Screening Of Prostate Specific Antigen (PSA) For The Detection Of Prostate Cancer And Its Prevalence In Jammu Region

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CERTIFICATE

This is to certify that the present thesis entitled "***Screening of Prostate Specific Antigen (PSA) for the detection of Prostate Cancer and its Prevalence in Jammu region"*** is the outcome of the original piece of work carried out by Mrs. Pratibha Rani (Registration No: 11300728) herself under my guidance and the contents of her thesis did not form a basis of the award of any previous degree to her and to the best of my knowledge to anybody also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further, certified that the candidate in habit and character is a fit and proper person for the award.

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Certified that the data included by Miss. Pratibha Rani in the Thesis titled "***Screening of Prostate Specific Antigen for the detection of Prostate Cancer and its prevalence in Jammu region***", is genuine and relates to her work done under our supervision and guidance. To the best of our knowledge and belief, the work presented here, it has not been submitted in part or in full for the award of any degree or academic distinction to any learned body and is in accordance with the approved plan of thesis.

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Declaration

This is to certify that, the work embodied in this thesis was carried out by me in the Department of Biochemistry at Govt. Medical College and Hospital Jammu. Under the direct supervision of Dr. A.S. Bhatia HOD of Biochemistry, Mr. Lakhbir Singh Demonstrator in Biochemistry(Govt, Medical College and Hospital Jammu) and Mr. Harpreet Singh (Lecturer) at Lovely Professional University

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List of Abbreviations

PSA – Prostate Specific Antigen

BPH- Benign Prostatic Hyperplasia

PZ - Peripheral zone

CZ - Central zone

TZ - Transition zone

DNA- Deoxyribonucleic acid

PIN – Prostatic Intraepithelial Neoplasia

PCa – Prostate Cancer

DHT- Dihydrotestosterone

EGF- Epidermal Growth Factor

IGF- Insulin Growth Factor

VEGF- Vascular Endothelial Growth Factor

KGF- Keratinocyte Growth Factor

TSG- Tumor Suppressor Gene

HAs- Heterocyclic amines

PIA- Proliferative inflammatory neoplasia

RITA- Radiofrequency interstitial tumor ablation

HIFU- High intensity focused ultrasound

DRE- Digital rectal examination

TRUS- Transrectal ultrasound

CGH- Comparative genomic hybridization

Abstract

Prostate cancer is the most commonly diagnosed non-cutaneous cancer in men. Prostate specific antigen is the biomarker used for the screening of prostate cancer and other prostate related problems. Not only the genetic factors are involved dietary factors, environmental factors also responsible for the development of prostate cancer. Risk factors like family history, age, chemical exposure, infection, and smoking are at the peak point for the development of prostate disease. Advanced age is one of the main risk factor. Radical prostectomy is the most common therapy for small group of patients with high grade tumors. Early screening of PSA reduces the incidence rate of prostate cancer. Mostly prostate abnormalities are seen in among male patients above the age of 50 or older. In worldwide population the epidemiology of prostate cancer is high in western countries and less in Asian countries. In this project I had detect the prevalence of prostate cancer in Jammu region. Data was collected from the year Aug 2014 – April 2015. A total of 150 males had screened with PSA and out of total population 70 were found high level of PSA. Digital rectal examination and biopsy was performed on abnormal those patients sample whose PSA is raised. After examine their test I came to know that out of 70 samples 10 got prostate cancer. After getting these test results I assumed the prevalence of prostate cancer in Jammu region. During my training period and the reports which I got from Superspeciality hospital Jammu, I assessed that there will be 15-16% (1 year) hike in prostate cancer cases in Jammu district.

CHAPTER-1

INTRODUCTION

1.1 Prostate Gland

1.1.1 Anatomy

The human prostate is a male accessory sex gland. It is chestnut shaped like structure, located in the basement of the pelvis and surrounds the neck area of the bladder and urethra. [1] Urethra plays a role in two main purposes urination and ejaculation. The healthy prostate weight is approximately 11grams, ranging between 7-16grams. [2] A thin vascularized fibrous sheath with encloses prostate gland along with a fibro muscular layer continues smooth muscle that surround the bladder. This fibro muscular layer extends and divides the prostate gland into different zones.[3-4] It allows running the prostatic fluid into urethra during ejaculation. The milky color fluid that is secreted is rich in citric acid, fibrinolysin and enzymes, especially acid phosphatase. These prostatic secretions are responsible for liquefying semen and trigger the sperm motility. [5] The proteins rich prostatic secretions change the environment of the vagina and hold the sperm in the female reproductive part for survival. [6] The main hormone which is secreted by the male reproductive organ is testosterone which is responsible for synthesizing Dihydrotestosterone in the peripheral tissue. Dihydrotestosterone responsible for supervise the prostate gland.

1.1.2 Prostate Function

The main function of the prostate gland is to store the seminal fluid. The prostate gland secretes small amount of alkaline fluid that makes 25% seminal fluid which allows the sperm to swim freely. Due to the alkalinity, it changes the vaginal tract environment which is acidic in nature and allows the sperm to stay viable in female reproductive part. The rich constituents present in prostatic secretions are Prostate specific antigen (PSA), along with citrate (18.7mg/ml), zinc (488 μg/ml), spermine (243 mg/ml) and cholesterol (78 mg/ml). [7]

1.1.3 Prostate Structure

The structure of prostate is divided into two different regions zones or lobes. The zones are further divided into four different regions. Peripheral

zone (PZ): About 70% part of the prostate are develop in this zone that encircles the urethra. In the peripheral zone there is 80% chances to develop prostatic cancer. 25% part of the prostate are formed in the central zone (CZ) that surrounds the ejaculatory ducts and only 2.5% chances of prostatic cancers develop in this region. Cancers that establish here are more intrusive. [8] Remaining 20% of prostatic cancers develop in transition zone (TZ) which encircles the proximal urethra. Sometimes enlargement of the transition zone arise benign prostatic hyperplasia. Anterior fibro-muscular zone is the final zone consists of muscle and fibrous tissue only. Zones of the prostate gland 1= Peripheral zone, 2= Central zone, 3= Transition zone, 4= Anterior fibro-muscular zone are shown in fig no (1.1). B= Bladder, U= Urethra, SV= Seminal vesicles.

The lobes of the prostate are classified into four different parts, the anterior lobe (roughly the same as the TZ), posterior lobe (comparable to the PZ), lateral lobes (spans all zones) and the median or middle lobe (CZ) are shown in fig no. (1.2).This classification is mostly used to describe anatomy of the prostate, [9] whereas Zonal classifications are mostly used in pathology. [8]

1.2 **Prostate carcinogenesis (PCa)**

Cancers are defined as uncontrolled production and subsequent spreading of cells to other parts of the body. All types of cell in the body that sustains such malignant changes and develops into cancers. Due to the unregulated cell division, the normal cell convert into the tumor cell that invades firstly into the localized area then spread into the surrounding tissue then spread via lymphatic system and vascular system to various other parts of the body. [10-11] Balance between the proliferation and cell death cycle is disturbed by unregulated division of cancer cells. This process is disrupted by mutation in DNA that causes cell to divide rapidly and multiply at higher rate. The arising mass can either be benign or malignant.

