HISTOPATHOLOGICAL STAGING IN PUTATIVE PROSTATE CANCER TISSUES AND REVIEWING LITERATURE OF CORRELATION BETWEEN PROSTATE SPECIFIC ANTIGEN LEVELS AND PROSTATE CANCER INCIDENCE

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CERTIFICATE

I declare that the dissertation entitled “HISTOPATHOLOGICAL STAGING IN PUTATIVE PROSTATE CANCER TISSUES AND REVIEWING LITERATURE OF CORRELATION BETWEEN PROSTATE SPECIFIC ANTIGEN LEVELS AND PROSTATE CANCER INCIDENCE” has been prepared by me under the guidance of Dr. Sanjeev K. Thakur, Assistant Professor, Centre for Biosciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

"**Histopathological staging in putative prostate cancer tissues and reviewing literature of correlation between prostate specific antigen levels and prostate cancer incidence"**

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Prostate cancer (PCa) remains the most significant cause of cancer specific mortality in elderly men. Asymptomatic behavior, non-modifiable risk factors and metastatic nature is the main problem of PCa. It remains clinically silent and presents itself only at advanced stage. Thus, diagnosing PCa at an early stage can result in increased chances of better treatment and hence, increased survival rate. An accurate biomarker can detect the cancer at an early stage and hence, at curable stage.

Clinical parameters can only suspect prostate cancer. Whereas, histopathological examination can establish definite diagnosis of PCa. Various histological patterns are associated with cancer aggressiveness. Therefore, better understanding of clinical relevance of these histopathological findings can help to evaluate a robust biomarker for early detection of PCa. Present study was divided into two parts. First part was aimed to study the histopathology of putative prostate cancer tissue specimens. In second part, the literature of association of pre-operative serum prostate specific antigen levels with cancer detection and aggressiveness was reviewed.

Protocol for histopathology of prostate tissues was established. Results of histopathological findings in putative PCa specimens were evaluated. Prevalence of histological PCa was not found in putative PCa tissues. Image library of results of the study was prepared for future analysis.

Review of literature of correlation of serum PSA levels with PCa incidence suggests that PSA screening for PCa is a two-sided debate. No doubt that PSA holds the probability of detecting early PCa before development of symptoms; certain

limitations are associated with it. First, it is not reported to be 100% accurate. Second, it generates false positive and false negative results. A false positive result leads to over-treatment whereas a false negative result generates false sense of security against PCa in patient, both affects quality of life. Another main concern with PSA screening is its inability to differentiate between indolent and aggressive cancer.

Therefore; accurate and economical molecular biomarkers for early detection and distinction of indolent versus aggressive cancer are urgently required. Until such biomarkers are developed and more convincing evidences regarding efficacy of PSA screening becomes available, research should focus on improving the diagnostic clinical utility of PSA by utilizing novel PSA isoforms. Identifying and validating correlation of serum PSA with tissue PSA and histological grade would be beneficial in terms of requirement of less invasive diagnostic methods to be used to measure PSA expression level as well as to confirm PCa. Future research may focus on evaluating the histological expression of other putative biomarkers and comparative serum proteomic profiling in different PCa stages.

Shweta Thakur Dr. Sanjeev K. Thakur

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LIST OF ABBREVIATIONS

S.No	Full form	Abbreviation
1	Prostate Cancer	PCa
2	Androgen Receptor	AR
3	Dihydrotestosterone	DHT
4	Prostate Specific Antigen	PSA
5	Serum PSA	s.PSA
6	Gleason Score	GS
7	Trans urethral resection of prostate	TURP
8	Transitional zone	TZ
9	Peripheral zone	PZ
10	Central zone	CZ
11	Prostatic Intraepithelial neoplasia	PIN
12	High grade, Low grade	HG,LG
13	Benign Prostate Hyperplasia	BPH
14	Metabolic syndrome	MetS
15	Body mass index	BMI
16	Prostate acid phosphatase	PAP
17	Digital rectal examination	DRE
18	Trans rectal ultrasound	TRUS
19	Proliferative inflammatory atrophy	PIA
20	World Health Organization	WHO
21	College of American pathologists	CAP
22	Haematoxylin and Eosin	H&E
23	International society of Urological pathology	ISUP
24	Tumor, Node, Metastasis	TNM
25	Neutral Buffered Formalin	NBF
26	Formalin-fixed paraffin embedded	FFPE
27	With respect to	w.r.t
28	Indian council of medical research	ICMR

CHAPTER ONE

INTRODUCTION

1.1 General Introduction to Cancer and Cancer Biology

"Cancer" is that terrible word that has become a curse and epidemic in our society because it is not only the disease that is deadly but the sufferings of its treatment are painful and not admissible. Cancer is a hard battle to beat because of its asymptomatic nature and aggressive metastatic behavior. Every survival story in cancer depends upon its early detection. Therefore, sincere research directed on the concept of early detection of cancer is required, so that it can help to make everyone live freely.

An approximate calculation of 12.7 million cancer cases and 7.6 million cancer deaths were reported in the year 2008 (Ferlay et al., 2010). Frequency of cancer is reported to increase in India also due to unhealthy lifestyles and immigration of rural population to the urban (Sinha et al., 2003).

Cancer can be defined, as uncontrolled and unmanageable growth of abnormal cells. Genetic alterations such as point mutation, translocation, or gene amplification elicited through an internal or external signal leads to malignant transformation of normal cells by providing them inheritable replicative advantage (Couch, 1996).

Physiologically, many changes takes place for carcinogenesis such as continuous uninterrupted growth signals, insensitivity to growth inhibitory signals, eluding apoptosis, enduring angiogenesis and invading surrounding tissue to establish metastasis (Hanahan and Weinberg, 2011). Replication of normal cells is requisite to maintain the homeostatic balance in the body. This homeostatic balance can be disrupted through both unnecessary increased rate of cell proliferation as well as decreased rate of apoptosis (Hanahan and Weinberg, 2011). Replication is tightly controlled via certain checkpoints within the cycle that initiates termination or apoptosis, if something goes wrong during the normal cell cycle. However, if such

checkpoints remains unchecked and the cell continue through the cell cycle despite errors, the cell acquires its first step towards the process of carcinogenesis.

Ideology of tumor cell is growth for the sake of growth. For growth, it needs good nourishment and oxygen to be provided by the blood vessels and capillary network. They may enter a dormant state due to lack of nutrients and oxygen. The last stage of the carcinogenesis is invasion of surrounding tissue by ignoring the constraints of the neighboring cells (Hanahan and Weinberg, 2011). This wandering tumor cell may enter the blood stream and can be carried out to other anatomic locations in the body where it can start all over again to form a new tumor, process known as “metastasis”.

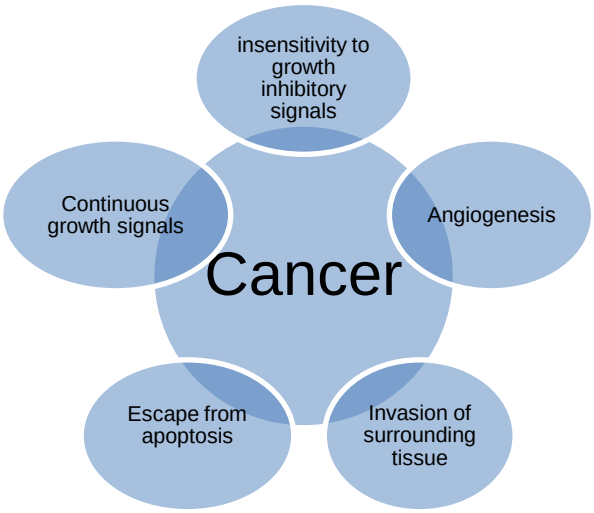

Fig 1. Physiological changes in carcinogenesis

Another important change linked with cancer is shortening of telomeres which affects the integrity of the chromosome and leads the cell to apoptosis. Telomerase enzyme prevents the shortening and these enzymes are expressed only in human stem cells. By activating this dormant function, most tumor cells make certain their maintenance through indefinite replications. Length of telomeres has been reported to correlate with disease outcome (Joshua et al., 2007). PCa and high-grade PINs have shown to display increased telomerase activity that suggests that telomeres length is changed during the process of carcinogenesis (Koeneman et al., 1998).

Pathologically, a cell first receives a mutation that provides it the advantage of increased proliferation producing new subsets of cells having similar appearance to the normal cell, but with the same defect. This stage is termed as hyperplastic stage. Hyperplasia is then followed by the metaplastic stage in which a tissue type transforms to another. This metaplasia is then further followed by the dysplastic stage in which these transformed cells further lose cellular control. Consequently, their orientation, size, nuclear size and shape alter. Finally, they enter anaplastic stage in which they acquire more structural and functional defects. If these cells stay confined to their site of anatomic origin, they are termed as “in-situ cancers” or “benign” cancers. However, if they detach from their site of anatomic origin and starts invading the surrounding or distant tissue, they are termed as “malignant” cancers. Thereafter, they may enter the lymphatic system or blood supply to invade new locations in the body.

1.2 Prostate, Prostate Cancer and Prostate Cancer Statistics

In 2008, PCa accounted for 6% of the total cancer deaths. It is the most frequently diagnosed cancer and sixth leading cause of cancer death among men. Highest incidence rates are recorded primarily in the developed countries due to increased utilization of screening programmes such as prostate-specific antigen (PSA), digital rectal examination (DRE) and histological evaluation of biopsies. Asian countries reported lowest incidence rate in the world. Whereas, males of African descent in the Caribbean region are reported to have the highest mortality rates of PCa in the world due to genetic susceptibility (Jemal et al., 2011). Consequently, worldwide research community is aimed at trying to understand more about the pathology and risk factors of the prostate cancer.

One of the major problems in diagnosis of prostate gland is its anatomic location in the body. It is deep seated in pelvic cavity, located inferior to the urinary bladder infront of the rectum. It wraps around the urethra that pass vertically through and carries urine out. Certain treatment options and clinical examinations can cause side effects to the nearby organs. Prostate is a small walnut-sized accessory sex

gland. In fetus, it is merely of pea size and then grows upto puberty. After puberty, its growth slows down and stops near the age of thirties. However, it starts to re-grow at later life also. It weighs about 20gms in normal adult male. It is approximately 3-4 cm long and 3-5 cm wide. McNeal (1988) described prostate into three major anatomical zones; the peripheral zone, central zone and transitional zone accounting for 65%, 25% and 10% of total normal volume of prostate. Most cancers have been reported to arise in the peripheral zone, whereas benign prostatic hyperplasia (BPH) arises from transitional zone only (McNeal, 1988).

Histologically, prostate comprises of glandular part and fibromuscular stroma. Glandular part consists of acini and ducts which contains a pseudostratified epithelium with three major cell types; basal cells, luminal secretory cells and neuroendocrine cells. Basal cells are present continuously. They separate the luminal cells from the basement membrane. They are highly proliferative. Luminal cell layer contains tall polarized columnar or cuboidal cells that produce secretions (Shah and Zhou, 2012). The neuroendocrine cells are not found commonly and hence, rarely seen in H&E stained prostate tissue sections. They regulate in paracrine signaling fashion (Hansson and Abrahamsson, 2001). Fibromuscular stroma consists of smooth muscle fibers. These muscle fibers squeeze out the prostatic secretions from ducts and acini into urethra on stimulation. This secretion makes about 33 % of ejaculation fluid. It contains fibrinolysin that aids in liquefaction of semen coagulum. It also contains amylase, prostate specific antigen (PSA), acid phosphatase, calcium and zinc (D'Antonio, 2008). Prostate gland is enveloped by a pseudocapsule formed by the peripheral layer of fibromuscular stroma .Pathology of prostate includes benign prostatic hyperplasia (BPH), prostatitis and PCa. BPH is nodular enlargement of prostate gland due to hyperplasia of stromal and glandular components. In PCa, adenocarcinoma is the most common histology. It results in loss of basal cells and perineural invasion. Changes cause overall loss of normal glandular architecture.

Growth and function of prostate is regulated by the androgens, specifically testosterone. Testosterone circulating in the blood passes through the cell membrane and enters luminal epithelial cells where it is converted into dihydrotestosterone

(DHT) by the enzyme 5 alpha reductase. DHT possess 10 times more affinity than testosterone for androgen receptor (AR). DHT binds to androgen receptors (AR's), targets androgen response elements (ARE) and subsequently leads to cell proliferation and growth (Chang et al., 1995; Feldman and Feldman, 2001) .Some studies suggest that transit amplifying population (TAP) which corresponds to stem cell population specifically located in the basal layer, is responsible for PCa. These cells are androgen sensitive for growth but not androgen dependent for their survival (Litvinov et al., 2003; Collins and Maitland, 2006).

1.3 Risk Factors for Prostate Cancer

Definite causes of PCa are not known but few major risk factors have been identified (figure 2). Until date, the only established significant risk factors are old age and positive family history. PCa is considered cancer of the elderly men as manifestations are usually diagnosed in men over the age of 65 years (Haas and Sakr, 1997) .A population-based study showed 2 times risk of PCa for family members of PCa patient (Steinberg et al., 1990). A study showed that hereditary PCa accounts for only 9% and reported majority of them sporadic (Carter et al., 1992). These studies shows that PCa can be attributed to shared genetic traits and shared environmental conditions. Other exogenous risk factors studied are high fat diet, chemicals used in paint, phytoestrogens, exposure to cadmium, vasectomy, smoking and alcohol consumption (Bostwick et al., 2004). Serum level of insulin growth factor (IGF) is also an established predisposing factor which may be connected to intake of high fat diet that increases the production level of IGF. This hypothesis is further supported by the investigations revealing association between basal metabolic rate (BMI) and PCa (Giovannucci et al., 1998; Chan et al., 1998; Barnard et al., 2002; Gennigens et al., 2006).

Fig 2. Risk factors for Prostate cancer (PCa)

1.4 Prognostic Factors and Predictive Factors for PCa

One of the greatest challenges in PCa treatment and management is developing reliable predictive and prognostic factors for early detection of tumor stage and prediction tumor progression. According to College of American Pathologists (CAP), prognostic factors can be classified mainly into three categories. Histological grade along with tumor, node, metastasis (TNM) staging and pre-operative s.PSA values are type I prognostic factors that are well supported by literature and are used in clinical practice (Bostwick et al., 2000). PSA is no doubt the most useful serum biomarker whose elevated concentration is of proven value in detecting early stage cancer. Histological microscopic examination can only establish definite diagnosis. It includes grading. Gleason scoring is specific for grading prostate adenocarcinoma (Gleason and Mellinger, 1974) .

1.5 Gleason Grading/Scoring System

Grade defines the degree to which the tumor cell looks different from the normal cell. Grading tells about cancer aggressiveness and hence, helps in deciding the appropriate treatment options. Gleason system is considered best and it is recommended by the WHO. It was developed by Donald F. Gleason in 1960 (Gleason and Mellinger, 1974; Gleason, 1981) .It is based on the observation of degree of differentiation and glandular architecture in H&E stained slides of prostate tissue

sections at low magnification. It describes the two most common patterns of tumor growth; primary and secondary. Primary is predominant whereas secondary is the second most prevalent pattern. Sum of both gives a score between 2 and 10; in which 2 is the least aggressive and 10 is the most aggressive. Since it identifies both patterns it is considered a reliable and good system as it takes into account tumor heterogeneity (Epstein, 2010).

