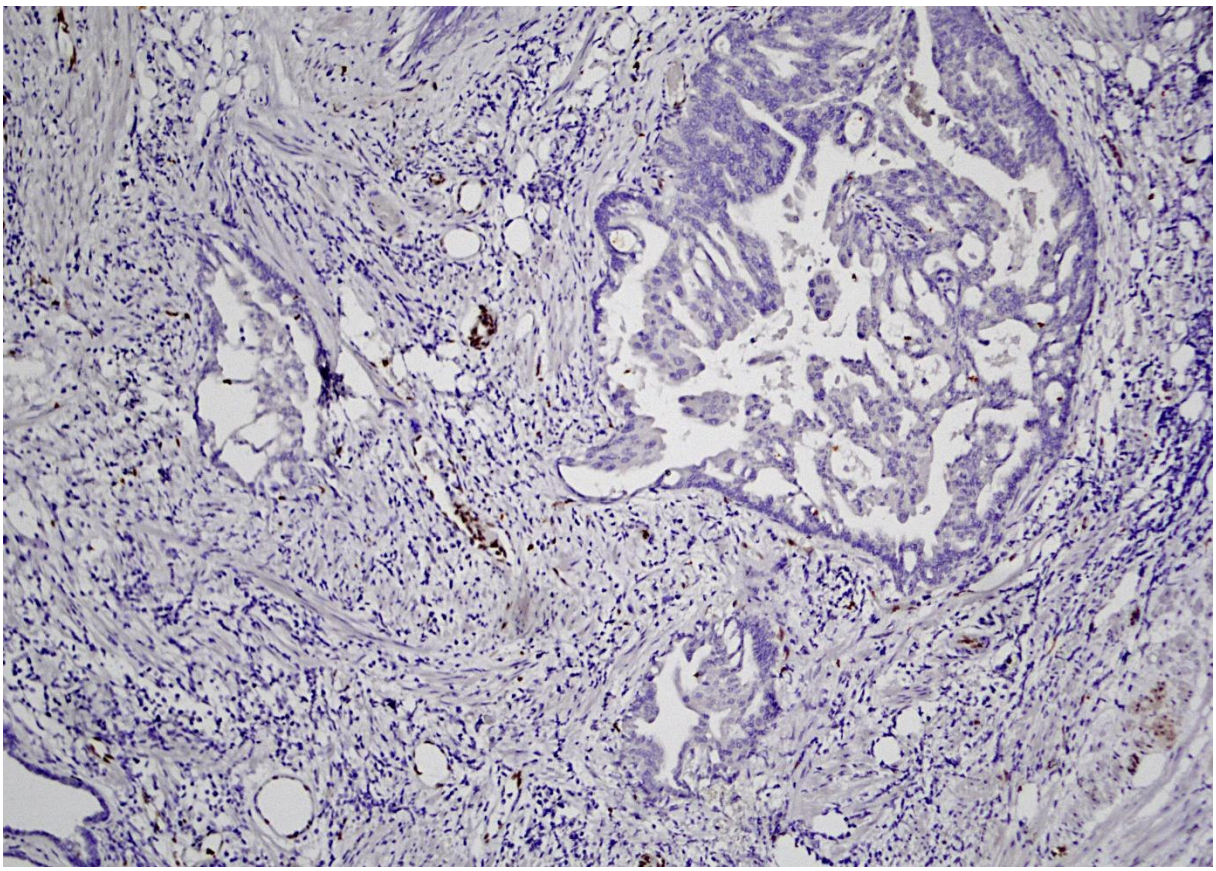


Figure 4.23: (Case no.# 1302) Gleason Grade Group 3 prostate carcinoma showing negative ERG expression (10×)