PIN (Prostatic intraepithelial neoplasia) is the possible precursor of prostatic carcinoma. It is responsible for the abnormal growth of epithelial cells that line the prostate gland. The irregular spaced of epithelial cells are characterized low grade of PIN. Nuclei becomes hyper chromatic (with elevated chromatin) and pleomorphism (variation in size and shape). Higher level of hyper chromatism and pleomorphism are found when the PIN is in high grade. Cluster round cells simulating a raspberry shape that distinguish the PIN from adenocarcinoma.[12] The increase risk for

adenocarcinoma can be advice by presence of PIN. Although adenocarcinoma can be up to 10 years before prior prostate carcinoma presents. [13]

1.3 Development and Progression of prostate cancer

In developed countries, prostate cancer is the second most common analyzed cancer and the third most common cancer lead to death in men. [14] In 2006 it was reported that near about 1 in 8 men at the age of 75 years and 1 in 5 at the age of 85 years will develop prostate cancer accordingly.[15] A study conducted in 2007 by Collins and its coworkers described the origin of prostate cancer from the glandular epithelium and the origin of tumor cells from luminal although both are dependent upon androgens and represent luminal cell marker but increasing evidence from the research studies depict the derivation of cancer cells is less from differentiated stem cells.[16-17] Most prostate tumors are heterogeneous and multifocal, suggesting that multiple neoplastic foci have emerged and evolved independently.[18] Development and progression of prostate cancer is a multistep process. Malignant cells develop due to the genetic alteration. Prostatic intraepithelial neoplasia, premalignant lesions considered as intermediary phase from benign epithelium to carcinoma and it is quiet comparable to prostate cancer with the exclusion that the basal layer is irregular but still presents. Due to additional alterations, malignant tumors expand that firstly constrained to the prostate, but ultimately enter the prostate capsule, attack on neighboring tissues and eventually form metastases.[19]

1.4 Molecular Changes

Cancer always generates from single somatic cells and by the action of many genetic changes it leads to a change in both phenotype and genotype.[20]Cancer leading to mutations mostly rise in the genes that are associated in the cell growth or cell death regulation.[21] Complexity or more than 100 types of cancer and their difference subtypes make it more difficult to point out the origin of the disease. Extensive research done from the past two decades on molecular, biochemical and cellular process depicts how normal cells transforms or changes into the malignant cells. The enumerable majority of cancerous cell comprises of entirely six different capabilities namely self sufficiency in growth signals, insensitivity to anti growth signals, evasion of apoptosis, infinite replication ability, sustained angiogenesis and ability to invade tissue and metastasis.[22] Cell division of normal

cells is under limitation that is, they first monitor the suitable external environment and then undergo cell division if necessities, in contrast to this cancerous cell have their own signals which set them free from the growth limitation of normal cells, so they divide and grow abnormally. Second capability is somewhat same as the previous stage, these cancerous cell have antigrowth signals i.e. they don't receive signals to inhibit or to stop growth. Third feature or character of cancerous cell is the capability of assisting the growth since normal cells after complete cell division stops replicating, this phenomenon is controlled by telomeres. Telomeres are the segment of DNA or shortened by each round of DNA replication. This shortening of DNA doesn't allow cell to undergo further cell division and finally leads to cell death (apoptosis), but this phenomenon cannot be found in cancerous cell because of the ability to maintain the length of telomere. This allows them to replicate infinitely. The next capability is the evasion of apoptosis done by gene P53. Gene P53 is often found mutated in cancer cells, thus does not leading to normal apoptosis. Angiogenesis which is the formation of new blood vessels, have the role for supplying or providing oxygen and nutrients to the tumor cells and the last but not the least capability is invasion in tissue and metastasis, in this cancerous cells get attached to other cells and spread throughout the body. [23] Cancer genes can be categorized into three main types: Oncogenes, Tumor suppressor gene and cells involved in DNA repair.

Oncogenes are the first cancer causing gene which causes unregulated cell growth. [24] They arise from proto-Oncogenes which in turn responsible for normal cell growth. They usually remain dominant and cause mutations like increase in protein activity or loss of regulation, this increase in protein concentration or chromosomal translocation causes gene expression of different cell types. Some examples of Oncogenes are RAS mutated in about 15% of cancer. [24-25] Antioncogenes (Tumor suppressor gene) are inactivated by loss of function mutation. Knudson in 1971 studied sporadic and familial retinoblastoma and based on his studies, he formulated two hit model of carcinogenesis which gives brief explanation of the loss of function changes. In familial retinoblastoma there is 50% chance to a child of inheriting this condition from one of the affected parent but in sporadic retinoblastoma no additional chance can be found. [26]

The inherited form cannot cause predisposition of tumor development because of the germ line mutations in one of the copy of tumor suppressor gene.[27] In second copy of tumor suppressor gene there occurs somatic

mutation that will cause tumor progressions. Sporadic retinoblastoma is involved in two different hit models that are required for the same cell to develop into a tumor P53 (TP53) gene being one of the most important tumor suppressor gene is found tobe involved in key cancer control pathways such as cell cycle control apoptosis (cell death), angiogenesis (formation of new blood vessels) and genetic stability. The last category comprises of the genes responsible for DNA repair mechanisms for normal DNA replication. In a single mutation in the whole process can result in genetic instability thus leading to abnormal chromosome number or breaks. [28] Moreover certain other mutations in Oncogenes and tumor suppressor genes leading to other type of cancers are very rare in primary prostate cancer, but specific mutation for prostate cancer is yet to be discovered and therefore needs further research.[23]

1.5 Benign prostatic hyperplasia

Benign prostatic hyperplasia also called BPH (benign prostatic hypertrophy), a condition in which the prostate gland become enlarged. It is the most common prostate problem, BPH is not the cancer but the symptoms of BPH is quiet similar to those of prostate cancer. Due to the over growth of epithelial nodules and stroma tissue in the transition zone of the prostate this leads the gland enlarged and the condition called BPH.[29] The two risk factor advanced age and circulating hormone (androgen) are responsible for developing for BPH.[30] Due to the enlargement of prostate gland, the urethra becomes compress and pressure will increased within the bladder causing frequently contraction and less amount of urine is present, by this the bladder is not able to emptifying itself and cause many other problem.

Androgen and estrogen are two sex steroid hormones are responsible for regulation of prostate and these are important for the prostate cell growth. Estrogen is originated through the stromal aromatization of androgen and estrogen: androgen ratio increases in BPH patients. [31-32] By the advanced age of men, testosterone conc. become lower and estrogen become high in blood. According to the studies, higher concentrations of the estrogen increases the smooth muscle cell proliferation and differentiation, this may lead to BPH. [33] Aromatase inhibitors such as testolactone are anti-estrogens, which is used for the treatment of BPH patients. These anti-estrogens have a role for preventing the androgen to estrogen. [34] Androgenic steroid testosterone is converted into Dihydrotestosterone (DHT) which is important for the

function of secretory epithelial cells; this conversion is catalyzed by 5α-reductase isoenzyme type 1 and 2. 5α-reductase type 1 is most often found in liver and skin but less amount in prostate. Type -2 is most prevalent in prostate. 5α-reductase type-2 is responsible for converting the androgenic steroid testosterone to Dihydrotestosterone (DHT) in prostate gland. In BPH condition, higher activity of 5α-reductase has been demonstrated as compared to normal tissue. [35] Finasteride, a 5α-reductase type2 inhibitor has been used for the treatment of BPH patients. [36-37]