1.6 Prostate Specific Antigen (PSA)

PSA is a serine protease of kallikrien family produced by the epithelium of acini and ducts of prostate. Wang et al. (1979) first identified and purified human PSA. Raised s.PSA is an indication of abnormal prostatic behavior under the influence of physiological and pathological processes. It is released into circulation when cell integrity is destroyed and thus, it can be used as a non-invasive means of screening. PSA levels are shown to increase with advancing clinical stage. It was found proportional to the prostatic volume, patient's age and volume of tumor (Stamey et al., 1987; Oesterling et al., 1993). PSA screening when introduced in the late 1980s lead to dramatic increase in the number of diagnosed early-stage PCa (McKean-Cowdin et al., 2000). But, there are controversies regarding screening using s.PSA due to chances of over-diagnosis and under-diagnosis .Until date, no s.PSA cut-off is identified with both high specificity and high sensitivity for detection of PCa.

PCa and its aggressiveness can only be confirmed by histological examination. The present work is focused on studying the histopathology of putative prostate cancer tissue specimens and reviewing the literature regarding association of preoperative serum PSA levels with PCa incidence and aggressiveness.

CHAPTER TWO

REVIEW OF THE LITERATURE

Prostate cancer is the leading cause of death among elderly men. Since risk factors are not modifiable, only early detection can reduce the PCa specific mortality rate. Concept of this chapter revolves around early detection. This chapter reviews the prevalence, risk factors, pathology, predictive/prognostic factors and screening of prostate cancer. Review is focused on equivocal issue of PSA screening for PCa.

2.1 Trends in Prostate Cancer (PCa) Incidence and Mortality Rates

Incidence and mortality rates for PCa all over the world are difficult to interpret due to rise and falls over the time, which can be accounted on different reasons. Introduction of PSA screening resulted in sudden increase in incidence rate between late 80's- mid 90's. Employment of lower PSA thresholds, usage of TURP for BPH along with histopathological examination of TURP specimens and biopsy cores resulted in sudden change in detection of PCa worldwide (Potosky et al., 1990; Haas et al., 2007). According to Surveillance, Epidemiology and End Results (SEER) cancer database statics, there has been a detectable change in PCa incidence and mortality rates after the employment of PSA for PCa screening (NCI, 2012).

Frequency of PCa is reported higher in developed countries as compared to developing countries (Hsing et al., 2000; Quinn and Babb, 2002; Jemal et al., 2011).These differences are due to exposure to exogenous risk factors, different lifestyles and genetic susceptibility. Recently, PCa incidence rates are reported to rise in Asia significantly (Pu et al., 2004; Sim and Cheng, 2005).

In India, numbers of cancer cases are increasing at a rate greater than diabetes and cardiovascular diseases. In 1981, ICMR started "The National Cancer registry programme" in which the number of cancer cases are based upon population-based cancer registries that hardly cover 5% area and population. Cancer registry in India is voluntary, not compulsory. Only 27 hospitals all over India provide data about number of cancer cases. However, it gives some idea about extent and magnitude of

cancer problem in India. PCa incidence rate has been reported to rise over the past 20 years (Yeole, 2008).Only few epidemiological studies have been conducted in India for PCa research.

Mortality and survival rates are affected by the stage diagnosed (Hankey et al., 1999). Changing trends of PCa incidence and mortality rates are might be consequences of early detection of PCa through PSA testing, DRE, increased radical prostatectomy (RP) rates and employment of hormonal therapy. PSA screening also resulted in reduction in the average age at diagnosis and the number of advanced stage cancer. Consequently, the treatment options and patterns of cancer care management have also changed accordingly. It cannot be concluded that PSA testing or DRE solely has affected the PCa incidence and mortality trends, as these changed trends are results of variations in diagnostics over the time. This issue can be only solved by randomized screening trials (Kramer et al., 1993; de Koning et al., 2002) .

2.2 Pathology of Prostate Cancer

Pathogenesis of prostate cancer is complex and not completely known. Transformation from benign to malignant is a continuous process.

2.2.1 Progression from Pre-cancerous Lesion to PCa

Possible precancerous lesions are proliferative inflammatory atrophy (PIA), low grade prostatic intraepithelial neoplasia (LGPIN) and high grade prostatic intraepithelial neoplasia (HGPIN) (Montironi et al., 2011). PIA was the term proposed by De Marzo to describe the proliferative luminal epithelial cells with the morphological appearance of atrophy associated with acute and chronic inflammation. It was observed that inflammation can promote PCa (De Marzo et al., 1999; De Marzo et al., 2007; Sciarra et al., 2008).Histologically, PIA is characterized by the reduction in number of basal cells (in contrast to their absence in PCa), hyperplasia of luminal epithelial cells and enlargement of nuclei (De Marzo et al., 1999). Its connection to the development of PCa remains unclear; possibly it is associated through inflammation. Second possible precursor is HGPIN (simply referred as PIN sometimes) is pre-malignant lesion with nuclear atypia in luminal secretory cells (Egevad et al., 2005). Tufting pattern of HGPIN is predominant. Correlation between HGPIN and PCa is well

supported by the evidences. HGPIN occurs in peripheral zone (PZ) of prostate and is found strongly associated with PCa based on morphometric, phenotypic, genetic, epidemiological and molecular evidences (Gokden et al., 2005; Netto and Epstein, 2006). Basal layer is fragmented in HGPIN but is completely lost in PCa. HGPIN incidence also increases with age. It was also shown that many proliferative cells with immature secretory cell morphology were common in PIA, PCa and HGPIN (Platz and De Marzo, 2004; Wang et al., 2009). HGPIN does not always progresses to PCa. It does not raise the PSA levels and can only be diagnosed by histopathological examination. It is predictive of PCa in future. Approximately 90-97 % of PCa is adenocarcinoma specifically acinar adenocarcinoma. Other histological subtypes are ductal adenocarcinoma, mucinous carcinoma, signet ring carcinoma (Grignon, 2004).

Natural history of PCa is complex and least understood. It is heterogeneous ranging from indolent benign cancer to aggressive malignant cancer. Some are multifocal which means genetically distinct multiple independent histological foci of tumor are present (Shah et al., 2004). Out of them, some progress to clinically presentable disease whereas some remains silent clinically. Differences are may be due to various pathogenic programmes. Multifocal PCa are found associated with high grade and high stage cancer as compared to unifocal PCa (Djavan et al., 1999; Arora et al., 2004). It can be concluded from these findings that during the process of cancer development and progression, some selective advantage might be provided to individual clones, which results in metastatic PCa.

2.2.2 Risk factors of Prostate cancer

Still the causes of PCa are not known. Although it is clear from the epidemiological studies that both environmental as well as genetic factors contribute to the development of PCa. First obvious risk factor is male gender. Other established and significant risk factors are age and positive family Differences in race mortality suggests that PCa can be attributed to shared genetic traits and shared environmental conditions (Gann, 2002). PCa is considered a cancer of the elderly men as manifestations are more common in men over the age of 60-65 years, however precancerous changes can be observed in young men of 20-30 years

(Yatani et al., 1982; Sharma et al., 2010). Autopsy studies reported 4-8 % of young men in between 30-40 years had PCa while prevalence increased to 75 % after the age of 80 years (Sanchez-Chapado et al., 2003; Soos et al., 2005).These studies suggest that PCa initiation may has taken place at an early age.

Men with positive family history for PCa were found at double risk for developing PCa (Steinberg et al., 1990). A 32 population-based study showed 2.46 fold risk of PCa for family members of a PCa patient (Steinberg et al., 1990). Hereditary PCa accounts for only 9% and majority are sporadic (Carter et al., 1992).There are also other proposed risk factors such as vasectomy (Giovannucci et al., 1993) obesity and a fat-rich diet (Amling, 2005; Hsing et al., 2007). Association of adiposity-related leptin with PCa is found controversial (Chang et al., 2001; Stattin et al., 2003; Stattin et al., 2001). Increased levels of androgens are also an important risk factor for PCa (Pienta and Esper, 1993). Obesity and leptin could be linked possibly through steroid and peptide hormones which are related to the risk of PCa. Some studies have observed association between high body mass index (BMI) and increased risk for PCa (Giovannucci et al., 1998). Increased consumption of tobacco was also reported a possible risk factor in one study (Hsing et al., 1990) .Metabolic syndromes are also found to have role in PCa development and progression (Giovannucci and Michaud, 2007; Hsing et al., 2007). Inflammation is another factor found to be involved in PCa development (Nelson et al., 2004; Platz and De Marzo, 2004). Some has also suggested that genetic polymorphisms and genetic mutations contribute to the development of PCa (Buchanan et al., 2001; Hsing et al., 2002; Hsing et al., 2000). Hence, we can conclude that PCa occurs due to interplay between genetic factors, environmental factors, dietary factors, influence of metabolic syndromes, inflammation and endogenous hormones. Further studies are needed to establish the risk factors and to evaluate their role in PCa development.

2.3 Screening of Prostate Cancer

Screening is necessary to provide patients best possible treatments as early as possible .According to WHO, screening includes the implication of simple tests across a healthy set of population in order to recognize individuals who have a disease but

do not yet have clinically presentable symptoms (WHO, 2012). An ideal screening test should be highly specific, sensitive, cost-effective and non-invasive. It should diagnose and treat PCa as early as possible, but at the same time, it should diagnose PCa as late as possible to keep patients away from the suffering of the negative side effects of treatment. Some investigators does not support screening in asymptomatic men as it may result in poorer health outcomes and increased cost factor (Krahn et al., 1994). Screening is advantageous only in the case of those patients whose cancers would have been incurable if it had been detected clinically, but is curable if detected early through screening, such as in case of slow growing latent PCa. Screening is not beneficial in case of very slow latent growing tumors with a very long phase that can never become clinically significant. In such cases, men die with PCa but not due to PCa. PCa screening is a very controversial issue. Screening methods generally used for PCa are: Digital Rectal Examination (DRE), transrectal ultrasound (TRUS), Prostate acid phosphatase (PAP) and Prostate specific antigen (PSA) (Zoorob et al., 2001). In DRE, clinician feels only the backside of the prostate through the rectum .Sometimes it detects cancers before rise in PSA. But sometimes, PSA detect many cancers where the DRE results are not found suspicious for PCa (Catalona et al., 1999). It is considered that DRE alone cannot be recommended for PCa screening (Gerber et al., 1993). A study about PCa screening by DRE and PSA concluded large differences in DRE performance suggesting DRE shows poor performance and it should not be used as a sole screening test for PCa (Schroder et al., 1998). TRUS gives ultrasound generated image of prostate gland. It is reported not a useful screening test because of its poor specificity. It is not useful for early diagnostics because early stage tumors are not palpable. It is only valuable to guide sampling of biopsy specimen (Brawer, 2000). PAP was the first biomarker identified for PCa detection. A study reported positive correlation between its serum level and advancing PCa (Gutman and Gutman, 1938).However, other studies show that analytical and physiological characteristics of PAP is not so good when compared to PSA (Schifman et al., 1987; Brawer and Lange, 1989; Brawer, 2000) .PSA performs better than all other tests but there are problem of false-positives and false-negatives. Still it is the best studied marker for PCa screening (Pienta, 2009).

2.3.1 Prostate Specific Antigen (PSA) Screening

PSA is a 34 k Da proteolytic glycoprotein of kallikrein family of proteases. It is composed of 237 amino acids. Gene encoding PSA is located on chromosome number 19 and is 6 Kb pairs long. Its function is to liquefy human semen. It was first identified in 1969 in seminal fluid. At that time, it was known as "Y-seminoprotein"(Hara et al., 1971). In 1979, it was discovered as a potential biomarker for PCa (Wang et al., 1979). Thereafter, it was detected in adult male serum in 1980(Papsidero et al., 1980). Later on, it was demonstrated that its levels are raised in pathological processes such as hyperplasia, inflammation and PCa. It was also reported that every gram of cancerous tissue raises the serum PSA level ten times more than equal amount of benign tissue (Stamey et al., 1987). However, elevation of the serum PSA values are not specific to PCa, they may also be caused by BPH, prostatitis and instrumentation (Tchetgen and Oesterling, 1997). Studies indicate that there is overlap between s.PSA levels of patients with BPH and organ-defined cancer (Oesterling, 1991).

PSA is produced from the epithelial cells lining the acini and ducts of prostate gland. It circulates in the serum as free or bound/complexed form. Serum half-life of PSA is approximately 2-3 days. It is complexed with two serum proteins; α1 antichymotrypsin and α2macroglobulin. But when it is complexed with α2 macroglobulin, it is completely encapsulated and hence not detected in PSA assay as no epitopes are available to antibodies of assays. Whereas, in case of bound α1 antichymotrypsin, three out of five epitopes are available. Thus, only free PSA, PSA complexed with α1 antichymotrypsin and total PSA values are recorded in PSA assay. Free PSA is less in proportion as compared to bound PSA and less stable than bound. It was found that the concentration of free PSA and total PSA may vary with the extent of cancer (Christensson et al., 1993). Total serum PSA measurement define one's risk for PCa. In general, normal diagnostic range used is 0-4 ng/ml. If this cut-off value is lowered, more cancers are detected but correspondingly more chances of false-positive results. Less than 4ng/ml value is thought to be in normal range. But a study reported that 15% of men diagnosed with PCa had a s.PSA level of <3 ng/ml and 15% of these had a Gleason score of 7 (Thompson et al., 2004)

.Chances of under diagnosis are more than over diagnosis in 4.0-10.ng/ml range (Graif et al., 2007; Pelzer et al., 2007).

Several other concepts have been developed to make the most of favorable diagnostic value of serum PSA. These are PSA density, PSA velocity, age-specific PSA references and quantifying free PSA (Nixon and Brawer, 1997). Ratio of free PSA to total PSA helps in distinguishing patients whose PSA levels are raised due to BPH from those with PCa. It was reported that free/total PSA improves PCa detection significantly (Djavan et al., 1999; Catalona et al., 2000) . Most of the PSA assays are standardized according to WHO 90:10 standard and Hybritech® assay (Loeb et al., 2008).

To address the issue of benefits of PSA screening, two large randomized clinical trials (RCT) were launched in 1990's. They are still ongoing and will provide their complete results in the coming years. Design of both trials is different. Current preliminary results of "European Randomized Study of Screening for Prostate Cancer" (ERSPC) are in favor of PSA screening. Study reported that men who were assigned to be screened showed 20% less death rate from PCa than men who were not assigned to be screened. However, it also concluded that screening do carried a high risk for over-diagnosis and over-treatment (Schroder, 2005).Whereas, RCT from the US trial named (Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) reported that PSA screening with a diagnostic cutoff of 4 ng/ml did not decrease the PCa death rate (Andriole et al., 2005). These trials suggest that PSA screening for PCa saves few lives. Problem is only that to save one life, large number of men needs to be screened. But, we cannot deny that PSA testing is potentially life-saving. What we can do is more research on effectiveness of screening tests for PCa by designing RCT and intelligent use of PSA testing.