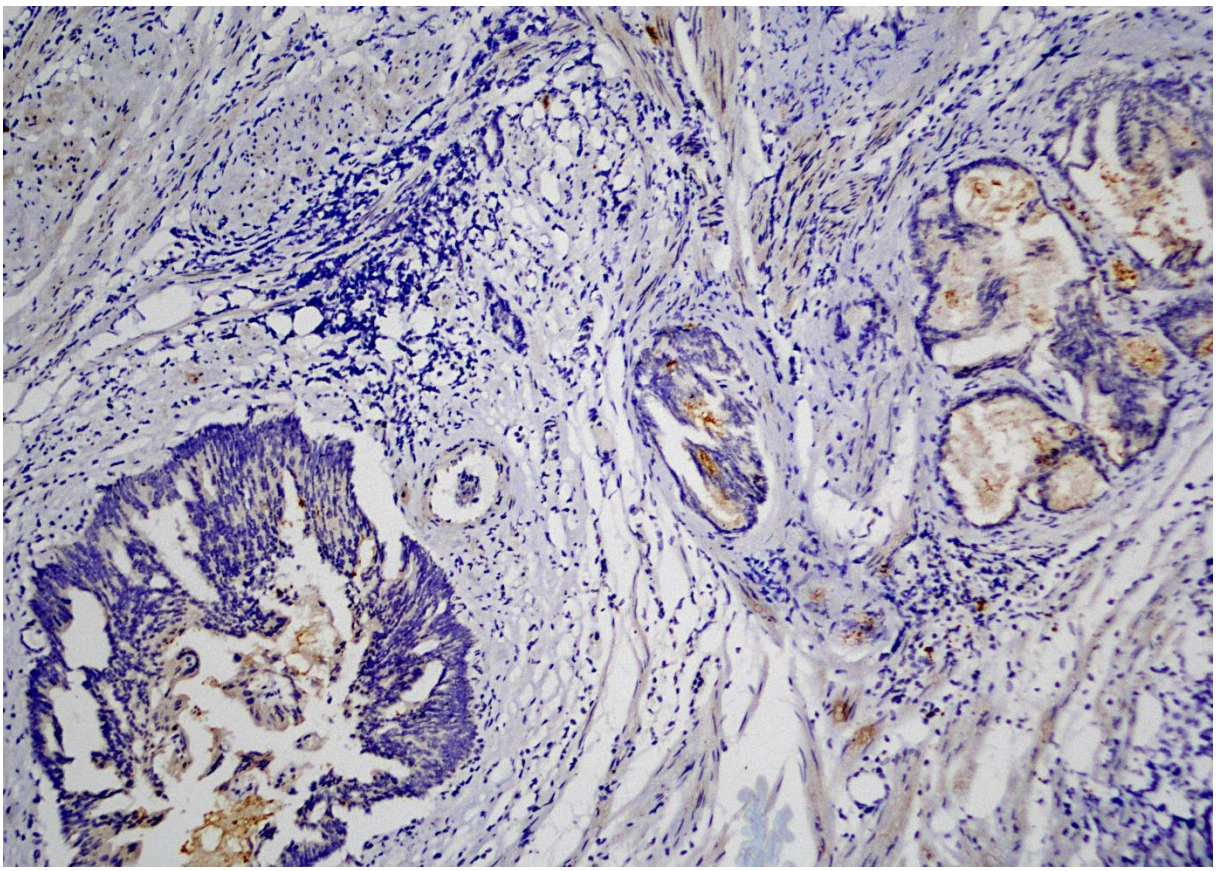


Figure 4.24: (Case no.# 1302) Gleason Grade Group 3 prostate carcinoma showing negative SPINK1 expression (10×)

CHAPTER 5

DISCUSSION

Prostate cancer is one of the major types of cancer that is prevalent in males globally. According to the WHO Global Cancer Observatory (GLOBOCAN) 2020, 1414259 cases of newly diagnosed prostate cancers were found globally, and 375,304 prostate cancer related deaths were reported in 2020. Whereas in Pakistan, around 4550 new cases were reported and 2188 deaths observed in 2020. It is the second most emerging cancer worldwide, according to estimated global epidemiology of prostate cancer, approximately 1.7 million new cases and about 499,000 mortalities will be reported up till 2030 (GLOBOCAN 2020).