1.6 Expression of peptide growth factor in benign prostatic hyperplasia and prostate cancer

Prostate cancer poses a significant clinical challenge both in terms of its prevalence and its complexity. [38] Several prostate cancer associated genes including c-myc, insulin-like growth factor-I, P27 and peptide growth factor are highly expressed across all of the tumor types. [39] Peptide growth factor, proteins that is responsible for regulating the cellular growth, differentiation and programmed cell death (apoptosis). Most important families such as epidermal growth factor (EGF) family, insulin growth factor (IGF) family, the transforming growth factor beta (TGFB) family and the vascular endothelial growth factor (VEGF) family, these families are involved for the progression of prostate cancer.[40-41] EGF family have further two members EGF and TGFA. EGF responsible for promoting the proliferation of cell and also involved in embryogenesis, angiogenesis and cellular differentiation. [42] This EGF protein is over expressed in benign and malignant. [43-44] FGF (Fibroblast growth factor) have several members FGF (bFGF OR FGF2), acidic FGF (aFGF or FGF1) and Keratinocyte growth factor (KGF OR FGF7), these families are highly expressed in varying levels by prostatic cells.[45] FGF2 families are over expressed in mRNA and its presence implicated the development of BPH.[46-47] TGFB belong to TGF-beta family,[48] the level of TGFB increase in prostatic neoplasia[49-50]and this finding associated with development of tumor and progression[50-51] because TGFB biological activities are exploited by cancer cells. TGFB promotes angiogenesis [52] along with this TGFB are immunosupressor. [53, 54, 55] TGFB protects the cancer cells from the host immune system, [56] it plays a key role in extracellular matrix by enhancing the invasiveness and metastatic ability of malignant cells. [57-58] VEGF (Vascular endothelial growth factor) is also involved for developing tumor and metastasis. [59] VEGF expressed in benign prostatic hyperplasia (BPH) and

prostate cancer epithelial cells [60-61] and its appearance plays a role for its tumor growth, inducing angiogenesis. [62] IGF1 productions in epithelial cells of the prostate have a role for the development of prostate adenocarcinoma. [63] In prostate malignancy the level of IGF1 is increased in blood [64-65] but not with BPH.

1.7 Biomarkers for evaluating prostate cancer

1.7.1 Prostate Specific Antigen (PSA)

Prostate belong to human kallikrein gene family, is a serine protease with chemotrypsin-like activity. Prostate specific antigen is a single chain glycoprotein made of 237 amino acids containing oligosaccharide side chain. PSA molecular weight is approximately 30,000 Dalton. [66-69]The glandular epithelium of prostate gland formed major portion of PSA. The breast cancer, salivary gland neoplasm, periurethral and anal gland, cells of male urethra, breast milk, blood and urine originate PSA. [70, 71]The prostate gland contains acinar cells that formed glycoprotein also known as PSA. [72] Main function of PSA is to dissolve the seminal clot that is formed after ejaculation and help to transport the spermatozoa in female reproductary tract. PSA has two types:

1) Complex PSA formed by complex combination of serum protein.
2) Free PSA are made by free combination of serum protein.

When these two combine together they formed total PSA. Seminal fluid contains high percentage of PSA (0.5 to 2.0 mg/ml) where as in blood, PSA concentration is quite less i.e. 1000 times. The changes in concentration are independent of other protein; its changes depend upon serum testosterone levels. [73] If the leakage of PSA from the prostate gland, the level of PSA becomes low and high level of PSA are allied with prostatic pathology, including prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer. [66, 74-78]In 1982 PSA was used to describe as prostate cancer marker, PSA first screening report came into existence in 1991. [79-80]The occurrences of prostate cancer have had a less increase in western countries over last 30 years. [81] The high grades of PSA Level are found in 50 years of age or in older males.

Age (years)	Reference ranges (ng/dl)
50-59	0-3.5
60-69	0-4.5
70-79	0-6.5

Table 1.1 PSA Reference ranges (Oesterling JE et al., 1993)

1.7.2 Digital Rectal Examination (DRE)

Digital rectal examination is the other method that is used for the examination of prostate abnormalities. [82-83]Enlargement of prostate gland can be found in BPH patients. DRE is performed via rectum by finger to feel the hardness of the gland or irregular or hard lump indicates the presence of tumor. This method is not able to examine all prostate abnormalities via rectum, so DRE method was not the best method for diagnosis of all prostate problems. [84]

1.7.3 Transrectal ultrasound (TRUS)

TRUS can be used for guidance of needle biopsies of the prostate gland. This method examines the enlargement of gland, cancer nodules and tumor invasion to the seminal vesicles. [85]Other molecular markers which is used for finding prostate abnormalities such as prostate stem cell antigen, early prostate cancer antigen, hepsin, enhancer of zeste homolog gene 2, human glandular kallikrein 2, transforming growth factor-1, chromogranin A have been suggested as potential promising biomarkers for prostate cancer. [86]

1.8 Symptoms and Diagnosis

Symptoms are not found for many years in prostate tumor because it is usually slow growing disease. In early stage of prostate cancer, symptoms ore not found. Its symptom mostly affect the urination because its location surrounding the urethra. In prostate cancer, symptoms like frequent urination. Nocturia (urination in night), hematuria (blood in urine), difficulty in maintain a steady stream of urine and dysuria (painful urination). These symptoms also found in other prostate disease like BPH. It also affects the sexual function, difficulty in achieving erection or painful ejaculation. If the prostate cancer is in late stage, it can

spread to other organs such bone, lymph nodes and causing bone pain in pelvis or ribs region. Diagnosis of the prostate cancer must be proofed by the needle biopsy. The International Classification of Diseases version 10 (ICD10) classifies malignant neoplasm of the prostate as code c61. [87]

1.9 Risk Factors

(1) Age

According to the ages, the risk of developing prostate disease is increased. Age is the one of the most common risk factor. Prostate cancers will significantly increased by the advanced ages and by the age 70, approximately 65% men have at least microscopic evidence of prostate cancers. . There is a positive correlation between time and prostate cancer progression. [88]

(2) Family History

It has been suggested that heredity may play a role in prostate cancer. Men with a family history of prostate cancer may have a higher risk of having this disease. [89-91]

(3) Ethnicity

In developed countries like Africa, America men of these countries have a high risk of developing prostate cancer, [89, 92, and 93] but in Asian countries lower risk of developing prostate cancer and if they move to North America risk will increases. [94]

(4) Dietary Factors

Dietary factors play an important role in African and American males and have a higher risk of prostate cancer in comparison to Asian males. High fat intake may increase the risk of prostate cancer. According to the researchers study they suggest that fat elevated the production of hormone testosterone that may promote the prostate cancer cell growth. Specific subtypes of fat are responsible for influencing the prostate cancer cell growth. HAs (heterocyclic amines), a group of carcinogens known as PhIP (2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine), is

found in grilled beef, pork, chicken, lamb, and fish. It has been indicated that PhIP relates to prostate cancer incidence. [95]

(5) Smoking

Cigarette smoking is one of the risks of prostate cancer. The circulatory level of steroidal hormone is altered by smoking. [96] A few hypothetical mechanisms were proposed to enhance the risk of prostate cancer. It has been suggested that smoking can increase the circulating levels of bioavailable testosterone and lower levels of bioavailable estradiol in men. [97] There are significant positive correlations between cigarettes smoked/day and serum total androstenedione as well as total and free testosterone in men. [98] Data from a population based case-control study suggest that smoking is a risk factor of prostate cancer Current smokers appear to be at moderately increased risk for this disease compared with non-smokers. [96]

(6) Exposure to Chemicals

Prostate cancer and chemical exposure relation are not fully understood but it is reported that, males who were indulge with heavy labor and work with certain metals and chemicals, including cadmium, dimethyl formamide, and acrylonitrile, may be at higher risk for prostate cancer. [99-100]Some studies indicate farmers are at higher risk of prostate cancer. [115]

(7) Infection and Inflammation

Genetic factors that affect the body's response to viruses can also associate with inherited prostate cancer. Some relation between the prostate cancer and bacterial or viral infections are seen in such infections like herpes virus, human papillomavirus, and cytomegalovirus. It implied that genetic susceptibilities in men could develop a chronic inflammatory condition in the prostate by viral infection and possibly initiate cancerous changes. It has been suggested that exposure to environmental factors such as infectious agents and dietary carcinogens, and hormonal imbalances could lead to prostate injury and develop chronic inflammation and regenerative risk factor lesions, referred to as proliferative inflammatory atrophy (PIA), which could progress to PIN (prostatic intraepithelial neoplasia) and eventually invasive carcinoma. [101] However, some recent studies have shown that there is no link between viral infections and prostate cancer development. [102]

1.10 Treatment

1. Surgery

Radical prostatectomy is the surgery procedure in which the entire prostate gland and surrounding tissue such as seminal vesicles are removed. Some amount of removed organ like lymph nodes are performed for biopsy to examine the cancer has metastasized or not. This therapy is used in early stage of prostate cancer and after surgery the patient can survive for 10-20 years. The main purpose of this therapy is to remove the whole cancer and prevent its spread to other parts of the body. There is some risk after surgery like impotence, heart attack, stroke, blood clot, infection.