2.3.2 Histological Grading/Scoring:

Outcome of screening tests is histopathological examination of prostate tissue biopsies. Once PCa is suspected, it can only be confirmed and diagnosed by histopathological examination of tissue samples. Tumor grading and staging is fundamental in this context. For this, we get biopsies from transurethral resection of

prostate (TURP) and radical prostatectomy (RP). TURP biopsy samples very small proportion of total prostate. RP gives accurate description of pathology of sample. Grading is based on histological patterns appearance. It helps to know whether a cancer is aggressive or not. It reflects how much cancer cells differs from normal healthy cells .Many grading systems have been proposed in the last 75 years. Till now, Gleason system is considered predominant method of grading throughout the world (Rajinikanth et al., 2008). It was developed in 1966 by Dr. Donald Floyd Gleason (Gleason, 1981). After that, it was modified and refined in 1974 (Gleason and Mellinger, 1974). It is categorized as class I prognostic marker by CAP (Bostwick, et al., 2000). Gleason system is based on the observation of degree of differentiation and glandular architecture of the tissue. It defines five grades/patterns with decreasing differentiation. This system is officially recommended by the WHO. It describes the two most common patterns (grade 1-5) of tumor growth; first most prevalent (primary grade) and second most prevalent pattern (secondary grade) giving a score between 2 and 10; in which 2 is the least aggressive and 10 is the most aggressive score. It is also known as Gleason sum. Gleason score can be translated into histological grade ; score 2-4 as grade I (well-differentiated),score 5-7 as grade II (moderately differentiated) and score 8-10 as grade III (poorly differentiated).

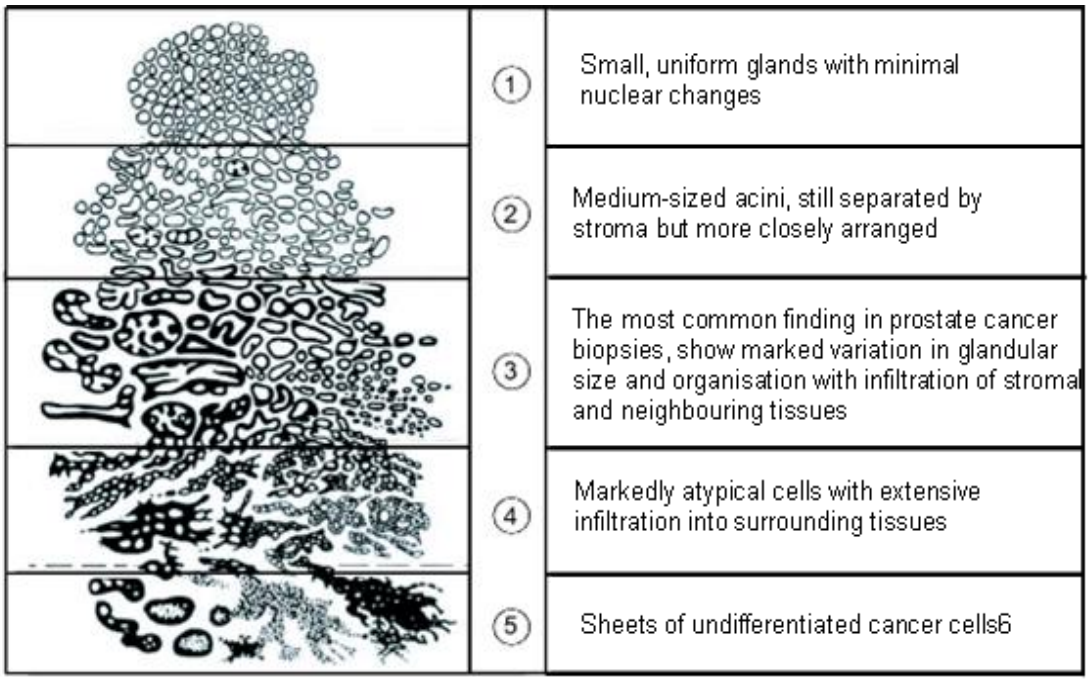

Fig 3 : Gleason grades of the prostate (Gleason & Mellinger, 1974)

Poorly-differentiated is most aggressive and well-differentiated is least aggressive form of PCa. Primary grade carries more weight than second grade. For example; for 7 Gleason score 4+3 is considered more aggressive than 3+4. But there is not much difference between the aggressiveness of score 9 and score 10. Primary pattern is doubled if only one pattern is present. This system follows the 5% rule i.e. second grade should be atleast equal to 5% of cancer, it is not included in the score. Although this system allows only two different patterns in an individual specimen, it was observed that more than two patterns can be present. They are often found in TURP chips (McGowan et al., 1983).

In 2005, International society of Urological pathology (ISUP) provided new modifications in the Gleason grading patterns mainly in pattern three and four (Epstein et al., 2005). According to ISUP, low grades 1 and 2 should be assigned with much caution in case of needle biopsies. They are rarely found in corresponding TURP and RP specimens. So practically, Gleason grading starts from pattern 3. Cribiform architecture rarely represents pattern 3. They should be assigned pattern 4. Even the glands are not found fused; these ill-defined clusters always represents pattern 4. If different cores contain different grades, overall score should be given. This new convention gives the modified Gleason scoring of PCa which consists of the Gleason grade of the predominant pattern plus the highest grade irrespective of its extent that means no 5% rule. It recommends tertiary pattern to be included in the final Gleason scores in case of needle biopsies. But still we have not much supporting evidences for ISUP Gleason score modified system. A study about comparison of classical and modified (ISUP) Gleason scoring system reported that modified system shifts the scores upward due to reclassification of classical pattern 3 into modified pattern 4 (Delahunt et al., 2012).

Some papers consider score 7 as high grade cancer, not included in moderately differentiated cancer. According to them, it should be considered a specific prognostic category (Tefilli et al., 1999). Increasing Gleason grade is correlated positively to histopathological endpoints such as tumor size, extraprostatic extension and lymph vascular space invasion by cancer (Humphrey, 2004).

Gleason grade determination provides greater prognostic significance than available biomarkers (True et al., 2006). Since the system depends upon human interpretation and is prone to subjectivity, it has limited inter and intra pathologist reproducibility. Another limitation is poor agreement on RP specimens and biopsies Gleason score (Mills & Fowler, 1986; Lattouf & Saad, 2002). However, modified Gleason grading system is reported to improve the agreement on RP specimens and biopsies Gleason score (Helpap & Egevad, 2006).

Other scoring system commonly used are Mostofi system, Mayo grading and Bocking combined grading. An important criterion that defines a good histological grading system is inter-observer and intra-observer reproducibility of results. A study compared the agreement between the observers for Mostofi's system, Bocking's system and Gleason grading system. They found Gleason system more reliable in comparison to other systems (Cintra & Billis, 1991). Gleason grading system showed relatively greater interobserver and intra-observer reproducibility and high prognostic significance than available biomarkers (Allsbrook et al., 2001; True et al., 2006). Stage and grade are correlated to each other. Less differentiated grade corresponds to more advanced stage.

Table 2.3.2 Stages and grades of PCa (Lattouf and Saad, 2002)

Stage	**Extent of cancer**	**Gleason grade**	**Histological grade**
Stage A	Confined to prostate and cannot be found by DRE	Gleason sum 2-4	Well Differentiated cells
Stage B	Still confined to prostate	Gleason sum 5-6	Moderately differentiated cells
Stage C	Spread beyond the prostate, but locally spread.	Gleason sum 7	Moderately poorly differentiated cells
Stage D	Spread to lymph nodes, bones etc,	Gleason sum 8-10	Poorly differentiated cells

2.4" Pros- PSA Screening" versus "Con- PSA Screening"

PSA screening helps in diagnosing PCa at a stage that can be cured .It is recommended that PSA screening should begin at the age of 40 years and biopsies are recommended for those males who have persistently rising s.PSA, not returning to the baseline level. PSA derivatives such as PSA density, PSA velocity and % of free PSA should be considered for screening PCa. (Catalona & Loeb, 2005).Data from various studies showed that PCa death rates have fallen in countries where PSA screening is performed (Hankey et al., 1999) and increased in countries where it is not practiced. At the same time, PSA screening is also reported to give false-positive and false-negative results. Till now, various studies have been performed on the use of PSA as a screening test for PCa. They all show different results. Still, it is a controversial issue as there is no agreement among investigators. Some studies are in favor of PSA testing as screening for PCa (Table 2.4.1).

Table 2.4.1: Studies in favor of PSA screening

Year	Reference	Recommendations
1991	Oesterling, 1991	Levels of PSA correlate well with tumor status.
1994	Catalona et al., 1994	Multi-institutional trial of PSA & DRE based screening .Led to U.S FDA approval of PSA as an aid for early detection of PCa using a threshold of s.PSA value of 4.0 ng/ml.
1994	Blackwell et al., 1994	Preoperative serum PSA level has significant predictive value in determining the pathological stage.
1996	Kupelian et al., 1996	Serum PSA profile correlated well with the pathological factors. Preoperative PSA and Gleason score both act as independent predictors of biochemical relapse.
1999	Horninger et al., 1999	High total PSA level with low % free PSA level correlates well with high Gleason score and hence, can predict more aggressive PCa.

2001	Herman et al., 2001	Gleason pattern showed positive correlation with clinical predictors of disease progression including pre-operative PSA values. Primary pattern showed having predictive value for disease progression in score 7.
2003	Punglia et al., 2003	Lower threshold PSA used to recommend prostate biopsies increases the clinical significance of PSA testing.
2004	Catalona et al., 2004	Pro-PSA can detect the more aggressive cancers; can act as more specific marker than total PSA.
2005	Jemal et al., 2005	Studied relation between PSA screening, PCa mortality rate and stage at diagnosis in US cancer registries. Concluded that more the widespread PSA testing, less the advanced stage disease and hence, less the mortality rate.
2005	Nadler et al., 2005	Found that men with pre-operative serum PSA value of 2.6-4.0, 4.1-7.0, 7.1-10.0 and > 10 ng/ml corresponded to 81, 74, 72 & 60 % rate respectively of locally confined cancer. Chance of locally confined cancer at radical prostatectomy correlates to PSA level at diagnosis.
2005	Zhu et al., 2005	PSA progression rate and pathological outcomes were found correlated. PSA level between 2.6-4.0ng/ml can detect significant PCa more frequently.
2006	Galper et al., 2006	Recent PSA era has resulted in reduction in preoperative s.PSA failure and increment in PSA doubling time. Hence, it minimizes the PCa mortality.

2006	Efstathiou et al., 2006	Annual PCa screening appeared more likely to result in indolent recurrence and less likely to cause prostate cancer specific mortality.
2007	Kundu et al., 2007	PSA density (serum PSA/prostate gland volume) helps in determining the PCa aggressiveness.
2007	Thompson and Ankerst, 2007	Increasing PSA levels reflects the increasing aggressiveness of PCa
2008	Loeb et al., 2008	Validated PSA velocity as a marker for PCa aggressiveness. Reported PSAV as an independent predictor of Gleason score.
2009	Gomez-Guerra et al., 2009	PSA screening along with DRE increases the diagnosis yield of aggressive PCa (specifically high Gleason grade PCa)
2009	Schroder et al., 2009	Preliminary data from ERSPC trial reported that PSA screening caused 20% reduction in cancer specific mortality. Early detection saves lives.
2010	Smith et al., 2010	Recommended PSA along with DRE for > 50 year old men with informed decision process, who have at least 10 year life expectancy.

However, despite the evidences supporting the direct positive correlation between serum PSA levels and aggressiveness of PCa, there is concern of false-positive and false-negative results. It is difficult to differentiate indolent tumor types and the aggressive tumor types. PSA screening in this context is not supported by the following investigations presented in table 2.4.2.

Table 2.4.2: Studies against PSA screening

Year	**Author**	**Recommendations**
2000	Schrder et al., 2000	Half of the cases with PSA 0-4ng/ml were aggressive (Gleason score≥ 7). Hence, PSA not correlated with Gleason score
2001	Barry, 2001	This study found that 75% of patients with PSA levels 4-10 ng/ml who undergo biopsy do not have PCa. Hence, PSA screening with this threshold value results in unnecessary cost and inconvenience.
2004	Thompson et al., 2004	PSA screening found 70% false-positive and 25 % false negative prone.
2005	Nadler et al., 2005	Lowering of PSA cut-off (in between 2.6ng/ml to 4.0 ng/ml) resulted in detection of PCa at a rate of 16.2 %. Gleason grade were same.
2008	Smith et al., 2008	Potential benefits of PSA screening not certain, side effects are reported.
2008	Lin et al., 2008	PSA screening causes psychological harms. Benefits not proved.
2009	Andriole et al., 2009	Preliminary results from the first report showed that PSA screening causes no significant reduction in cancer specific mortality.
2009	Draisma et al., 2009	Used data from ERSPC, concluded that PSA screening led to over diagnosis
2010	Wolf et al., 2010	Widespread PSA testing led to increase in diagnosis of patients with locally confined low

		grade Gleason cancer that may not require treatment. Suggests less frequent PSA screening.
2011	Vickers et al., 2011	PSA screening generates frequent false positive results. Its value remains unclear.
2011	Chou et al., 2011	PSA screening leads to non-significant reduction in PCa specific mortality and is associated with over diagnosis.
2011	Drazer et al., 2011	PSA screening in elderly men with less life expectancy is a significant problem.

After reviewing the literature, it can be said that PSA remains the unsolved issue due to imbalance between evidences of potential harms and benefits of PSA screening for PCa. However, we cannot deny the fact that PSA is the only significant available biomarker for PCa detection we have for now. Presently, histological examination is considered “gold standard” for PCa diagnosis and is used in conjunction with PSA test (Chun et al., 2010).There is need to research further to bring more evidences to clarify the correlation between pre-operative s.PSA values and histopathological stages of PCa . Also, patients should be informed and well-educated about risks and benefits of PCa screening (Greene et al., 2009). Intelligent use of PSA testing along with improvement in its clinical performance can minify PCa specific mortality rate with tolerable and admissible side effects (Loeb and Catalona, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Clinical material

The study included formalin-fixed non-biohazardous putative prostate cancer prostatectomy and TURP specimens, procured from Department of Pharmacology, PGIMER, Chandigarh. Tissue specimens were transported at 4° C in ice-box.

3.2 Procedure

3.2.1 Histological Processing of Provided Samples

3.2.1.1 Sample Collection

Fixed non-biohazardous prostate tissue samples from patients who underwent observation and prostatectomy (n=2) and patients who underwent TURP (n=2) were collected from PGIMER, Chandigarh in the month of March, April and May, 2012. These tissue samples were received coded as per the approval of protocol by IEC (Institutional Ethical Committee).