According to the annual cancer registry report 2019, of The Shaukat Khanum Memorial cancer hospital & research center, Pakistan, total of 7,044 new cases of neoplasms were diagnosed and treated in Pakistan, from January 1, 2019 to December 31, 2019. Prostate cancer was the second most common malignancy reported in adult men after colorectal cancer.

In the current study majority of the cases (42.4%) presented with age group 61-70 years, this was followed by males in the age group 71-80 years with the frequency of 36.4%. Similar results were seen in another study conducted by Shabbir et al. (2019) at Jinnah Postgraduate Medical Centre, Karachi, which included patients from 41 to 90 years among which most of the patients were between 61-70 years of age. A study conducted at GC University Lahore, on cancer prevalence in Punjab, Pakistan, over a period of 5 years, showed that the male population aged above 60 years were more predisposed to prostate cancer. The prevalence and incidence rate increased throughout the study period (Hafeez et al.,2020). Similarly, a retrospective study was conducted by Qureshi et al. (2013) to identify prevalence of cancers from 2007 to 2012 at the Oncology Department,

Ayub Teaching Hospital, Abbottabad, Pakistan. They found 8.3% of prostate cancer incidence and further emphasized on increased predominance of cancer in individual more than 60 years of age. A cross-sectional study was conducted by Badar et al. (2020) at Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH&RC), Lahore, Pakistan, to determine the incidence rates of malignancies in Lahore. 33,028 new malignancies were recorded between 2010 and 2015, in adult males. Age-specific incidence rates for prostate cancer were found to increase at the age of 55 years and reached a peak at 75 years.

The mean age and standard deviation in the present study was 72 ± 7.4 years. This is in concordance with a study conducted by Owen et al. (2019) on prostate cancer which showed a mean age and standard deviation of 71 ± 6 years. Another study observed mean age of 66.2 ± 8.6 years, and median age 66 years in the overall cohort (Bechis et al., 2017).

In the present study, the most common clinical presentation of prostate cancer patient was nocturia and dysuria (27.3%) followed by prostatic enlargement (24.2%) and urinary retention (21.2%). A study conducted in UK, by Hamilton et al. (2016) reported urinary retention, impotence, frequency, hesitancy and nocturia as most common clinical presentations in prostate cancer followed by haematuria and weight loss. Another study carried out at Shifa International Hospital, Islamabad by Masood et al. (2018) also found obstructive lower urinary tract symptoms in majority of the cases (49.11%).

Present study observed that majority of the cases 17 out of 33 (51.5%) belong to a high Gleason score (9 and 10). Similarly a study conducted by Liu et al. (2020) over a period of 10 years observed the majority of cases belonging to Gleason score of 8-10 and reported in the Surveillance, Epidemiology, and End Results (SEER) database. Likewise another study conducted by Shabbir et al. (2019) at JPMC also observed prostate cancer with Gleason scores 8-10 to be more common.

The most frequently observed Gleason grade group in present study was Gleason grade group 5 (51.5%) followed by grade group 4 and grade group 1 (15.2% each). A study conducted by Bukhari et al. (2020) at Dow University Hospital from 2016-2018, also reported majority of

the tumors (40%) in Gleason grade group 5, which had increased mortality rate and was indicative of an aggressive disease. Whereas a study done by Gul et al. (2017) during a period of 3 years from January 2014 to January 2017 at Benazir Bhutto Hospital, Rawalpindi, Pakistan found Gleason grade group 4 in majority of the cases (39.5%). Another study conducted in Nigeria by Abubakar et al. (2018), showed Gleason grade group 1 as most common (32.2%) pattern in patients. Higher frequency of high-grade group points towards the tendency of patients presenting late with the disease in our population.