2. Radiation therapy

Radiation therapy is one of the treatments for prostate cancer for several decades. [103] Prostate cancer is a radiation-sensitive neoplasm that determines a classic sigmoid dose response curve to X-rays. Higher volume tumors need higher radiation doses. Bladder and rectum are at risk when radiation is performed.

3. Hormone therapy

Hormone therapy causes blocking hormonal action due to which the growth of cancer cells stops, e.g. Luteinizing hormone-producing hormone against, which are able to inhibition of gonadotropin secretion. Following an early stimulation of gonadotropin, chronic administration of leuprolide acetate causes suppression of testicular steroidogenesis. This proves and shows that luteinizing hormone-releasing hormone agonists and results inhibition of the growth of certain hormone which promote tumors (such as prostatic tumors). Examples are leuprolide, goserelin, and buserelin. Antiandrogens exert its action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen to the androgen receptors on prostatic cells such as flutamide and nilutamide.

Studies are still being carried on to find the ideal therapy for localized prostate cancer. Currently, the two most common therapies used in the United States to treat prostate cancer remain radical prostatectomy and radiation therapy. [104-105] The newer focal therapy consists of cryoablation techniques and heat energy–based treatments [high intensity focused ultrasound (HIFU), radiofrequency interstitial tumor ablation (RITA), and thermal brachytherapy]. [106] Radioactive seeds were first used by Dr. Anthoy D'Amico at Harvard Medical School to treat early stages of prostate cancer. Magnetic resonance imaging (MRI) was used to place 100 radioactive seeds into

tumors inside prostate to destroy cancer cells. For some patients, it may be superior to the usual methods of surgical removal of the gland.

1.11 Epidemiology

In western populations, prostate cancer is the most common cancer in men. In 2005, the new cases were expected beside 232,090**.** [107]From the early 1990's the incidence rates was greatly increased worldwide. [108]In western countries, due to the increased detection of BPH with surgical procedure particularly TURP, this may lead the incidence rate was high in late 1970's and early 1980's. [109]The sharpened incidence rate was rise in between 1986 and 1992 by the more use of PSA. [110]In US, the incidence rate was usually dropped down in mid 1990's, but recently the rate was start and slowly rises again. [111]Generally in Asian countries, the incidence rate is low but recently found that the rate was raised more than the western countries.

1.11.1 Incidence and Mortality

The variation in incidence rate and mortality rate, by the differently appearance of prostate cancer was directly impact on world's population, while the examination of these rates, it provide initiation about the disease and also helpful for the generation of hypothesis for further research.

Incidence

Prostate cancer considers incidence rate are variable in whole world. African and American countries rates are at highest in the world (185.4 per 100,000 person-years), displacing by Caucasian-Americans (107.8 per 100,000 person-years) (fig.1.11.1). The Caribbean and in Brazil rate falls on (92-96 per 100,000 person years). Rates are much lower (28-42 per 100,000 per years) in Central America and other parts of South America. In Europe rates are (15-100 per 100,000 person years), but Western Europe rate are higher than Eastern Europe (15-36 per 100,000 person years). The highest incidence rate reported in such countries; Australia, New Zealand, Northern and Western Europe and Northern America.

Moderate rates were reported in South America and Eastern Europe and lowest rates were found from South – Central Asia. In Asian countries the incidence rate found lower than the other countries. But widely the western countries like Japan, Israel and the Philippines (22-47 per 100,000 person years) were found more incidence rates than Thailand, India, Pakistan, Shanghai, China (3-7 per 100,000 person years). [112-113]Part of the difference in worldwide incidence rates is related to the extent of prostate cancer screening, especially the less-frequent use of prostate-specific antigen

(PSA) testing in developing countries. However, screening practice differences alone are unlikely to explain the nearly 60-fold difference in prostate cancer risk between high- and low-risk populations.

1.11.1 Mortality

Only one in six American men diagnosed with prostate cancer will eventually die from it. Nevertheless, 30,350 prostate cancer deaths are expected in the U.S. in 2005, making prostate cancer the second leading cause of cancer death among U.S. men, after lung cancer. [107]Age adjusted prostate cancer mortality rates from 38 countries in 1998 are shown in (fig.1.11.2). Overall, the pattern of mortality worldwide reflects that of incidence, although the mortality rates show less variation between countries. Nevertheless, mortality rates are still higher in Western nations than in lower-risk, Asian countries. Interestingly, the world's highest mortality rates (30.3 to 47.9 per 100,000 person-years) were seen in the Caribbean nations of Barbados, the Bahamas, and Trinidad and Tobago, where there are large populations of men of African descent. Mortality was higher in Scandinavian countries and parts of northern Europe than in the U.S. (18.7-23.6 versus 14.0 per 100,000 person-years), and lowest of all in the Asian countries of South Korea, Philippines, and Japan (1.6-4.4 per 100,000 person-years).The patterns of incidence and mortality worldwide provide a number of interesting leads. The pronounced excess risk of prostate cancer in western nations suggests that factors associated with westernization, such as diet and obesity, may be positively associated with prostate cancer etiology. In addition, prostate cancer's disproportionate impact on African-Americans and Caribbean men suggests that factors associated with African ancestry may also play a role in prostate cancer etiology. While it is not known whether the risk factors explaining the observed patterns are environmental, lifestyle, or genetic, it is likely that a complex interplay of these factors is associated with prostate cancer development. [114]

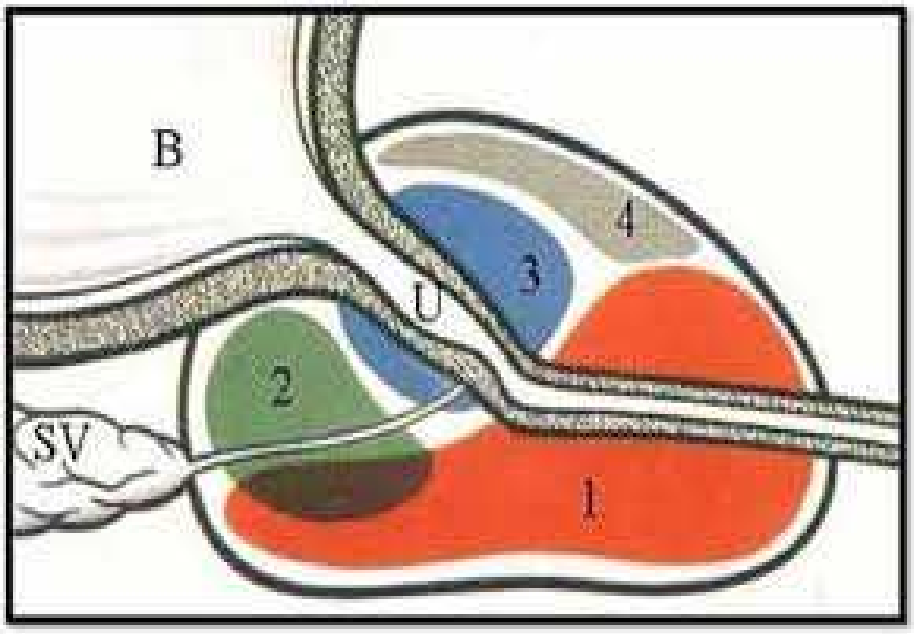

Figure 1.1 zones of prostate gland U-Urethra, B-Bladder, SV-Seminal vesicles

(www.google.com)