Codes received were: CUP_P1, CUP_P2, CUP_T1 and CUP_T2 respectively.

3.2.1.2. **Fixation** (Bhagwan and Kandasamy, 1988)

After tissue acquisition, they were immediately first preserved in fresh 10% NBF. Fixing the tissue as soon as possible was necessary to prevent autolysis and putrefaction. Fixation preserves the tissue close to its natural state. 10% NBF (Neutral Buffered Formalin) with a pH of 6.0-7.0 was used as fixative. Fixation in formalin is best suited because of induced protein-nucleic and protein-protein cross linkages that preserve the tissue and retain morphology intact. Then after, tissue can be stored at room temperature and used for 3-6 months.

3.2.1.3 Grossing (Rosai, 2004)

Measurement in three dimensions (length ×width ×height), weight (when fresh) and color-appearance of specimens were recorded.

3.2.1.4. **Slicing** (Rosai, 2004)

Partial sampling was performed as it is time and cost-effective. CUP_P1 and CUP_P2 specimens were rendered into slices through transverse sectioning. One single mid-anterior section from both left and right sides, 2 left and 2 right posterior sections, random transverse section and section from the site of entry of ejaculatory duct were taken at a thickness of about 3-4mm. Landmarks that guided the identification of posterior, anterior, apex, base, left and right sides :

- Contour of gland used to recognize anterior and posterior part. Posterior side is relatively flat whereas anterior side is convex and round.
- Apex is narrow and cone-shaped, whereas base is broad and flat.
- U shaped prostatic urethra is located near the centre of each slice (transverse); its arms point posterior part whereas convexity corresponds to anterior part.

Slicing was done from apex to base and was designated as A, B, C and D from the same direction. It helped to understand the orientation.

3.2.1.5. **Tissue Processing** (Bhagwan and Kandasamy, 1988)

- **Dehydration:** Dehydration was performed with series of graded alcohols (70 %, 80 %, 90%, and 3 changes of absolute alcohol for 1 hour each) in order to remove water from the tissue and to replace it with alcohol. It was done with series of graded 2-propanol (from SD Fine Chem) to minimize distortion of tissue. Volume of alcohol used was 50-100 times to that of tissue.
- **Clearing**: Next step followed was clearing which is the process of replacing the dehydrating fluid with the solvent that should be completely miscible with both dehydrating fluid and embedding medium. It was achieved by using xylene (from Loba Chemei). Two changes of xylene for 1 hour duration were done.
- **Infiltration:** It was infiltrated by melted paraffin wax which provided sufficient external support for sectioning. Paraffin wax used in present research was of melting point between 58-60 °C, procured from HIMEDIA. Infiltration was done using two changes of molten paraffin wax for 1 hour each maintained at 60ºC. Left overnight in molten paraffin wax at 60ºC.

- **Blocking:** L types of mould systems were used for blocking. Mould was first filled with melted paraffin wax. Then, by using warm forceps tissue was selected. Tissue was carefully oriented and gently firmed into the wax with forceps. This ensured that the correct orientation is maintained. . A small paper slip with label was also gently firmed in one side. Blocks were allowed to cool at room temperature and then kept in refrigerator for 30 minutes.
- **Sectioning:** The blocks were sectioned in 4-6 µ thickness with the help of microtome. We used rotary microtome YD-2508 from Biocraft scientific systems. HIMEDIA slides used for mounting sections were thoroughly cleaned to make it grease free. Applied little egg albumin for firm attachment of the sections. The ribbons were stretched in the warm water bath maintained at 56ºC. Minimum 3-4 sections were prepared from each paraffin block. Dewaxed on hotplate after drying.
- **Staining:** Staining included treatment with H&E stain to increase the contrast of the tissue and to highlight the specific features of interest. Harris's haematoxylin and eosin yellow from HIMEDIA were used. Both are charge-based stains. Haematoxylin is blue colored and it stains acidic molecules whereas eosin stains basic molecules shades of pink, orange and red.

Table 3.2. Staining protocol as follows

Steps	**Medium**	**Time**
Step-1	Xylene	10 minutes, two changes
Step-2	Transferred to Absolute alcohol	2 minutes
Step-3	Transferred to 80% alcohol	2 minutes
Step-4	Transferred to 50% alcohol	2 minutes
Step-5	Dip in distilled water	1 minute
Step-6	Staining with Harris's Haematoxylin	5 minutes
Step-7	Dip in 1 % acid alcohol	1 dip only
Step-8	Washed in running tap water	15 minutes
Step-9	Counter stained with Eosin Yellow	10-20 seconds.
Step-10	Wash in distilled water	1 minute
Step-11	Dehydration in 90% and Absolute alcohol.	2 minutes each

- **Mounting:** Stained slides were cleared in xylene for 15 minutes in two changes. Allowed to dry and then mounted with HIMEDIA DPX. Mounting medium DPX is colorless and fast-drying. It prevents moisture from developing under the coverslip and also prevents the clouding of the specimen. H&E staining produced :
  - Nuclei as bright blue
  - Smooth muscles as bright pink
  - Collagen and cytoplasm as pale pink
  - Erythrocytes as orange red
- **Observation under light microscope:**

  Then examined and studied under Olympus light microscope with digital E-450 DSLR camera with resolution of 10 megapixels. Photomicrographs were taken at 4X, 10X and 40 X resolutions. Detailed evaluation and revision of diagnostic features of each slide was done. After that images were stored and maintained an image library for analysis and future records.

3.2.2 Evaluation of H&E stained prostate tissue section slides (Rosai, 2004)

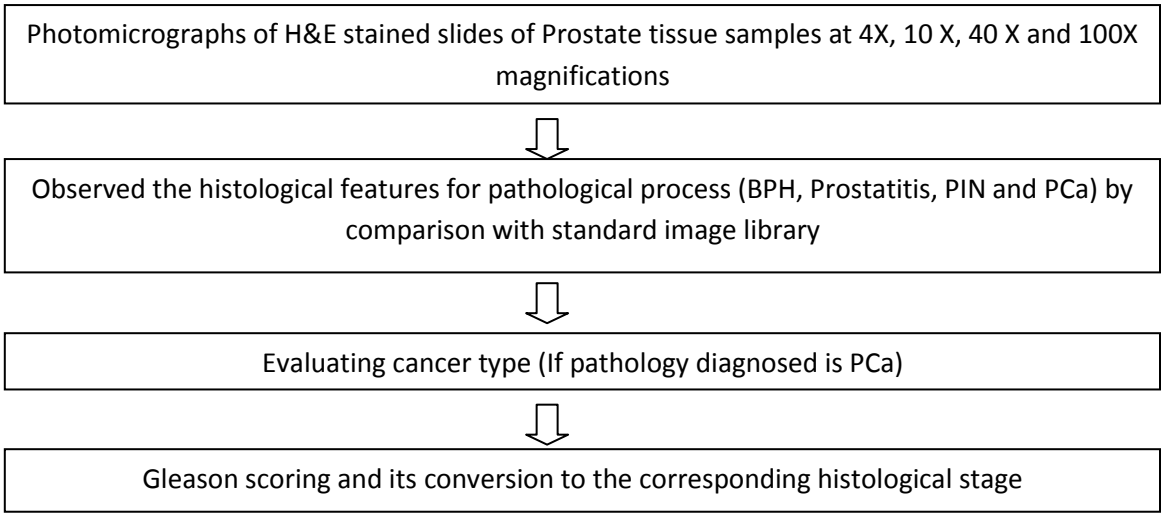

Fig 4. Methodology followed for evaluating pathology of H&E slides of prostate tissue specimens.

Image library of reference images were used to improve the reproducibility of results. A standard image library of normal prostate slides was used as a control. Image library of different pathological types and PCa Gleason grades was prepared in order to improve the understanding (Fig. 5a and Fig. 5b).

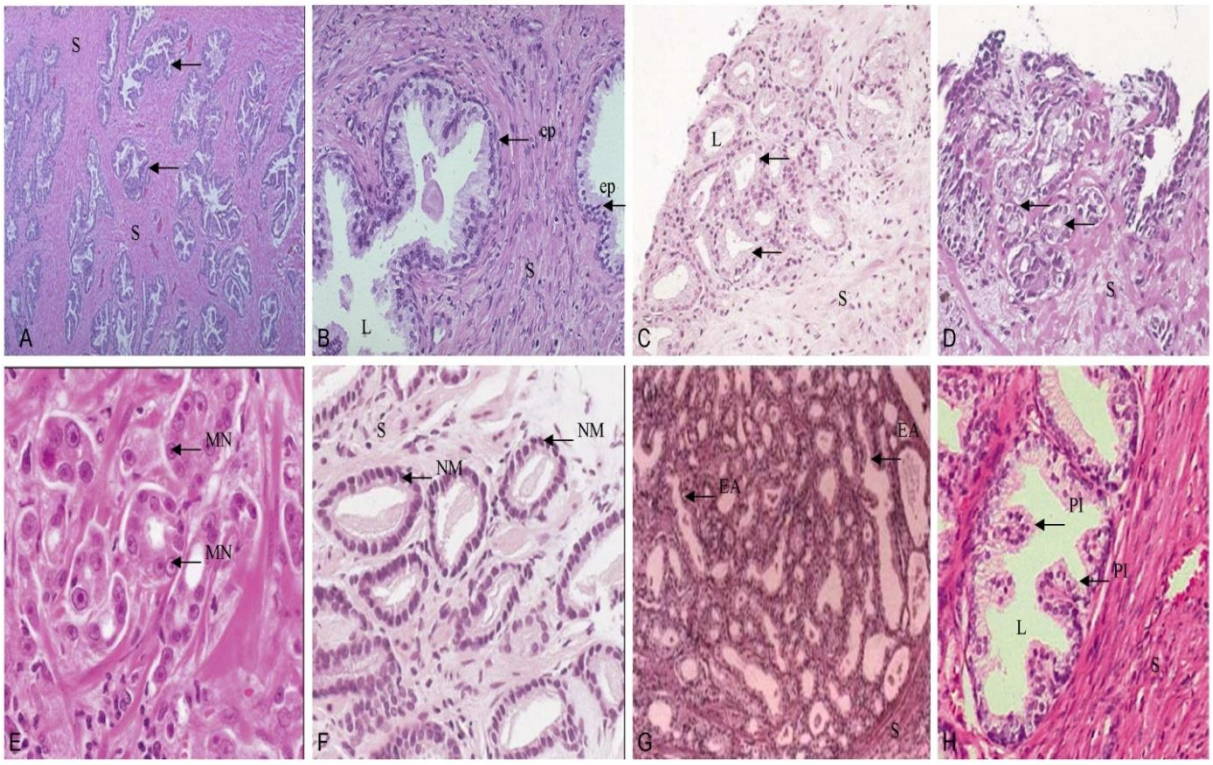

Fig 5(a). Photomicrographs of H&E sections of normal prostate, prostate adenocarcinoma & benign prostatic hyperplasia (BPH)

(A), normal prostate section with glands (arrows) & fibromuscular stroma in equal proportion , 40X (NHI, 2012) **(B)**, Normal prostate with well-defined epithelial cells (arrows), 400X (NHI, 2012) .**(C),** aggregate of closely packed small acini (arrow) in well-differentiated prostate adenocarcinoma, 300X (Thorson and Humphrey, 2000). **(D)**, small glands (arrows) invading large glands with absence of basal cells in prostate adenocarcinoma , 300X (Thorson and Humphrey, 2000). **(E)**, prominent macronucleoli (arrows) in prostate adenocarcinoma nuclei at 500X (Thorson and Humphrey, 2000). **(F)**, hyperchromatic enlarged nuclei (arrows) in prostate adenocarcinoma, 400X (Thorson and Humphrey, 2000). **(G)**, circumscribed elongated nodules (arrows) of glands in benign prostate hyperplasia, 40X (Bostwick, 2005) **(H)**, increased papillary infoldings (arrows) with stromo-glandular hyperplasia in benign prostate hyperplasia, 400X (Roehrborn, 2008).

S: stroma, L: lumen, MN: macronucleoli, NM : Nucleomegaly, EA: elongated acini, PI: papillary infolding, ep : epithelial cells.

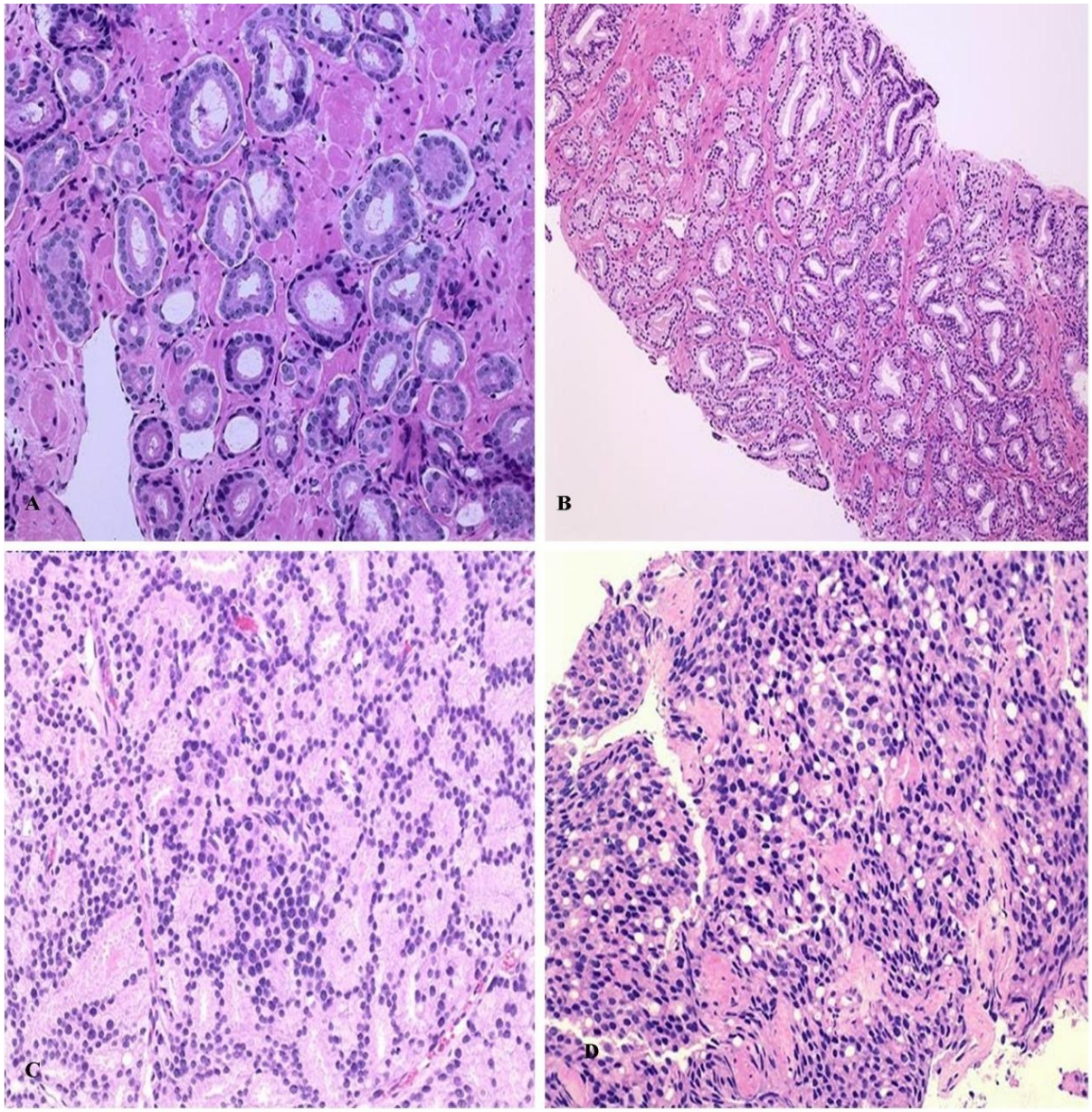

Fig.5(b). Photomicrographs of Gleason grades of Prostate Cancer. **(A),** grade 2 showing round glands ,relatively uniform sized, no shape distortion, 100X. **(B),** grade 3 showing variation in shape, size and spacing, angular distorted glands, 40X. **(C),** grade 4 showing glandular fusion, mostly occluded lumen, 100X. **(D),** grade 5 showing loss of glandular lumen, 100X. (Source : WP, 2012).