Lymphovascular invasion is defined as the presence of tumor cells in an endothelium-lined space. According to the International Society of Urological Pathology (ISUP) recommendation, lymphovascular is part of the standard examination of radical prostatectomy specimens. Since in the present study all samples were obtained by TRUP and TRUS, the lymphovascular status of 27 out of 33 cases (81.9%) could be affirmed. Among the known cases 45.5% were negative for lymphovascular invasion whereas 36.4% were positive. Most of the lymphovascular invasion was seen in grade group 5. A study conducted in Korea by Jeong et al. (2019) also found negative lymphovascular status in 49.1% of cases. Some authors suggest that the presence of lymphovascular invasion in Prostate cancer is associated with adverse oncological outcomes and higher recurrence rates, whereas others argue that lymphovascular invasion is not an independent predictor for prognosis (Jaing et al. 2018). It was also suggested that there is insufficient evidence to recommend the routine use of lymphovascular invasion for clinical prognostication (Ng. et al. 2012). A possible reason for the variation in frequency of lymphovascular invasion may be due to small sample size in present study and also because samples were obtained by TRUP and TRUS only, as lymphovascular invasion and perineural invasion cannot be affirmed on these biopsies.

Prostate cancer has been recognized to show a propensity to invade and grow along prostatic nerves, this is because nerves provide cancer cells a low resistance path out of the prostate. Regarding status of perineural invasion in present study, 6 were unknown, among 27 cases with known status 45.5% were positive for perineural invasion and 36.4% were negative. Perineural invasion in present study was more common in grade group 5. Similarly a study conducted by Zareba et al. (2017) observed perineural invasion to be much more common in high Gleason grade disease. The association between perineural invasion and lethal prostate cancer was

found to be apparently even among men with high grade cancers and indicative of an independent mechanism by which cancers cells can leave the prostate. Another study by Jeong et al. (2019) showed no perineural invasion in 23.2% cases and positive perineural invasion in 76.8% cases. A multi-institutional study conducted by Kraus et al. (2019) observed perineural invasion in roughly 22%-65% of prostate cancer specimens in patients with organ-confined disease. Another study carried out by Barsky et al. (2020) reported 14% positive cases for perineural invasion while 86% were negative for it. A study conducted by Bukhari and colleagues (2020) reported perineural invasion in 35% of cases and lymphovascular invasion in 12% of cases. The presences of perineural invasion with higher grade lesions signifies a more aggressive disease

The term intraductal carcinoma refers to the location of the tumor within large ducts. Intraductal carcinoma of the prostate gland, which is now categorized as a distinct entity by WHO 2016 (Varma et al., 2019). The incidence of intraductal carcinoma varies widely depending on the patient cohort studied, as it is more commonly seen in association with high-grade, high-stage invasive prostate cancer (Varma et al., 2019). In the present study only status of 19 cases were known in which none was identified to have intraductal carcinoma. In one of the largest study by Watts et al. (2013) found intraductal carcinoma in only 2.8% of 1176 prostate biopsies.

This study revolves around the expression of ERG and SPINK1 and their relationship with various clinicopathological parameters. In prostate carcinoma specimens 60.6% of the cases expressed ERG, 42.4% with strong positivity, 9.1% with moderate and 9.1% with weak positive expression. None of the BPH cases showed expression of ERG and SPINK1. A study conducted at Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore Pakistan, from Jan 2018 to Jan 2019 reported ERG expression in 62% cases (Tabish et al. 2021). A study performed by Hashmi et al. (2019) found ERG expression in 39.7% of cases, out of which 3.8% showed low, 12.8% intermediate and 23.1% revealed high ERG expression. Abdel-salam et al., (2020) demonstrated 42.7% ERG positive prostate tumor in their cohort. ERG expression of 49% cases was documented by Verdu et al. (2016). Data regarding ERG positive prostate cancer showed wide variation. ERG expression was found to be more prevalent in western countries (54%) as compared to Asian countries (23%). Several studies have reported that Eastern Asian patients are two to five times less likely to harbor the fusion. Furthermore, a meta-analysis indicated that 27% of Asian

patients are fusion positive, which is approximately half the rate in the Western populations, exceptions were Indian and Turkish, of which the fusion rates were 52% and 46%, respectively (Rawal et al. 2013). Whereas recent studies show higher incidence of ERG expression in Asian population. This wide variation from the previously published data might be due to lifestyle changes and geographical variation even in Asian countries (kimura et al. 2018). Expression of ERG in 60.6% of cases and focal ERG expression observed in apparently normal looking glands adjacent to tumor area in 3 cases in present study suggests TMPRSS2-ERG fusion gene to be a driving molecular alteration in development of prostate cancer in our population, and therefor can be used as an effective diagnostic marker.