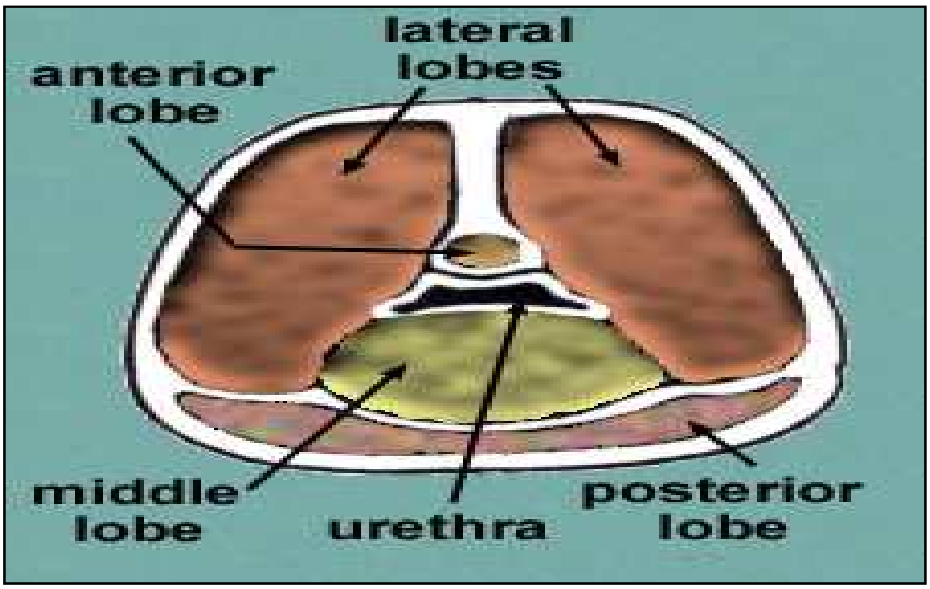

Figure 1.2 lobes of prostate gland

(www.google.com)

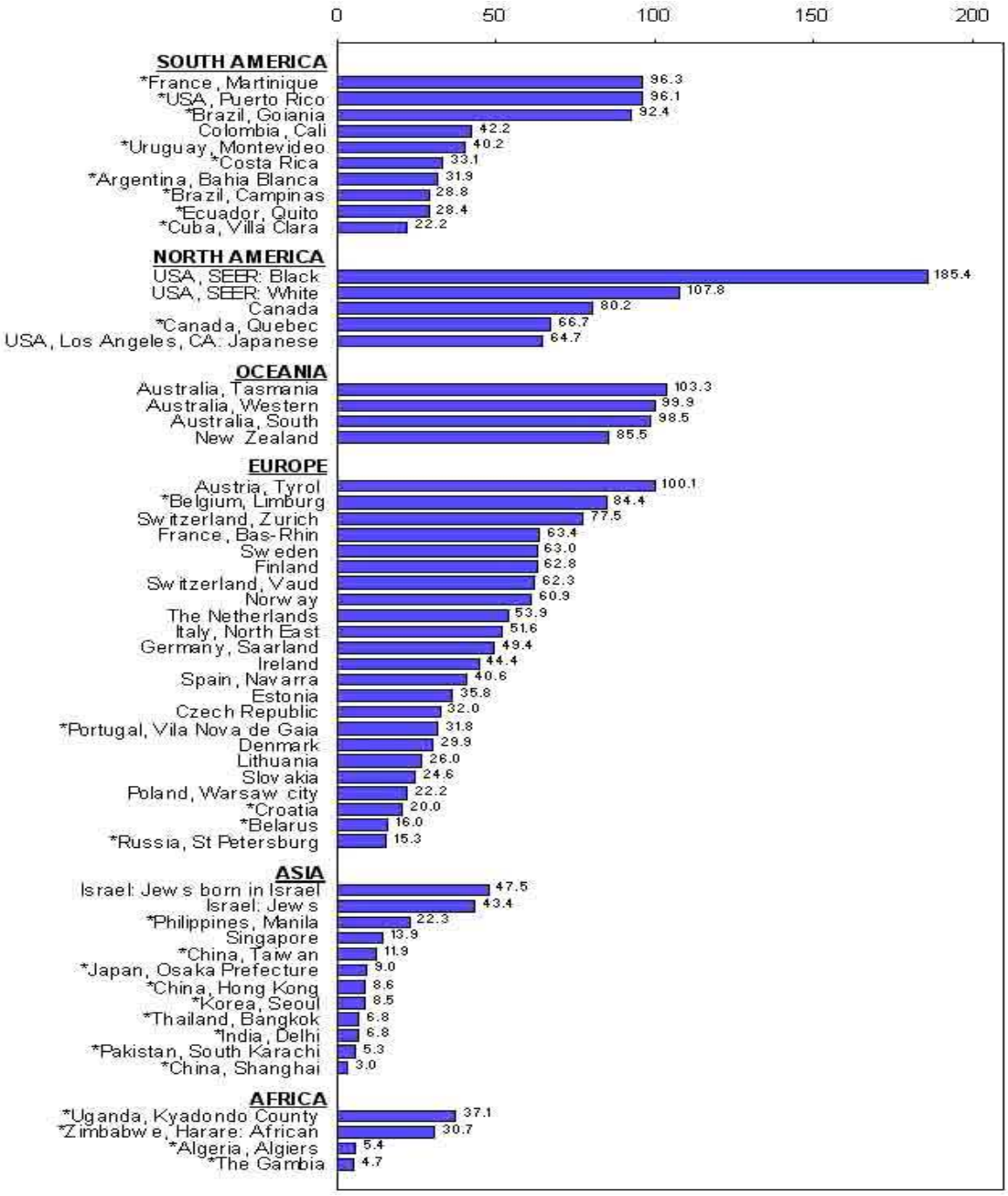

Figure 1.11.1 Age-adjusted incidence rates (per 100,000 person-years) for prostate cancer in 48 countries, 1993-1997. Reproduced with permission from (7). * Rates are from 1994