3.2.2.1 Identification of Certain Key Features and Histological Details of Pathology in H&E stained Slides of Provided Prostate Tissue Specimens

Standard images of H&E stained normal prostate tissue sections were used as control (Fig.5). Histological features of H&E stained normal prostate tissue sections are (Rosai, 2004):

1. Presence of uniform sized acini with uniform spacing (Fig 5a, A).

2. Presence of well-differentiated luminal and basal epithelial cell layers (Fig 5a, B).

3. Glandular and stromal component present in equal proportion (Fig 5a, A).

4. No nuclear enlargement and hyperchromatic nuclei (Fig 5a, B).

5. No prominent nucleoli present (Fig 5a, B).

6. No dilation or elongation of acini present (Fig 5a, A).

7. No infiltration of stroma by luminal epithelial cells (Fig 5a, B).

8. No inflammatory cells present (Fig 5a, A).

9. No sinusoidal spaces present (Fig 5a, A).

Slides were observed for diagnostic criterion histopathological features of the following pathologies of the prostate :

- **Benign Prostatic Hyperplasia (BPH)** (Bostwick, 2005)

1. Evident cell proliferation and increase in stromal component.

2. Presence of small sinusoidal spaces.

3. Uniform spacing with presence of more smooth muscle and less elastic tissue in stroma.

4. Dilated glands often containing multiple corpora amylacea (Fig.5a, G).

5. Well defined basal cell layer and columnar/cuboidal epithelial cell layer (Fig.5a, G).

6. Bland and small nuclei are present with no nucleomegaly (Fig.5a, G).

7. Small clusters of lymphocytes may be present.

8. Focal proliferation may be seen.

9. Increased papillary folding towards lumen (Fig.5a, H).

- **Prostatitis** (Rosai, 2004)

1. Aggregates of histocytes, lymphocytes and plasma cells are seen.

2. Scattered multi-nucleated cells centered on acinus.

- **Prostate Intraepithelial Neoplasia (PIN)** (Rosai, 2004)

1. Presence of abnormal epithelial cell proliferation.

2. In low grade PIN, marked variation in size and shape of nuclei.

3. In high grade PIN, small aggregates of cells protrude in the lumen.

4. Prominent basal cell layer present.

5. Large branching glands with papillary infolding.

- **Prostate Adenocarcinoma (PCa)** (Rosai, 2004)

1. Tall columnar epithelial cells disappear and start infiltrating the surrounding stroma.

2. Irregular crowded glands and nest of poorly formed glands.

3. Absence of basal layer is the hallmark of PCa (Fig.5a, F).

4. Straight luminal border.

5. Broken basement membrane.

6. Nuclear enlargement (Fig.5a, F).

7. Hyperchromatic nuclei (Fig.5a, F).

8. Perineural invasion.

9. Luminal obstruction (Fig.5a, E).

10. Less prostatic luminal secretions.

11. Prominent nucleoli (Fig.5a, E).

12. Presence of blue mucin and crystalloids

- **Gleason Grading/Scoring** (Rosai, 2004)

1. Gleason pattern 1 features are separate, closely packed uniform round glands with well-defined margins. They do not infiltrate into surrounding benign tissue. This pattern is rarely present.

2. Gleason pattern 2 must satisfy the three R's i.e. round, relatively uniform sized and regular spacing. They are loosely packed with loose margins. There is no shape distortion in pattern 2 and intervening stroma is present. There may be minimal invasion into adjacent benign tissue (Fig 5b, A).

3. Pattern 3 is the most common pattern with still recognizable glands having variable spacing and loss of intervening stroma. Glands are variable in size, shape and spacing. Some of the cells may have left the glands and started infiltrating the surrounding tissue (Fig 5b, B).

4. One of the most important key features for defining pattern 4 is acinar fusion forming chains and chords, no longer completely separated by stroma. Cribriform acini are feature of pattern 3 but when these sieves like masses of acini lose their round appearance and become solid, they indicate transition to pattern 4. Their lumen is not completely encircled by the epithelium (Fig 5b, C).

5. Absence of acinar lumens indicates pattern 5. Sheath of cells are present throughout the tissue. Single cells invading stroma are seen. Hallmark is complete loss of glandular lumina (Fig 5b, D).

CHAPTER FOUR

RESULTS

4.1 Results of Histopathology Study on Putative Prostate Cancer Tissue Specimens

Putative PCa prostatectomy specimens (n=2) and TURP specimens (n=2) were collected in the month of March, April and May, 2012 after informed consent. They were received coded as: CUP_P1, CUP_P2, CUP_T1 and CUP_T2 respectively. Standard images of normal prostate H&E slides were used as a control. Image library of H&E slides of PCa, benign prostatic hyperplasia (BPH), prostate intraepithelial neoplasia (PIN), prostatitis and Gleason grades was also prepared and used for understanding of diagnostic criterion histological features (Fig.5).

4.1.1 Macroscopic Description and Microscopic Description of CUP_P1 specimen

Received specimen was grossed.

Measurement: 2.9 cm × 2.8 cm × 1cm (Length× Breadth× Thickness) ; It was 2.8 cm from superior to inferior, 2.9 cm from left to right and 1cm from anterior to posterior

Appearance: Creamish brown in color. Left and anterior part was spongy. Right anterior part was slightly solid and firm.

Specimen was fixed in 10% NBF for 36 hours. It was rendered into thin slices (3-4mm) transverse alternate right and left posterior sections (P-AR, P-AL, and P-CR, P-CL) from apex, single mid section comprising anterior, posterior, left and right part (P-B), single mid left and right anterior section (P-D) and a random section (P-T). Site of entry of ejaculatory duct (P-E) was also sliced as it is one of the challenging sites for detecting PCa. All the sections were subjected to paraffin embedding and H&E staining. These slides were then examined at 4X, 10X, 40X and 100X resolutions (objective), imaged and histopathology was studied. Benign Prostatic Hyperplasia (BPH) is assigned on the basis of histological details given in table 4.1.1.

Table 4.1.1: Results of histopathological study on CUP_P1 specimen

Section Code	Region of sampling	Histological features suggesting BPH	Histological features that do not suggests carcinoma	Fig ref.
P-AR & P-AL	First posterior right and left sections from apex.	▪ Presence of distended acini and increase in diameter of lumen in acini. ▪ Many acini in nodular pattern along with intervening stroma. ▪ Tufted infoldings and increased secretions in lumen. ▪ Focal proliferation of secretory cells. ▪ Faintly eosinophillic cytoplasm at the luminal border.	▪ Cells making glands are normal in appearance with well-defined epithelial cells. ▪ Absence of basal layer is hallmark of prostate cancer. Intact basal layer present. ▪ No infiltration of stroma by epithelial cells. ▪ No uniform proliferation of small acini.	Fig.6(B,C,E)
P-CR & P-CL	Second posterior right and left sections from apex.			Fig.6(A,D,F)
P-B	Single middle transverse section comprising anterior, posterior, left and right regions. .	▪ Acinar elongation with prominent epithelial cells. ▪ Cystic dilation with significant secretions in the lumen. ▪ Many corpora amylacea. ▪ Papillary infoldings towards lumen. ▪ More smooth muscles.	▪ Significant stroma present. ▪ Well-defined epithelial cells present. ▪ No crowding of acini. ▪ Basal cells can be seen. ▪ No nuclear enlargement. ▪ No prominent nucleoli seen. ▪ No luminal obstruction seen.	Fig 7

Section Code	Region of sampling	Histological features suggesting BPH	Histological features that do not suggests carcinoma	Fig.ref
P-D	Left and right mid-anterior section.	▪ Elongated dilated acini present. ▪ Increased epithelial tufting. ▪ Mild inflammatory mononuclear cells present. ▪ Bland nuclei. ▪ Necrotic cells in lumens of acini. ▪ Focal secretory epithelial cells proliferation.	▪ Well-differentiated epithelial cells present. ▪ No hyperchromatic nuclei. ▪ Significant stroma present. ▪ No uniform proliferation of small acini. ▪ No nuclearmegaly. ▪ No prominent nucleoli.	Fig 8
P-E	Transverse section of site of entry of ejaculatory duct.	▪ Increase in stromal component. ▪ Capillary vessel proliferation. ▪ Papillary infoldings towards lumen along with some cells.	▪ No nuclear enlargement and hyperchromatic nuclei. ▪ No invasion of stroma. ▪ Basal cell layer present. ▪ No crowding of glands.	Fig 9
P-T	Random tranverse section.	▪ Capillary vessel proliferation. ▪ Infolded epithelium with cells in lumen. ▪ Cystic dilation of acini with intraluminal secretions inside.	▪ No hyperchromatic nuclei. ▪ Well-defined epithelial cells present. ▪ No infiltration of stroma by epithelial cells. ▪ No luminal obstruction is seen. ▪ No prominent nucleoli seen.	Fig 9

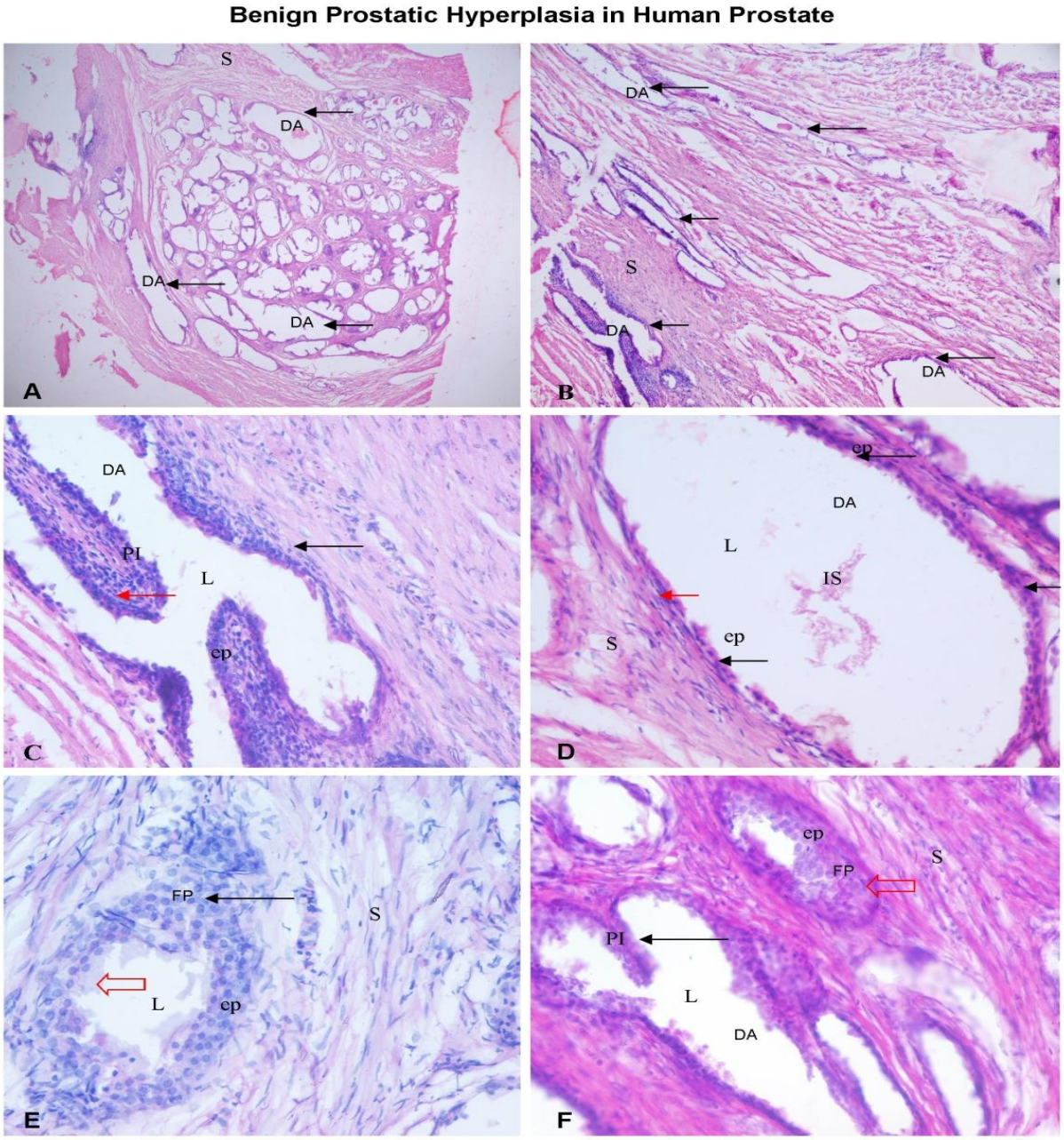

Fig. 6 Photomicrographs of H&E slides of posterior right & left transverse sections of CUP_P1 specimen demonstrating BPH. **(A),** distended acini (DA, arrows) ,increase in diameter of acini, 40X . **(B&C),** distended acini (DA, black arrows), more stromal component & intact epithelial cells (ep, red arrows),100X and 400X. **(D),** distended acini (red arrow) with intraluminal secretion & intact epithelial layers (black arrows),400X .**(E),** faintly eosinophillic cytoplasm at luminal border (red block arrow) & focal secretory cell proliferation (FP, black arrows),400X. **(F),** papillary infolding towards lumen (PI, black arrow) & focal proliferation (FP, block arrow) in acini ,400 X.

S:stroma,ep:epithelial cells, L:lumen, DA: distended acini, PI: papillary infolding, FP: focal proliferation, IS: intraluminal secretion.