Out of 33 cases of prostate carcinomas in present study only 3 (9.1%) cases showed expression of SPINK1, with 1 case (3.0%) showing 50-80% of cells staining and 2 cases (6.1%) showed > 80% of cells staining. None of the BPH cases expressed ERG or SPINK1. Terry et al. (2015) identified SPINK1 expression in approximately 5.9-15.3% cases of prostate carcinoma. SPINK1 expression was reported only in 6.5% of the specimens in a study done by Koide et al. (2019). Positive cytoplasmic staining of SPINK1 was only detected in 13.5% of cases in a study by Pan et al. (2106). Brooks et al. (2015) observed SPINK1 expression in 3.4% of cases. Likewise Flavin et al. (2014) observed only 8% of prostate tumors with SPINK1 positive expression. A study suggested that about 10% -15% of prostate cancer patients are positive for SPINK1 and this type of cancer is more aggressive (Zhang et al. 2017). According to Segura et al. (2021) expression of SPINK1 was observed in approximately 10–25% of prostate carcinomas. In present study expression of SPINK1 in only 3 cases undermine its significance as a diagnostic/prognostic marker. However the difference in the finding could most likely be due to small sample size.

Majority of the cases (36.7%) in the present study showed no expression for both ERG and SPINK1. 43.3% of the cases had strongly positive expression for ERG but negative for SPINK1. 9.1% cases had weak to moderate expression of ERG but did not express SPINK1. 18% of ERG negative cases expressed SPINK1, and only 1 case was observed to express both ERG and SPINK1. No statistically significant association was seen between ERG expression and SPINK1 expression (p-value: 0.91). Brooks et al. (2015) in his study observed 44% ERG positive cases and 3.4% SPINK1 positivity, while expression of ERG and SPINK1 proteins were inversely correlated,

it was not mutually exclusive since 3 (0.28%) cases showed high expression of both. According to Abdlesalam et al. (2020) 20% of prostate cancer were ERG positive, non-of which was SPINK1 positive. On the other hand, 80% were ERG negative, 4 of which (2%) were SPINK1 positive, which confirms mutual exclusivity of ERG and SPINK1. The differences in results is most likely because ERG expression varies and is lower among Arab population compared to North American and European population (Aldaoud et al. 2017). Lu et al. observed ERG overexpression in 56%, and SPINK1 in 40% of prostate carcinoma cases, 17% cases showing both ERG and SPINK1 overexpression and higher frequency of ERG expression was seen in Caucasian men whereas SPINK1 expression was more in African American men (Lu. et al. 2020). Most of the cases (43.3%) showed strong ERG expression and were negative for SPINK1, however one case showed expression of both markers in same focus of the tumor thus questing the mutual exclusivity of ERG and SPINK1 expression. Differences in the studies might be due to the fact that expression of these biomarkers differs in relation to racial background and genetics of prostate cancer.

Majority of the cases (26) belonged to age group 61-80 years, out of these 13 cases showed strong ERG expression while the 3 cases positive for SPINK1 also fall in this age group. No statistically significant association was found between age group and ERG expression or SPINK1 expression (p-value: 0.50 and 0.44 respectively). This is in concordance with studies conducted by Kong et al. (2020), Kimura et al. (2017) and Flavin et al (2015) which also showed no significant association between these parameters. Few studies have demonstrated a significant association with ERG positivity and age; Lu. et al. (2018 and 2020) and Brooks et. al (2015) observed a significant association of ERG expression with younger age group (≤ 45 years), while Rezk et al. (2019) showed an association with ERG positivity and older age (≥ 75 years). As most of the cases included in our study were those of patients more than 60 years of age therefore the association of ERG in prostate cancer with younger age group cannot be affirmed.