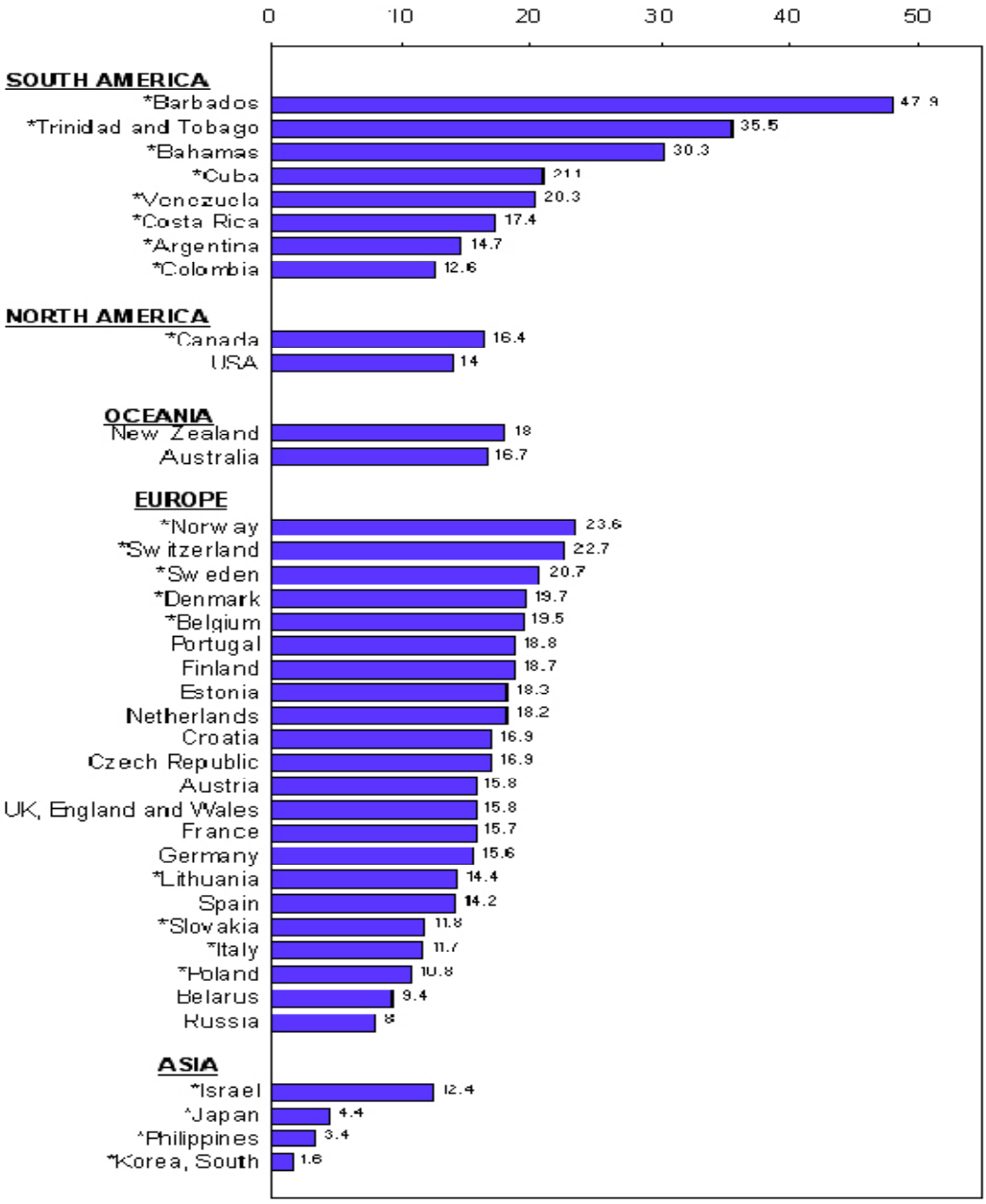

Figure 1.11.2 Age-adjusted mortality rates (per 100,000 person-years) for prostate cancer in 38countries, 1998.Reproduced with permission from (http://www.depdb.iarc.fr/who/menu.htm) Rates are from 1994.

CHAPTER-2

LITERATURE REVIEW

1.12 Review literature

Tapio Visakorpi in 1995, studied on genetic changes in primary and recurrent prostate cancer by Comparative Genomic Hybridization (CGH). In this study, comparative genomic hybridization (CGH) was used for screening the changes in DNA sequence copy number along with all chromosomes in 31 primary and 9 recurrent uncultured prostate carcinoma. The main purpose of the study to determine the chromosomal region containing genes that is responsible for the progression and development of prostate cancer. The result of CGH analysis showed that 74% of primary prostate carcinoma showed the changes in DNA sequence copy no. 8p, 13q, 6q, 16q, 18q and 9p are the most common chromosome region that are lost in primary prostate cancer. CGH losses data compared with LOH (Loss oh heterozygosity) studies by using 5 polymorphic microsatellite markers (D6S283, D8S265, D13S153 and D16S413) for 4 chromosomal arms. The result showed that 76% similarity was found between CGH and allelic loss results. During endocrine therapy, the local recurrence developed that there was more gains and losses of DNA sequences in primary tumor. 8q, X and 7 was gaining where as 8p was loss. In conclusion the CGH results showed that any losses of chromosomal regions that contain genes is responsible for cancer development and progression. By, CGH, losses were found in primary tumors involving 6q and 9p submitted the two new regions that have prostate cancer. TSGs (Tumor suppressor gene) in addition to the previously reported TSG loci 8p, 13q, 16q, and l8q. Gains of DNA sequences at 7 (7p13), and X appear important for prostate cancer progression. Further studies with specific probes are required to narrow down the critical regions in each chromosome and to identify the genes involved.

In 1997, CS Mantzores etal studied on the hormone Insulin like growth factor in relation to prostate cancer and BPH. In this year, they conducted the study from 52 incident cases that are histologically confirmed prostate cancer and 52 cases of BPH and equal number of control cases. By the use of Radioimmunoassay the steroidal hormone, sex hormone binding globulin and insulin like growth factor were analyzed in U.S centre. Androgen hormone plays a key role for the production of prostate cancer and BPH. Statistical analyses were performed by using multiple logistical regressions. IGF-1 is produced from the prostatic secretions. Prostate cells express IGF-1 receptors and induce the proliferation of cells that is directly depend on IGF

presence and is modulated by IGF- binding proteins. The investigation concluded that the expression of IGF-1 in relation to prostate cancer and BPH were adjusted for demographic and anthropometric factors as well as other measured hormones. This study increase the possibility that IGF-1 may increase the risk of prostate cancer but there is no clue between the IGF-1 and BPH. The increased value of this hormone associated with increased risk of prostate cancer. From this study there was also some evidence that found between the high level of testosterone and IGF-1 in relation to prostate cancer. These data suggests that the high value of testosterone, IGF-1 also play a key role for increasing the risk of prostate cancer.

In 2003, Lara A. Plaskon etal studied on the cigarette smoking is the risk factor of prostate cancer. Study conducted from the patients' resident of Africa, America, North western, Washington. 1055 patients were identified along with age, personnel history, height, weight, physical activity, history of PSA, BPH, family structure, cancer history. Out of these 753 patients between the ages of 40-65 year were diagnosed with prostate cancer. This study was conducted from the year 1993 to 1996 by using Seattle Puget Sound Cancer Registry. 703 patients were randomly diagnosed without a history of prostate cancer by using a digit dialing. These peoples were the residents of Washington belong to 40-64 years of age. According to the study, current smokers had 40% chances of prostate cancer risk. Men who smoked more than 40 years had a moderate risk of prostate cancer. However the odd rate in current smokers was similar for men with more aggressive disease and those with less aggressive disease. In conclusion this study results that the men who quit smoking appear to reduce their risk of prostate cancer 10 or more years after termination. These results have essential public health implications and should be useful for educating physicians and patients about the adverse health effects of smoking and to promote primary prostate cancer prevention and smoking termination strategies.

In 2006, Nikalaus Soulitzis etal, studied on the expression of peptide growth factors in prostate cancer and BPH. Peptide growth factors are responsible for various intracellular processes such as cellular growth and differentiation, angiogenesis and apoptosis as well as in carcinogenesis, their contribution also in malignant transformation. The study was conducted on 42 patients with prostate cancer, 42 with BPH and 10 normal prostate samples. The study conducted in order to determine mRNA expression levels of VEGF (Vascular endothelial growth factor), FGF2

(Fibroblast growth factor), TGFB1 (Transforming growth factor beta), EGF (Epidermal growth factor) and IGF1 (Insulin growth factor). The result conducted by using a RT-PCR method, results showed that, in prostate cancer VEGF, EGF and FGF2 are over expressed and reduced the mRNA levels by expression of TGFB1 and IGF1. TGFB1, VEGF and IGF1was down regulated in BPH samples where as FGF2 and EGF was normal in BPH samples. Patients with increased level of PSA (Prostate specific antigen) have decreased FGF2 expression. Increased EGF and IGF1 mRNA levels are found in cancer patients with low Gleason score. In old age, patients who suffered with BPH have decreased EGF expression but in young patients IGF1 was over expressed. Finding result suggest that factor VEGF, FGF2, TGFB1, EGF and IGF1 responsible for both BPH and prostate cancer development and these peptide factors also helpful for the early detection, potential therapy for prostatic disease.