Benign Prostatic Hyperplasia in Human Prostate

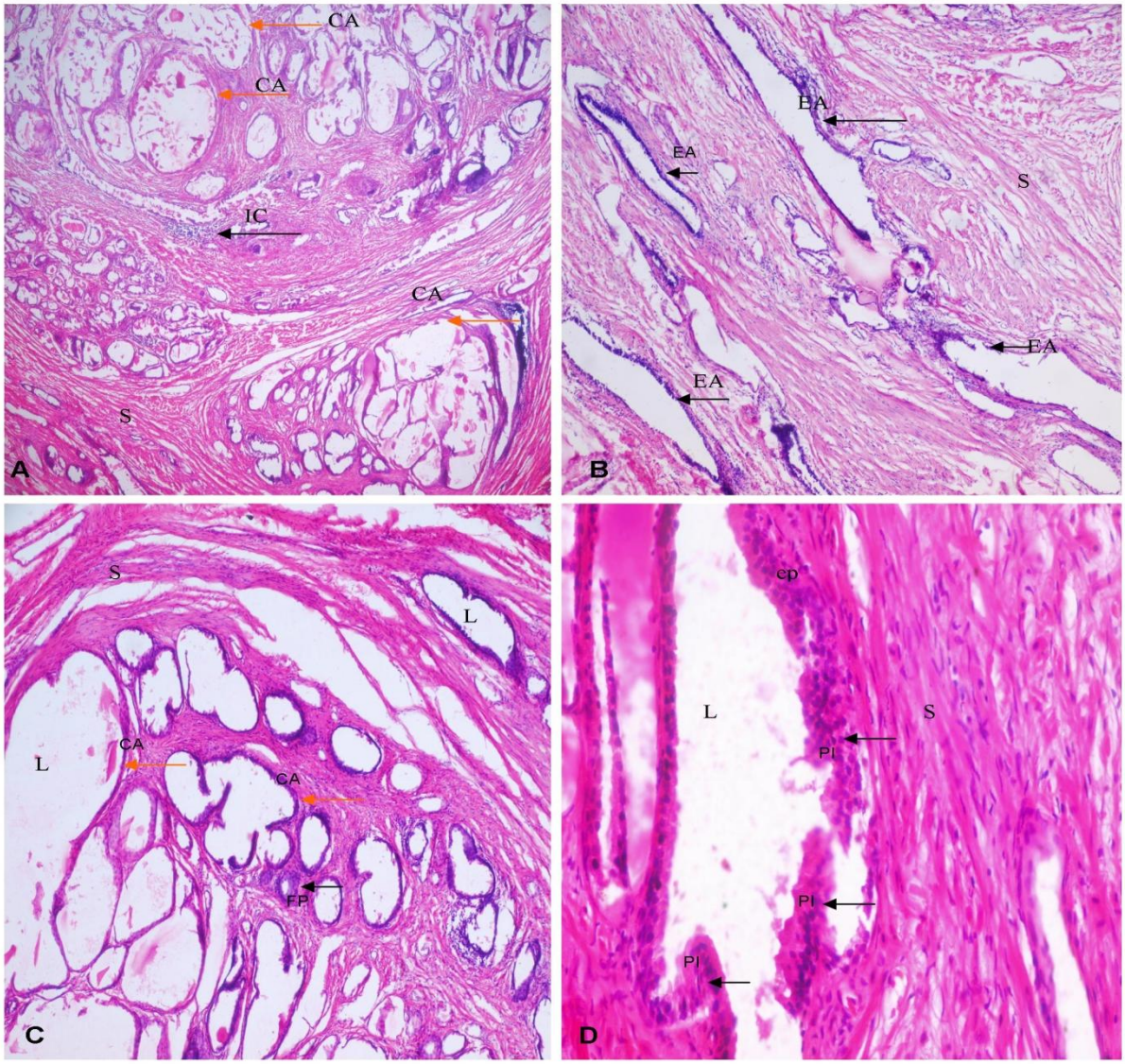

Fig.7 Photomicrographs of H&E slides of single mid-anterior transverse section of CUP_P1 specimen demonstrating benign prostate hyperplasia (BPH). **(A)**, cystic acinar dilation (CA, red arrows) with intraluminal secretions, increased stromal smooth muscles and mild inflammatory mononuclear cells present (IC, black arrows) ,40X . **(B)** , elongated acini (EA, black arrows) , increased stromal content with well-differentiated epithelial layers (ep) ,100X . **(C)** , cystic acini (CA, red arrow) and focal proliferation of small acini (FP, black arrow), 100X . **(D)**, papillary infoldings towards lumen (PI, black arrows) with secretions ,400 X.

S: stroma, L: lumen, ep: epithelial cells, CA: cystic acinar dilation, PI: papillary infolding, FP: focal proliferation, IC: inflammatory cells, EA: elongated acini.

Benign Prostatic Hyperplasia in Human Prostate

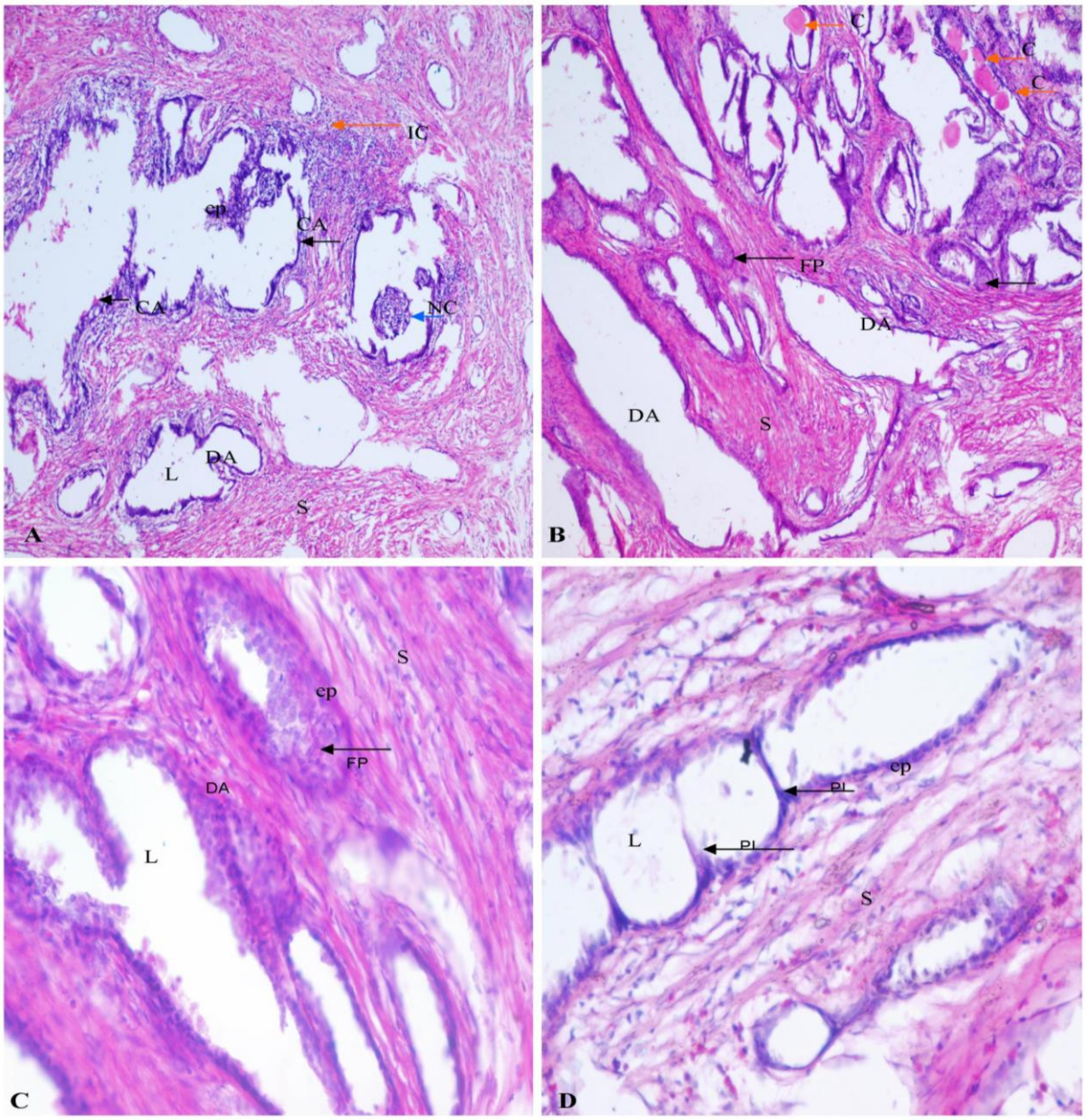

Fig.8 Photomicrographs of H&E slides of single mid transverse section of CUP_P1 specimen demonstrating BPH. **(A)** , Cystic acini (CA, black arrows), epithelial tufting, mild inflammatory mononuclear cells infiltration (IC, red arrow), necrotic cells debris in lumen of acini (NC, blue arrow) ,40X .**(B&C)** , dilated acini (DA), focal proliferation of secretory cells (FP, black arrows), multiple corpora amylacea (C, red arrows) 100X & 400X respectively. **(D)**, papillary infoldings (PI, black arrows) towards lumen, 400 X.

S: stroma, ep: epithelial cells, L: lumen, PI: papillary infoldings, FP: focal proliferation, C: corpora amylacea, NC: necrotic cells, IC: inflammatory cells

Benign Prostatic Hyperplasia in Human Prostate

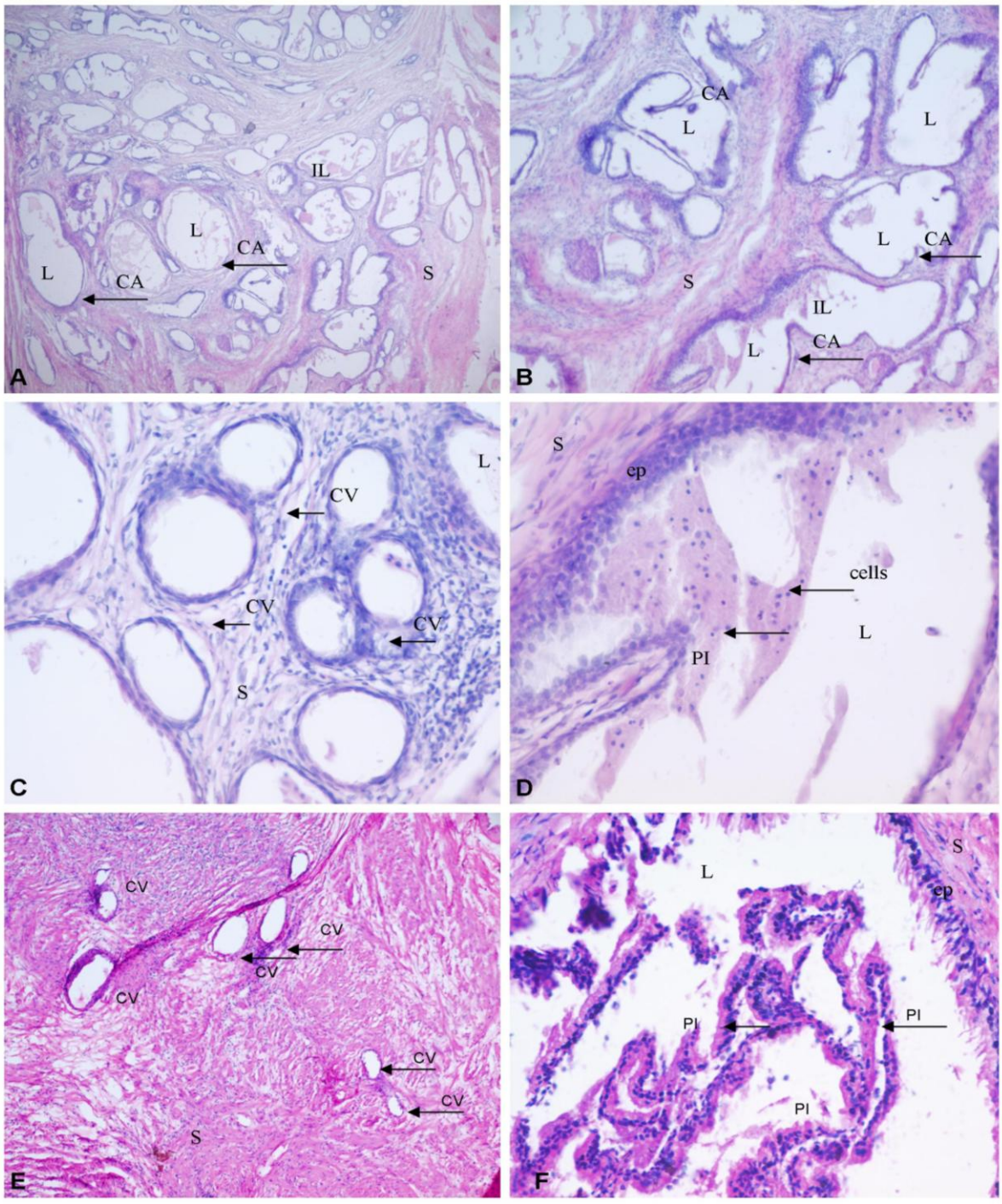

Fig. 9 Photomicrographs of H&E slides of random transverse section and transverse section of site of entry of ejaculatory duct of CUP_P1 specimen demonstrating BPH. **(A&B),** cystic dilation of acini (CA, arrows) ,with intraluminal secretions & epithelial infolding, 40X &100X . **(C),** increased capillary vessel proliferation (CV, arrows) ,400X. **(D),** infolded epithelium with cells in lumen (PI, arrows) ,40X .**(E),** increase in stromal component with capillary vessel proliferation (CV, arrows), 40X. **(F),** increased papillary infolding of epithelial layers (PI, arrow) ,400 X.

4.1.2 Macroscopic Description and Microscopic Description of CUP_P2 Specimen

Received prostatectomy specimen was grossed:

Weight: 7.0 grams

Measurement: 2.1 cm × 1.8 cm × 0.8cm (Length× Breadth× Thickness)

Appearance: Creamish brown color with yellowish grey bulging nodular region.

It was then followed by fixation in 10% formalin for 36 hours. Two transverse sections (P-T1 and P-T2) of 3-4mm thickness were taken from grossly visible light yellowish nodule from sample. Sections were subjected to paraffin embedding. After paraffin embedding, these sections were placed onto microscopic glass slides and stained with hematoxylin-eosin stain (procedure outlined in materials and methods). These slides were then examined at 4X, 10X, 40X and 100X resolutions, imaged and histopathology was studied. Benign prostatic hyperplasia is assigned on the basis of histological details given in table 4.1.2.