In the present study Gleason garde group 5 expressed ERG in 57.1% of the cases, grade group 4 included 5 cases out of which 4 expressed ERG and grade group 2 contained 2 cases and both expressed ERG. 2 out of 3 cases of SPINK1 were expressed in grade group 5. No statistically significant association was found between Gleason grade group and ERG expression and

SPINK1(p-value: 0.13 and 0.46 respectively). A study conducted by Baohang et al. (2019) observed significant association of ERG expression with lower Gleason grade (≤ grade 3). Whereas Aldaoud et al. (2019 and 2017) observed no significant association between Gleason grade group and ERG expression. Lu. et al. (2020) demonstrated significant association between ERG and Gleason grade group 1 and 2. Flavin et al. (2015) found no significant associations between SPINK1 status and Gleason grade group. Similarly a study by Abdlesalam et al. (2020) also reported significant association between ERG and grade group 1 and 2 and no association between SPINK1 and grade. As ERG expression is equally expressed in higher as well as in lower grade tumors, this signifies the TMPRSS2-ERG fusion in early prostate lesion and is persistent throughout the disease, so it can be used as an early diagnostic tool for detection of prostate carcinoma in our population.

The prevalence of lymphovascular invasion greatly differs (5% to 53%) in patients of prostate carcinoma. Lymphovascular invasion is believed to be a susbstantial biomarker for biochemical recurrence and many studies have suggested it to be an independent marker in prostate carcinoma (Jiang et al., 2018). In the present study, the status of lymphovascular invasion could be determined in 27 out of the 33 cases. 36.4% were positive for lymphovascular invasion whereas 45.5% were negative. In the presence of lymphovascular invasion, 42.9% of cases displayed strongly positive ERG expression and 50% of cases expressed SPINK1. There was no statistically significant association observed between lymphovascular invasion and ERG and SPINK1 expression (p-value: 0.24 and 0.78 respectively). This is similar to many studies which also saw no statistical significance between lymphovascular invasion and ERG and SPINK1expression (Kong et. al. 2020, Kim et. al. 2013, Noh et. al. 2016). Equal ERG expression was observed in cases with and without lymphovascular invasion and the same was observed with SPINK1 expression suggesting insignificant association of the expression of these markers with lymphatic and hematogenic spread.

Regarding perineural invasion in the present study status of only 27 were known. 45.5% of cases were positive for perineural invasion whereas 36.4% were negative. In the perineural invasion, 80% of cases expressed SPINK1 and 57.1% of cases expressed ERG. There was no statistically significant association was found between perineural invasion and ERG and SPINK1

expression (p-value: 0.39 and 0.41 respectively). Many studies showed similar results (Noh et al. 2016, Bahnushali et al. 2018). While Hashmi et al. (2019) and Kim et al. (2015) found significant association between ERG and perineural invasion. ERG expression was equally observed in cases with and without perineural invasion. However all 3 cases with positive SPINK1 expression showed presences of perineural invasion as well, this might indicate the association of SPINK1 expression with comparably more aggressive tumors.

In present study, status of intraductal carcinoma could be determined in only 19 out of 33 cases. In 57.6% of cases intraductal carcinoma was not identified rest of 42.4% of cases the intraductal carcinoma status was unknown. 66.6% of cases were intraductal carcinoma was not identified showed strongly positive staining for ERG. Majority of the SPINK1 staining was also seen in negative intraductal carcinoma cases. No statistically significant association was seen between intraductal carcinoma and ERG expression and SPINK1 (p-value: 0.39 and 0.67 respectively). This is in contrast with studies by Morais et al. (2015) and Schneider et al. (2014) which showed significant association of intraductal carcinoma with ERG. This difference might be due to small sample size in present study.