IN 2009,Jennifer Dwu.etal studied on those men who were referred for prostate biopsy with those men who were referred for prostate biopsy with the raised PSA level and abnormal DRE with usually found no cancer, but the risk of developing cancer in these men more than the general population of men. In this study, they investigated that DNA damage is one of the factors which inclined those men who were referred for initial prostate biopsy to a higher risk of prostate cancer. The level of 8-oxo-2-deoxyguanosine in the referred men was higher than control prostates of men. No. of referred men was 50 (n=50) no. of control men is 32 (n=32). According to the investigation 12 men from control belong to middle aged men and 20 of them belong to older age. These men have normal PSA and DRE with no prostate disease but their condition diagnosed with bladder cancer. They detected the phosphorylation of ataxia telangiectacia mutated kinase and expression of the immune stimulatory molecule. MIC in the prostate epithelium on those men who have positive prostate due to increased level of 8-oxo-2-deoxyguanosine. Elevated level of 8-oxo-deoxyguanosine in the referred men indicates that oxidative stress is responsible for damaging the DNA and this may predispose the prostate to neoplastic transformation. Due to the DNA damage, activation of DNA repair pathways represented by ataxia telangiectacia mutated phosphorylation and induction of the immune stimulatory MIC expression may prevent the cancer development.

CHAPTER 3

AIMS AND OBJECTIVES

1.13 Objectives

- To screen the PSA level in comparison to both BPH and Prostate Cancer.
- To detect the prevalence of prostate cancer in Jammu region.
- To study the effects of risk factors (smoking, age, infections etc) on prostate carcinoma.
- To study the incidence and mortality rate of Prostate cancer.

CHAPTER 4

PLAN OF WORK

1.14 Plan of work

Literature Survey	Completed
Selection of patients	Completed
Study of patients history	Completed
Collection of Sample	Completed
Screening of patients	Completed
Interpretation of results	Completed

Table 1.2

CHAPTER-5

MATERIALS AND METHODS

1.15 Material and Methods

1.15.1 Requirements

1) Blood sample
2) Biopsy sample
3) Needle
4) Test tubes or vacutainer
5) Cuvettes
6) Test tube rack
7) Tourniquet
8) Spirit
9) Cotton swab
10) Gloves
11) Lab coat

1.15.2 Instruments

1) Architect Autoanalyser (shown in fig.1.15.1)
2) Centrifuge machine

Fig 1.15.1 Architect Autoanalyser

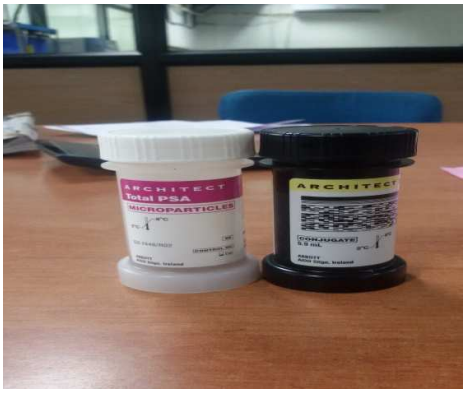

Fig 1.15.2 Reagents total PSA, Trigger solution, Pre-Trigger solution

1.15.3 Data collection

I had collected the data related prostate cancer and BPH from Super specialty hospital in Jammu. Data was collected from the year 2014 and 2015, approximately 150 male patients 50 years of age or older had screened till now who had prostate related problems and PSA is the initiator biomarker which is used for the detection of prostate abnormalities.

1.15.4 Present study

In my training period (Jan –April), a total of 60 men 50 years of age or older was participated in the study. These men had prostate related problems and PSA is the initiator biomarker which is used for the detection of prostate abnormalities. The level of PSA indicates that whether patients is normal or have BPH or prostate cancer. Level of PSA is diagnosed via blood. Blood sample was collected from vein and then incubate at room temperature or in incubator, serum is separated by centrifuge machine. Then the sample is proceeding for analyzing that was performed by ABOTT ARCHITECT AUTOANALYSER. Along with blood, person's personnel history was also collected and found that mostly the males are smokers or ex-smokers. Comparative data was shown in (fig.1.15.4).

1.15.5 Reagents (shown in fig. 1.15.2)

1) ARCHITECT Total PSA reagent kit (Microparticles, Conjugate)
2) Manual diluents
3) Pre-Trigger Solution
4) Trigger Solution
5) Wash buffer

Method – ARCHITECT Total PSA (Prostate Specific Antigen)

Principle –

The ARCHITECT Total PSA assay is a two step immunoassay determine the presence of total PSA (both free and PSA complexed to alpha-1-antichymotrypsin) in human serum, using chemiluminescent Microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as chemiilex.

In the first step, sample and anti-PSA coated paramagnetic microparticles are combined. PSA present in the sample binds to the anti-PSA coated microparticles. After washing, anti-PSA acridinium labeled conjugate is added in the second step. Pre-trigger and Trigger solution are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as reactive light units (RLUs). A direct relationship exists between the amount of total PSA in the sample and RLUs detected by the ARCHITECT optical system.

Protocol

Material provided

- 7K70 ARCHITECT Total PSA Reagent Kit (material provided)

Material required but not provided

- ARCHITECT i system
- 7k70-01 ARCHITECT Total PSA calibrators
- 7D82-50 ARCHITECT – Multi-assay manual diluents
- ARCHITECT – Pre- trigger solution
- ARCHITECT Trigger solution
- ARCHITECT Wash buffer
- ARCHITECT Reaction vessels
- ARCHITECT Sample cups
- ARCHITECT Septum
- ARCHITECT Replacement caps
- 7K70-10 ARCHITECT Total PSA Controls.

Procedure

- Load the ARCHITECT Total PSA reagent kit in the system make ensure that assay reagents are present.
- The minimum sample cup volume is calculated by the system and printed on the order list report. No more than 10 replicates may be sampled from the same sample cup.
- Press run the ARCHITECT System performs following functions.
  (Move the samples to the aspiration point).
- Load samples
- Press RUN, the ARCHITECT System performs the following functions:-

- Move the sample carrier to the aspiration point.
- Loads a reaction vessel (RV) into the process path.
- Aspirates and transfers sample into the RV.
- Advances the RV one position and transfers microparticles into the RV
- Mixes, incubates and washes the reaction mixture.
- Adds Pre-Trigger and Trigger solutions.
- Measures chemiluminescent emission to determine the quantity of total PSA in the sample.
- Aspirates contents of RV to liquid waste and unloads RV to solid waste.
- Calculate the results.

CHAPTER 6

RESULTS AND DISCUSSIONS

Results

A prospective study was conducted to demonstrate the usefulness of PSA in the detection of prostate cancer. A total of 150 men 50 years of age or older participated in the study. The result showed that 70 persons who had abnormal level of PSA. PSA level should not indicate the presence or absence of prostate cancer. In addition, PSA testing should be done in conjunction with DRE because PSA and DRE together detected the greatest number of cancers. Prostatic biopsy is required for the confirmation of cancer. 70 patients who were non malignant and 10 patients who had prostate cancer this result was confirmed by biopsy. In table 1.3 level of PSA are shown in normal, cancer and in BPH.

Table 1.3 reveals out of 150, 70 patients screened had high PSA level and these abnormal patients i.e. 70 had further screened then among the screened ones 10 patients had Prostate carcinoma comparatively high level of PSA.

Table 1.4 it indicates that out of screened 70 patients, having a history of smoking with comparatively high level of PSA.

Prevalence of prostate cancer in Jammu region

According to the study, data was conducted from the year Aug.2014-April 2015 and approx. 150 men had screened with PSA and out of total population 70 persons who had high PSA level.

Total population screened – 150

No. of malignant or cancer patients -10

Prevalence of prostate cancer in Jammu region is 15% (Fig.1.15.5) and by this the hypothetical prediction will develop for prostate cancer.