Table 4.1.2: Results of histopathological study of CUP_P2 specimen

Section Code	Histological features suggesting BPH	Histological features that do not suggests carcinoma	Fig ref.
P-T1 and P-T2	▪ Increased tufting of epithelial cell layers towards lumen. ▪ Some dilated acini with increased intraluminal secretions. ▪ Bland nuclei present. ▪ Multiple corpora amylacea present.	▪ Presence of well-defined epithelial cell layers ▪ No nuclear enlargement ▪ No stromal invasion ▪ No hyperchromatic nuclei ▪ No prominent nucleoli ▪ No infiltrative growth pattern	Fig 10

Benign Prostatic Hyperplasia in Human Prostate

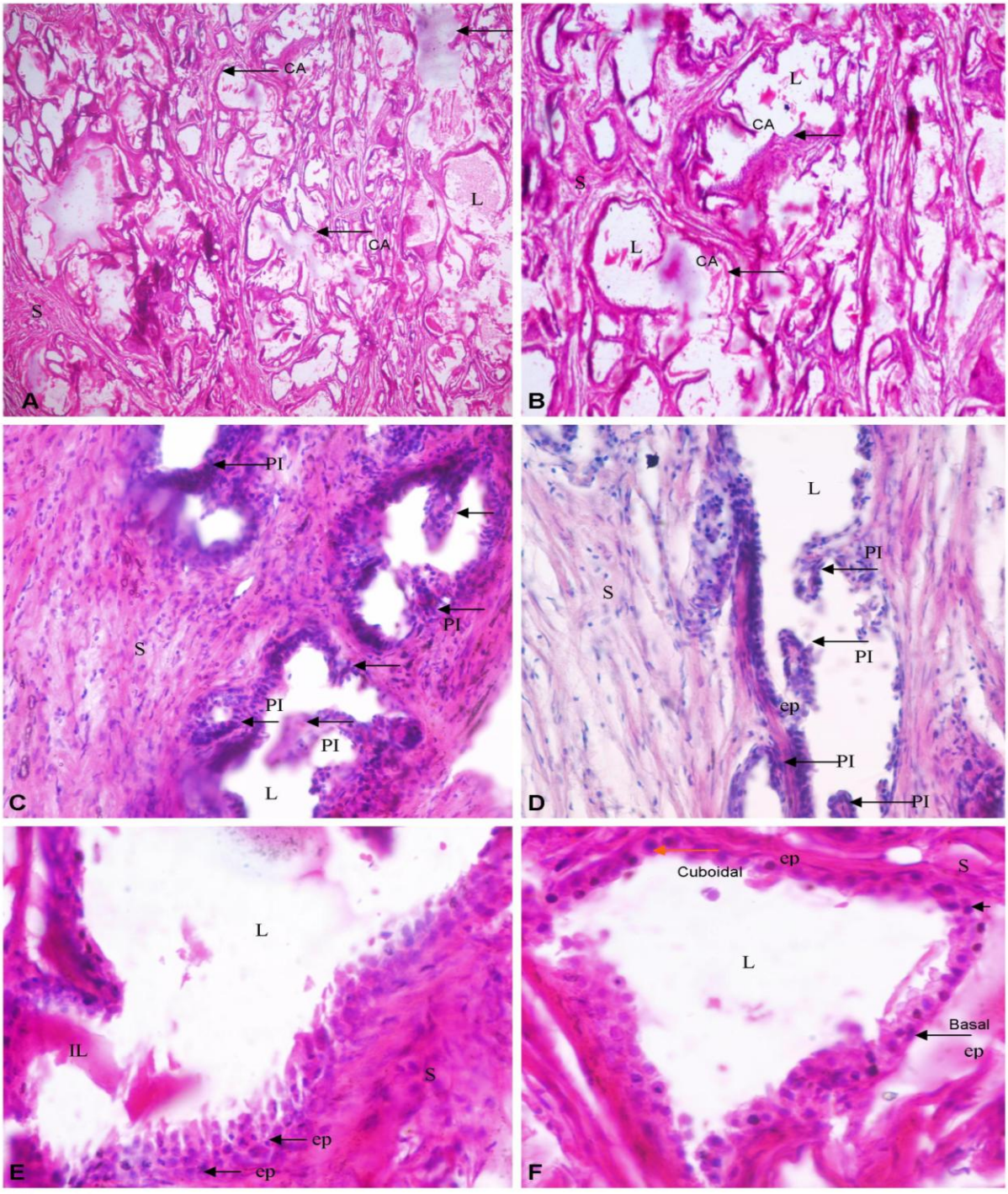

Fig. 10 Photomicrographs of H&E slides of transverse sections of CUP_2 specimen demonstrating BPH. **(A&B),** cystic dilation (CA, arrows) ,with intraluminal secretions & papillary infolding , 40X & 100X . **(C&D),** increased epithelium papillary infolding towards lumen (PI, arrows), 400X . **(E),** well-defined epithelial cell layers (arrows) with secretions in lumen, 400X. **(F),** well-defined cuboidal secretory epithelial cell layer (red arrow) with visible basal cells (arrow), 400 X.

ep: epithelial cells , S: stroma, L: lumen, PI: papillary infolding, CA : cystic acini, IL: intraluminal secretions.

4.1.3 Macroscopic Description and Microscopic Description of CUP_T1 Specimen

Received TURP specimen was grossed. Macroscopic description involves weight, measurement and appearance

Weight: 7.2 grams

Measurement: 1.6cm×1.2cm×0.8cm (Length× Breadth× Thickness)

Appearance: Creamish brown

No. of fragments: Small 12 bits

It was then followed by fixation in 10% formalin for 12-14 hours. Fragments were submitted for paraffin embedding. Two blocks were made. After paraffin embedding, these sections were placed onto microscopic glass slides and stained with hematoxylin-eosin stain (procedure outlined in materials and methods). These slides were then examined at 4X, 10X, 40X and 100X resolutions, imaged and histopathology was studied.

Table 4.1.3: Results of histopathological study on CUP_T1 specimen

Section Code	Histological features suggesting BPH	Histological features that do not suggests carcinoma	Fig. ref
CUP_T1A	▪ Bland nuclei present ▪ Dilated acini with significant prostatic secretions ▪ Multiple corpora amylacea ▪ Papillary infoldings towards lumen. ▪ Cells in luminal secretion.	▪ Presence of well-differentiated basal cell layer ▪ No crowded glands present ▪ No hyperchromatic nuclei.	Fig. 11(B,C,D)
CUP_T1B	▪ Bland nuclei present ▪ Corpora amylacea ▪ Papillary infoldings towards lumen. ▪ Dilated acini present.	▪ Continuous basal cell layer ▪ No luminal obstruction ▪ No hyperchromatic nuclei. ▪ No nucleomegaly.	Fig. 11(A)

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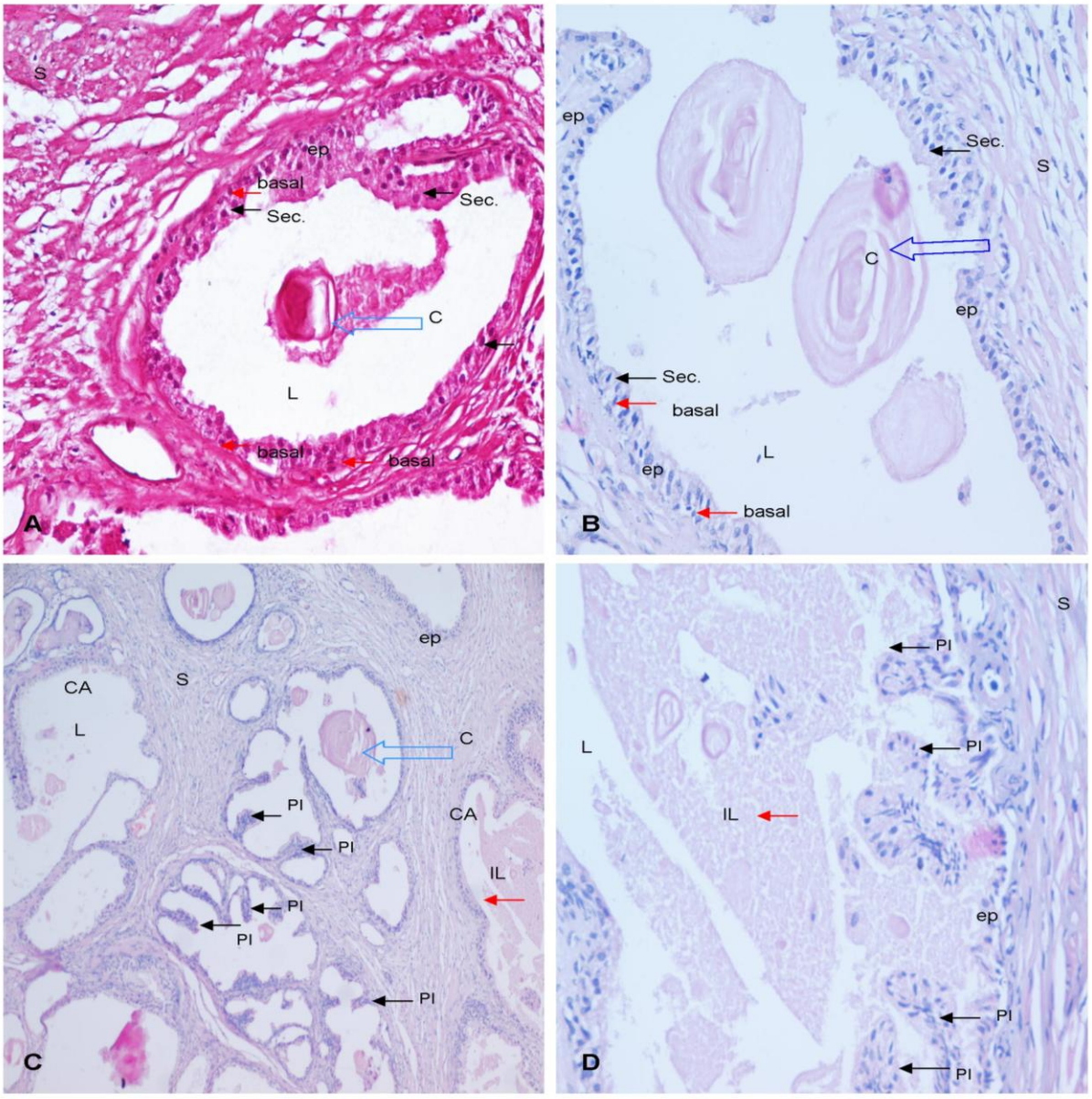

Fig. 11 Photomicrographs of H&E slides of CUP_T1 specimen demonstrating BPH. **(A&B)** , well -defined epithelial basal cells (red arrows) and differentiated epithelial secretory cells (sec., black arrows) with bland nuclei and corpora amylacea (C, blue block arrow) in centre, 400X . **(C)** , glandular hyperplasia showing papillary infoldings towards lumen (PI, black arrows), corpora amylacea (C, blue block arrow), cystic glandular structure with significant prostatic secretions (CA, red arrow), 100X . **(D)**, glandular hyperplasia showing papillary infoldings towards lumen (PI, black arrows) with secretions (IL, red arrow) ,400 X.

ep: epithelial cells, S:stroma, L: lumen, PI: papillary infolding, C: corpora amylacea, CA: cystic acini, IL: intraluminal secretions, sec : secretory epithelial cell.

4.1.4 Macroscopic Description and Microscopic Description CUP_T2 Specimen

Weight: 11.85 grams

Measurement: 5cm ×5.1cm ×1.0cm (Length× Breadth× Thickness)

Appearance: Creamish color and irregular shapes.

No. of fragments: 32

It was then fixed in 10% formalin for 12 hours. Fragments into cassettes of 4 were submitted for paraffin embedding. These sections were placed onto microscopic glass slides and stained with H&E stain. Examined at 4X, 10X, 40X & 100X magnifications.

Table 4.1.4: Results of histopathological study of CUP_T2 specimen

Specimen Code	Histological features suggesting BPH	Histological features that do not suggests carcinoma	Fig.ref
CUP_T2A	▪ Stromal hyperplasia ▪ Capillary vessel proliferation Inflammatory mononuclear cell infiltration ▪ Significant secretions in lumen.	▪ No crowded glands are present ▪ Well-defined basal layer present ▪ No nucleomegaly seen.	Fig. 12(A,B)
CUP_T2B	▪ More stromal component ▪ Capillary vessel proliferation. ▪ Increased smooth muscle	▪ No crowded glands are present ▪ No nuclear enlargement. ▪ No prominent nucleoli seen	Fig 12.(C,D)
CUP_T2C	▪ More stromal component with distended glands ▪ Mild clusters of Inflammatory cells present ▪ Bland nuclei present	▪ Intact basal cell layer present ▪ No infiltration of stroma by epithelial secretory cells. ▪ No luminal obstruction	Fig 13(B,C)
CUP_T2D	▪ Significantly increased stromal component ▪ Increased smooth muscle	▪ No crowded glands ▪ No loss of interveining stroma.	Fig 13(A,D)

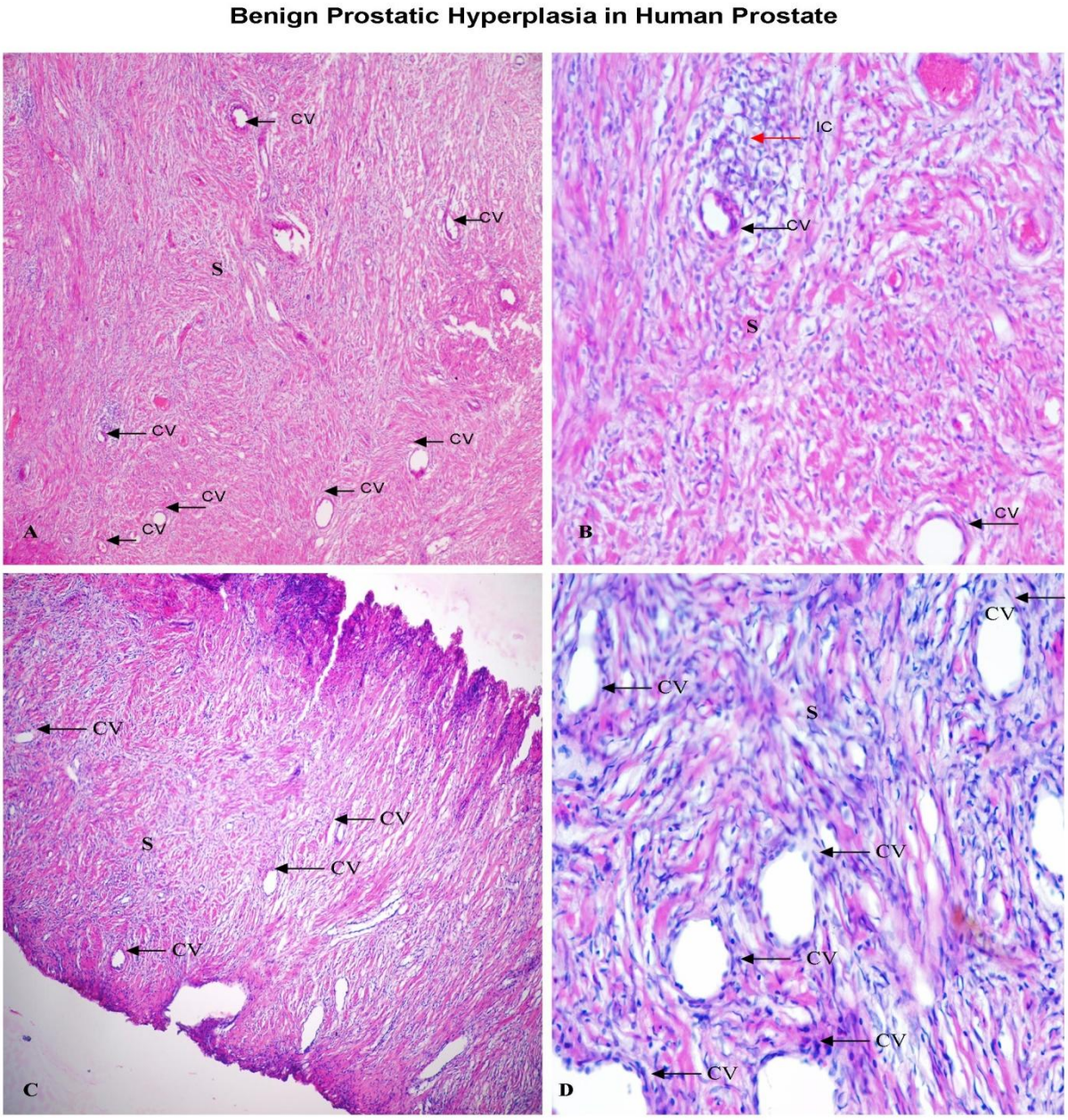

Fig. 12 Photomicrographs of H&E slides of CUP_T2 specimen demonstrating benign prostate hyperplasia (BPH). **(A)** , stromal hyperplasia and capillary vessel proliferation (CV, black arrows), 100X . **(B)** , inflammatory mononuclear cell infiltration (IC, red arrows) with capillary microvessels (CV, black arrows) and secretions (IL) , 400X .**(C)**, stromal hyperplasia showing more stromal component with capillary vessel proliferation (CV, black arrows), 40X. **(D)** capillary vessel proliferation (CV, black arrows), 400 X.