SPINK1 has been found to be expressed by multiple types of tumor cells, including breast, ovarian, prostate, pancreas, liver, and colon (Rasanen et al., 2016). Recently, SPINK1 has also been found to be expressed by the tumor stroma after chemotherapy, where it may contribute to chemoresistance and increased risk of recurrence (Chen et al., 2018). SPINK1 expression is linked with poor prognosis in many cancers and represent a distinct prostate cancer subtype. Zhang and colleagues found a prominent correlation between clinical outcomes and SPINK1 protein expression in metastatic prostate carcinoma. They suggested that the difference between metastatic and nonmetastatic diseases is the plausibility of expression of SPINK1 at different stages of cancer development (Zhang et al., 2017). Similarly, Huang and colleagues identified correlation of SPINK1 with ERG and PTEN by employing immunohistochemistry and fluorescence in situ hybridization and found upregulation of SPINK1 expression at primary sites of prostate carcinoma patients with nodal metastasis (Huang et al., 2017). Prostate tumors that express SPINK1 have been reported to show a significantly more aggressive phenotype and poorer progression-free survival (Tomlins et al., 2008 and Leinonen et al., 2010). Knockdown of SPINK1 might reduce

cell invasion only and not affect its proliferation. This finding might explain why SPINK1 was associated with adverse prognosis in aggressive prostate tumors. Ateeq and colleagues (2011) demonstrated that SPINK1-positive cancers may potentially be targeted therapeutically through humanized SPINK1 directed monoclonal antibodies and EGF receptor (EGFR) inhibition.

Even though the expression of both biomarkers were corelated with demographic and histopathological parameters, the association with clinical parameters special staging could not be evaluated due to the current pandemic and lack of access to the clinical records.

Many studies have provided strong evidence for the importance of SPINK1. The regulatory pathways that control SPINK1 expression and the direct targets of SPINK1 in the context of the tumor microenvironment, including both protease targets and cell surface receptors, remain largely unknown. The identification of these targets of SPINK1 will aid in development of therapeutic strategies to reduce tumor metastasis. For better understanding we need to further investigate the missing link between SPINK1 and EGFR signaling using modern methods and technologies. Resolute efforts are needed to discover SPINK1 targets and signaling mechanisms. These efforts will lead to the development of novel therapeutic strategies to reduce the impact of SPINK1 on tumors and improve patient prognosis.

CHAPTER 6

CONCLUSION

6.1 Conclusion of the study

- ERG expression was observed in 60.6% of prostate carcinoma whereas none of the BPH cases showed ERG expression
- ERG expression was seen in both high and low grade prostate carcinoma, suggesting TMPRSS2-ERG fusion as an early event in carcinogenesis of these tumors and its persistence throughout the disease
- Only 3 cases showed expression of SPINK1, thus undermining its significance as a diagnostic marker or a marker for advanced lesions
- One case expressed both ERG and SPINK1 in same tumor focus thus questioning the mutual exclusivity of expression of these markers
- This study is the first to assess the association of ERG and SPINK1 in prostate carcinoma cases in Karachi, Pakistan and is expected to pave way for further preferably prospective studies which might aid in evaluation of these markers in early detection of prostate carcinoma and also as prognostic indicators and targets for precision medicine

6.2 Recommendations

- Lack of comprehensive cancer registry stresses upon the need of effective data compilation and reporting of incidence and prevalence of common cancers in our population for designing effective health management plans and therapeutic intervention for the more prevalent tumors in Pakistan
- Future preferably molecular studies are required to assess the significance of ERG and SPINK1 as diagnostic/prognostic markers and as therapeutic targets for precision therapy
- There is a need to standardize detection methods and techniques for ERG and SPINK1 expression to obtain consistent results
- Designing a cost-effective techniques for identification of diagnostic and therapeutic markers are needed in order to facilitate effective and accessible screening and diagnosis of cancers

6.3 Strengths of the study

- This is the one of the first studies conducted on the association of ERG and SPINK1 in prostate carcinoma patients in Karachi, Pakistan. The results will aid in selection of proper targeted therapeutic drugs for treatment of one of the most common cancer in adult males in Pakistan, namely prostate cancer
- The cases taken were treatment naïve, hence were free from drug induced changes affecting of ERG expression and SPINK1 expression, if any

6.4 Limitations of the study

- The study was conducted at a single tertiary care hospital, hence cannot be generalized to the entire Pakistani population
- Sample size was small limiting the generalization of results
- There was no data available on lymph node status and stages
- Time for the study was limited

CHAPTER 7

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INFORMED CONSENT FORM FOR PATIENT

I am giving my consent to participate voluntarily and at my own will in this research project which aims to determine effective tumor markers of prostate carcinoma.