Category 1	PSA (mean)
NORMAL (n=80)	2.59
MALIGNANT (n=10)	21.18
BPH (n=60)	6.89

Table 1.3 Level of PSA in cancer, normal and BPH

Category	% of PSA level
Smokers	12.13
Non smokers	10.18

Table 1.4 Level of PSA in Smokers and Non smokers

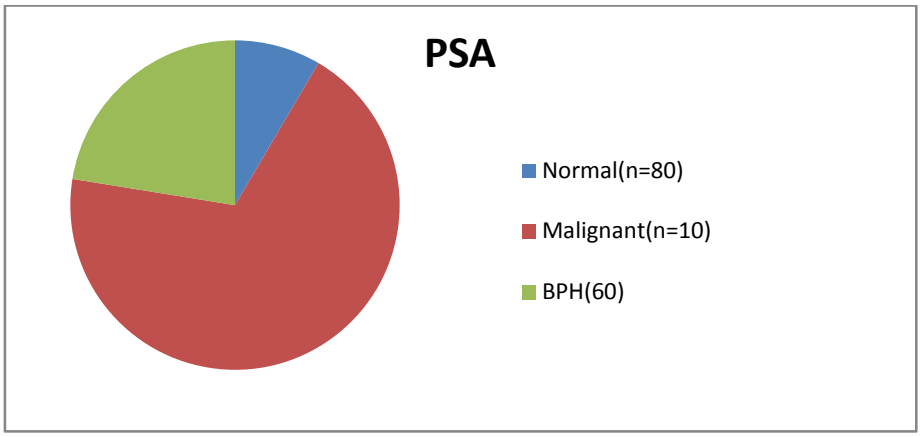

Fig.1.15.3 Level of PSA in normal, malignant and BPH

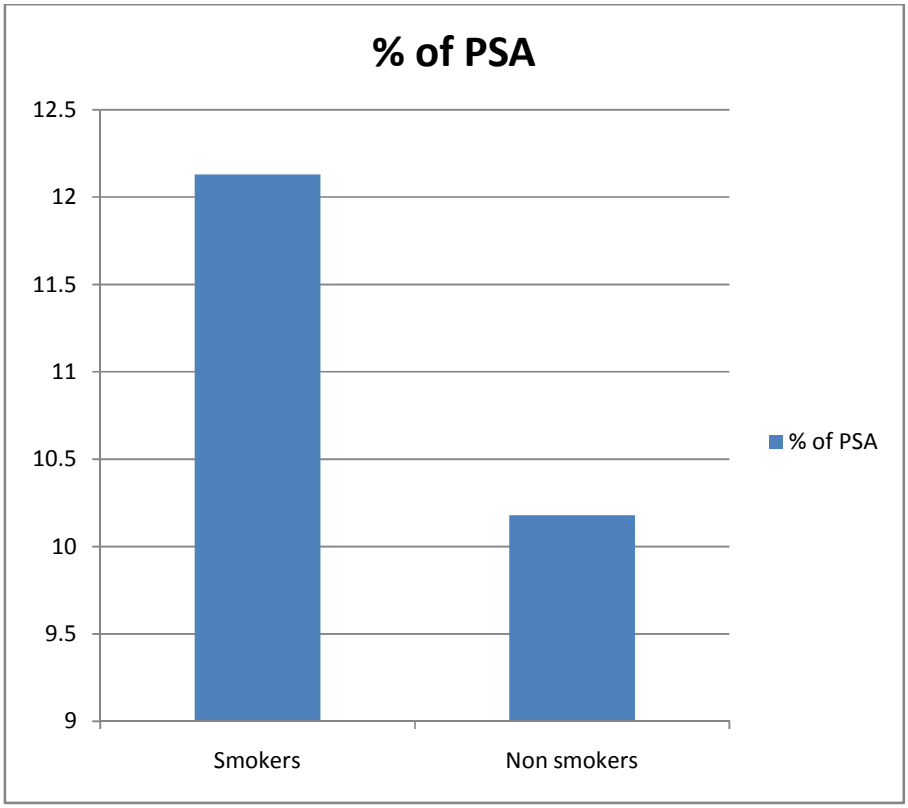

Fig.1.15.4 Percentage of PSA in smokers and non-smokers

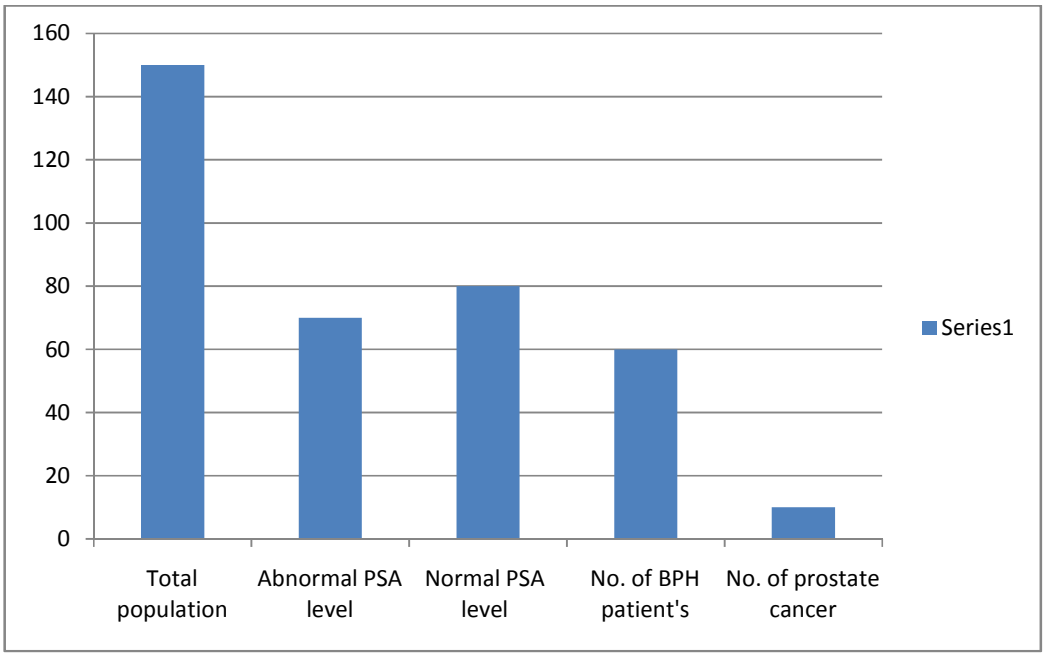

Fig 1.15.5 Prevalence of PSA in Jammu region

Discussion

Prostate cancer is the third most leading cause of death from cancer in men after colorectal and lung cancer. Prostate specific antigen testing is used for the early detection of prostate cancer or many other prostate related abnormalities such as benign prostatic hyperplasia, prostatitis. PSA belong to human kallikrein gene family, which is serine protease with chemotrypsin like activity. PSA is secreted in the epithelial cells of the prostate gland and can be demonstrated in biopsy samples or other histological specimens using immunohistochemistry. According to the study which was done in training session, it concludes that PSA is one of the best markers for diagnosis of prostate abnormalities and by biopsy prostate cancer was diagnosed. A total of 150 men were participated in this study and with the help of PSA marker the level was analyzed. The level of PSA rises in prostate abnormalities such as in benign prostatic hyperplasia, prostate carcinoma. Since level of PSA was comparatively higher in patients with history of smoking as observed from the present study. Thus the hazards of smoking (risk factor) cannot be ignored. So, by this study we concluded that the prostate cancer is a major health problem and the incidence is gradually increased. In Netherland, 20 men's were dying every day from prostate cancer. Curative treatment of prostate cancer reduces the disease specific mortality significantly. Early screening of disease by PSA marker reduces the mortality of prostate cancer. The prevalence of prostate cancer in the studied population was 15%. It is hoped that this work would help alert our adult male population on the need to go for early and routine screening for prostate disorders (from age 50). Early detection of BPH and prostate cancer makes management easy and lowers the impact of disease.

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