S : stroma, CV: capillary vessel, IC: inflammatory cells,. IL: intraluminal secretions.

Benign Prostatic Hyperplasia in Human Prostate

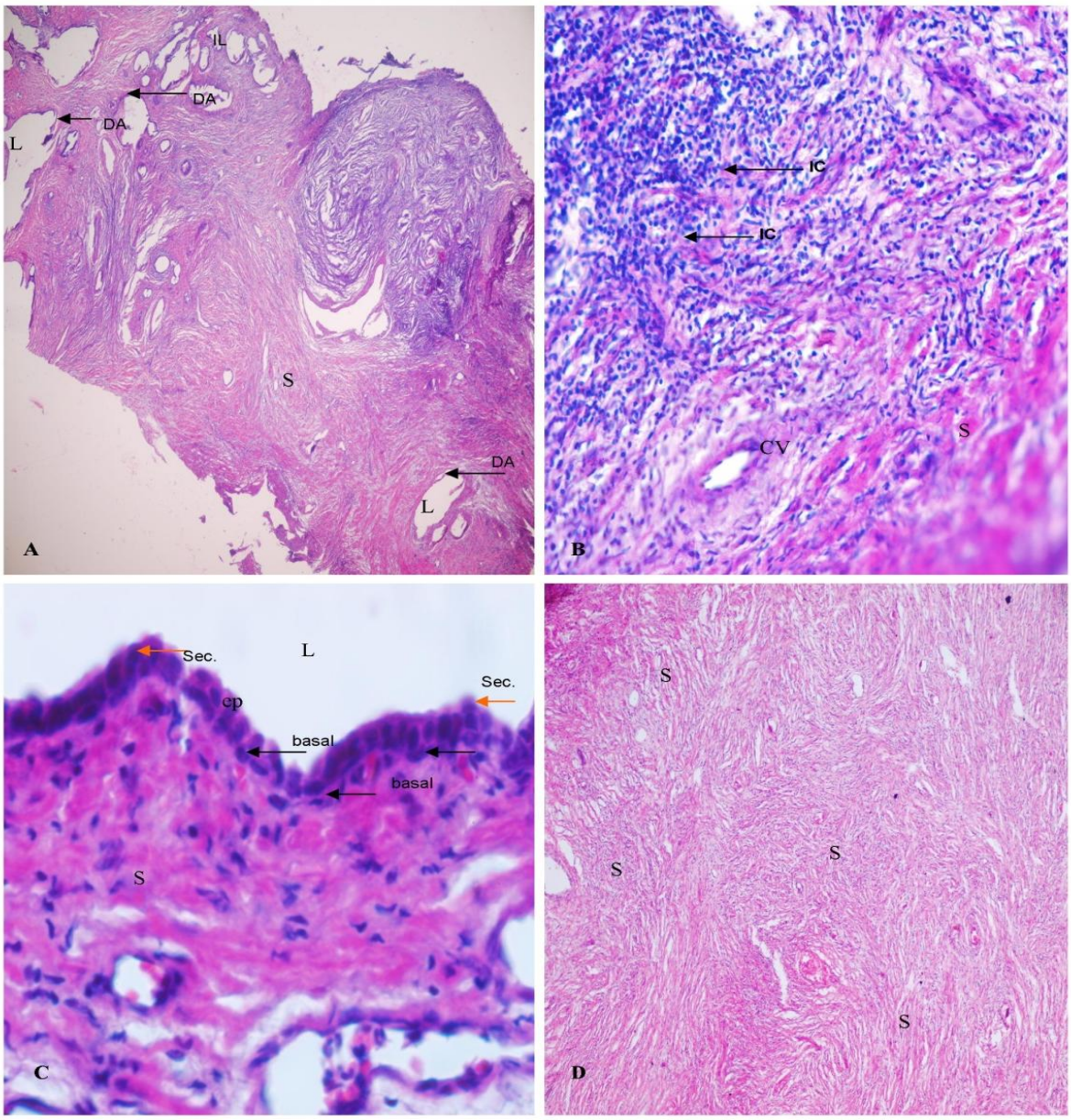

Fig. 13 Photomicrographs of H&E slides of CUP_T2 specimen demonstrating benign prostate hyperplasia (BPH). **(A)** , more stromal component and dilated acini (DA, black arrows) with secretions (IL) in lumen, 40X . **(B)** , inflammatory mononuclear cell infiltration (IC, black arrows) with microvessels (CV), 400X .**(C)**, well-defined basal cell layer (black arrows) and luminal secretory cells (red arrows) with stroma,1000X **D) ,** stromal hyperplasia (visible more interstitium) ,100X.

S: stroma, L: lumen, ep: epithelial cells, IC: inflammatory cells, IL: intraluminal secretions, CV: capillary vessel/ microvessels, DA: dilated acini, sec.: secretory epithelial cells.

4.2 Conclusion and Assessment of Reviewing Literature of Correlation Between Prostate Specific Antigen (PSA) and Prostate Cancer Incidence

After reviewing the relevant literature regarding correlation of PSA levels with PCa incidence, it can be concluded that PSA screening for PCa detection is an equivocal issue. There is no established balance between pros and cons of PSA screening. One side, there are many studies that reports that PSA screening results in reduction of PCa specific mortality rate and increase in early detection of PCa. On the other side, some recent studies indicate that PSA screening leads to over-diagnosis and under-diagnosis of PCa. PSA is no doubt widely used currently available biomarker for PCa screening. Potential drawback of PSA screening is its inability to differentiate indolent cancer from aggressive life-threatening cancer at the time of diagnosis. It is difficult to predict which tumors are aggressive and which are harmless. Actual concern is over detection and consequently over-treatment of PCa. Main difficulty is to recognize those cases that require immediate treatment options to prevent men from death than those that do not. Since we are not able to recognize such cases, our approach is to find all the cases.

It cannot be concluded that PSA is worthless for PCa screening. Firstly, we have evidences from many studies that favorably reports sharp decline in PCa specific mortality rates after introduction of PSA screening. If they were largely consisted of harmless indolent cancers as the reports against PSA screening says, how could it be possible that treating such harmless cancers resulted in reduction of PCa specific mortality rate? Secondly, it is well known fact that tumor cells holds the capability of acquiring further mutations and aggressiveness over the time, if left untreated. So, it is equally important to diagnose indolent tumors. Thirdly and obviously, saving lives is more important than preventing men from needless treatment side effects.

Studies aimed at improving the performance and clinical values of PSA testing are required. Future advances in PCa screening should focus on development of additional molecular markers with higher sensitivity and specificity for PCa detection.

DISCUSSION AND CONCLUSION

The burden of prostatic diseases is high among aging men. Malignant diseases in the form of prostate cancer (PCa), pre-malignant conditions in the form of prostate intraepithelial neoplasia (PIN) and non-malignant diseases in the form of benign prostatic hyperplasia (BPH) and prostatitis are quite common. Only histopathological interpretation is the reliable approach for establishment of diagnosis of PCa.

Some previous studies have reported prevalence of cancer in prostatectomy specimens operated for benign diseased conditions (Schwartz, 1986; Aghaji and Odoemene, 2000). In contrast to general assumption that cancer in prostate develops in peripheral zone, low grade cancers arising from periurethral transitional zone have also been described (De Marzo et al., 1998). In light of these evidences and established importance of histopathological examination in PCa diagnosis and staging, present study was an attempt to study the histopathological staging in putative prostate cancer tissue specimens and subsequently, evaluating the prevalence of cancer in them.

For the present work, four putative PCa specimens were obtained. Two were from prostatectomy and two were from transurethral resection of prostate (TURP). Lack of experience and thoroughness in evaluation of histopathological features could lead to misinterpretation. Therefore, standard image library of normal prostate, other malignant and non-malignant conditions of prostate was prepared and then used for comparison. Standard images of normal prostate were used as control. Protocol was established for histopathology of prostate tissues.

None of the putative prostate cancer tissue specimens was diagnosed with cancer on the basis of histopathological findings. Hallmark of prostate cancer is absence of epithelial basal cells and expansion of luminal epithelial cells (Humphrey et al., 2007). In contrast, all the four samples were observed with the presence of intact basal cells (fig.10, 11 and 13). Other minimal and major criterion key features used to diagnose prostate cancer, such as, presence of prominent large nucleoli,

nucleomegaly, obstruction of lumen, perineural invasion; infiltrative growth pattern and uniform proliferation of small acini were also not observed in the given four samples. Frequency of detection of nucleomegaly, infiltrative growth pattern and prominent large nucleoli in prostate cancer biopsy tissue specimens were reported to be 96%, 88% and 64% respectively based on findings of 50 cases (Thorson et al., 1998). None of them was characterized in the given samples.

However, all the samples were characterized by the histological features which are mainly diagnostic criterion of BPH. Constellation of histological features such as circumscribed elongated nodules of glands, increased papillary infoldings, dilated acini with necrotic debris and increased stromal component were considered for specific diagnosis of BPH to be made in each of four samples (fig.8 & fig.10). These histological features along with commonly associated findings such as mild inflammatory mononuclear cells and multiple corpora amylacea are established diagnostic criteria for BPH (Bostwick, 2005; Roehrborn, 2007). These were also found prominently in the given prostate specimens (fig. 11 & fig. 12).

Increased stromal component was found in almost all the samples (fig. 7, 9, 12 and 13). Deering et al. (1994) observed no significant variation in stromal component in samples obtained by different surgical procedures for BPH. However, given TURP specimens reported slightly more increase in stromal component than prostatectomy specimens. This finding is supported by the study of Mc Neal (1990), who demonstrated that majority of periurethral nodules contain increased stromal component. Other studies from small resected glands also demonstrated predominant fibromuscular stroma (Rohr and Bartsch, 1980). However, it is also suggested that an increase in stromal to epithelial ratio does not always indicate stromal disease; it may be due to epithelial disease (Roehrborn, 2007).

BPH was diagnosed in all the given specimens including two putative PCa TURP specimens (fig.11, 12 and 13). Our results are supported by the fact that BPH develops most commonly in periurethral transitional zone (Mc Neal, 1978). And this portion is resected in TURP which is a recommended treatment option for BPH.

Inflammatory cell infiltrates were also seen in some sections (fig. 11, 12 and 13). Kramer et al. (2007) also reported infiltration by T-lymphocytes in human BPH tissues. These inflammatory cells are known to produce various growth factors that promote hyperplasia.

There are always chances of incidental finding of early stage cancer in TURP specimens and prostatectomy samples operated for benign diseases. However, results from the present study reported no cancer in the given putative PCa specimens. It is supported by the study of Chokkalingam et al. (2003) as they found low incidence of PCa in BPH patients undergoing TURP. Rich (2007) also concluded that prostate cancers are not detected clinically so often, they are detected more commonly at autopsy.

High prevalence of PCa at autopsy compared to low prevalence in alive men is the main controversy that raises the question; is the prevalence of early prostate cancers is very less? If yes, then why it is? Answer lies in the fact, that still we do not have any accurate and robust PCa early detection marker that can possibly detect cancer at an early stage. Although, prostate specific antigen (PSA) is still widely used biomarker for PCa detection, its false positive and false negative results raises major concern because it affects the quality of life. PSA screening in PCa detection remains an equivocal issue. It is to be hoped that further research in this area regarding refining diagnostic clinical utility of PSA in context of PSA isoforms can help.

SUMMARY

Globally, prostate cancer remains the significant cause of death among elderly men. It remains clinically silent and presents itself only at advanced stage, when the treatment becomes problematic. Risk factors for PCa are non-modifiable. Therefore, only early detection can reduce this burden by providing better chances of cure and treatment options. Another major problem is its heterogeneous nature. Some are latent i.e. not potentially harmful, whereas others are aggressive which are life-threatening. Goal of an ideal screening program should be detecting PCa as early as possible before it becomes too advanced for treatment and bypassing the harmless latent cancers that are not programmed to become aggressive. Currently, there are no reliable biomarkers that can diagnose stage early and can accurately differentiate latent versus aggressive PCa.

PSA is used widely serum biomarker for PCa detection. Although, it is not cancer specific, but it is sensitive enough when compared to other available biomarkers. Previous studies support the fact that PSA has contributed in increase in early detection rate of PCa i.e. PSA holds the probability of detecting PCa at an early stage. However, recent studies reports false-positive and false-negative results of PSA screening which affects the quality of life. The issue whether PSA screening reduces PCa specific mortality or not is also being addressed by the ongoing randomized clinical trials whose preliminary results have also generated controversy. According to present review of literature of association of PSA with PCa incidence, PSA remains the debatable issue in both aspects; qualitatively and quantitatively. In spite of shortcomings of evidences regarding benefits and efficacy of PSA screening, it still holds the probability of detecting PCa early as well as differentiating latent versus advanced PCa. Research should focus more on improving the diagnostic clinical utility of PSA rather than replacing it altogether. Evaluating clinical relevance of histopathological stages is an important aspect because histological examination is considered as only confirmatory diagnostic method. Future research can be carried out in evaluating the correlation between expression level of putative biomarkers in serum and tissues of different stages of prostate cancer.

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