I have been explained that tissue specimen will be taken for the purpose of research.

I have been told that findings of my disease and my data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

I fully agree to give my samples (tissue) at the beginning and end of study and when required in between.
I also agree to give all relevant information when needed, in full and to the best of my knowledge to the researcher. It is clarified to me that no incentive will be provided to me for participating in the study, whereas I do have the right to withdraw from the study at any time.

I am advised to contact Dr. Mubina Qayyum on mobile number: 03313726837 or visit PNS Shifa Hospital in case of any query/ emergency related to my disease.

Name of Patient:_____ Sex _____
S/O_____ Age_____

Signature / Thumb impression of Patient: _____
Name of Researcher: _____
Signature of Researcher: _____
Date: _____

مریض کے لئے باخبر رضامندی فارم

رضاکارانہ طور پر اور پروسٹیٹ کارسنوما کے مؤثر ٹیومر مارکر کا تعین کرنے کا ارادہ رکھتا ہے جو اس تحقیق کے منصوبے میں میری اپنی مرضی سے حصہ لینے کے لئے میری رضامندی دے رہا ہوں
مجھے بتایا گیا ہے کہ ٹشو کا نمونہ تحقیق کے مقصد کے لئے لیا جائے گا۔

مجھے بتایا گیا ہے کہ میرے مرض اور میرے ڈیٹا کی کھوج کو سختی سے خفیہ رکھا جائے گا اور یہ صرف معاشرے اشاعتوں اور کاغذی پریزنٹیشن کے مفاد کے لئے استعمال ہوگا۔ ،

مطالعے کے آغاز اور اختتام پر دینے پوری طرح سے اتفاق کرتا ہوں اور جب ضرورت پڑتا(ٹشو) میں اپنے نمونے ہوں۔

میں بھی ضرورت پڑنے پر تمام متعلقہ معلومات ، مکمل طور پر اور اپنے بہترین معلومات محقق کو دینے پر متفق ہوں۔
یہ بات مجھے واضح کردی گئی ہے کہ مطالعے میں حصہ لینے کے لئے مجھے کسی طرح کی ترغیب نہیں دی جائے گی ، جبکہ مجھے کسی بھی وقت مطالعے سے دستبردار ہونے کا حق ہے۔
پر ڈاکٹر مبینہ قیوم سے رابطہ کریں یا میری 03313726837 :مجھے مشورہ دیا گیا ہے کہ میں اپنے موبائل نمبر
ایمرجنسی ہونے کی صورت میں پی این ایس شیفا اسپتال دیکھیں۔ / بیماری سے متعلق کوئ سوال

مریض کا نام:_____

جنس(مرد ، عورت): _____

عمر:_____

انگوٹھا تاثر: _____

محقق کا نام:_____

محقق کا دستخط:_____

SUBJECT EVALUATION FORM

Department of Pathology

PNS Shifa Hospital, Karachi

PROFORMA

Epidemiological Data:

S .No._____ Case/I.D No._____ Date._____

Patients Name_____ S/O. _____

Age._____

Address:-_____

Clinical Diagnosis._____

Gross Findings:-_____

_____

Histopathological Findings:-_____

_____

Tumor infiltrating lymphocytes_____**Morphology type** _____

Lympho vascular invasion_____

Necrosis_____

Invasion _____

Tumor Grade_____

Tumor stage_____

Histopathological diagnosis:_____

Location	**ERG**	**SPINK1**
Cytoplasmic		
Nuclear		
Membrane		
Intensity of Staining		
Weak		
Moderate		
Strong		
Positive cells (%)		
SPINK1		
No staining = 0		
< 50% of cells staining in scattered individual cells = 1		
< 50% of cells staining in complete glands = 2		
50-80% of cells staining = 3		
>80% of cells staining= 4		


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