# Chapter One

## Introduction

Prostate tumor signifies the growth of cancerous cells in the prostate gland. Prostate tumor is more widely observed in men who are around 70 to 80 years old (American Cancer Society, 2010). However, prostate tumor in young men has become a common observation, as many people around the age of 30 are experiencing this problem too. Prostate cancer is a form of cancer that develops in the prostate, a gland in the malereproductive system. Most prostate cancers are slow growing; however, there are cases of aggressive prostate cancers (American Cancer Society, 2010). The cancer cells may metastasize (spread) from the prostate to other parts of the body, particularly the bones and lymph nodes. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, or erectile dysfunction. Other symptoms can potentially develop during later stages of the disease(American Cancer Society, 2010).

Many factors, including genetics and diet, have been implicated in the development of prostate cancer. The presence of prostate cancer may be indicated by symptoms, physical examination, prostate-specific antigen (PSA), or biopsy. The PSA test increases cancer detection but does not decrease mortality (Djulbegovic et al., 2010).

Prostate cancer is the most commonly diagnosed noncutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. It has addressed this problem by characterizing the expression profiles of several benign and malignant human prostate samples, and identified several genes that are differentially expressed between benign and malignant tumors (Magee et al., 2001). Hepsin (serine protease hepsin) is over expressed in prostate tumors, and in situ hybridization demonstrates that hepsin is specifically over expressed in the carcinoma cells themselves. These facts, together with the molecular properties of hepsin, make it an ideal target for prostate cancer therapy (Magee et al., 2001).

## Objectives

### General Objective:

To detect hepsin gene in prostate tumors patients in Gezira state using polymerase chain reaction (PCR) technique.

### Specific Objectives:

To associate between the expression of hepsin gene and the behavior of prostate tumors.

To describe the relation between the age group and the behavior of prostate tumors.

# Chapter Two

# 2.Review of Literature

## 2.1 Anatomy, histology and physiology of prostate:

The prostate is a compound tubuloalveolarexocrine gland of the male reproductive system in most mammals, It differs considerably among species anatomically, chemically, and physiologically(Tsukise and Yamada, 1984).

The prostate is a part of the male reproductive system that helps make and store seminal fluid. In adult men, a typical prostate is about 3 centimeters long and weighs about 20 grams (Aumuller and Adler, 1979). It is located in the pelvis, under the urinary bladder and in front of the rectum. The prostate surrounds part of the urethra, the tube that carries urine from the bladder during

urination and semen during ejaculation(Dalley, 1999). The prostate gland contains three major glandular regions; the peripheral zone, the central zone, and the transition zone, which differ histologically and biologically. The central zone is relatively resistant to carcinoma and other disease; the transition zone is the main site of origin of prostate hyperplasia. There are also several important nonglandular regions concentrated in the anteromedial portion of the gland. Each glandular zone has specific architectural and stromal features. In all zones, both ducts and acini are lined by secretory epithelium. In each zone, there is a layer of basal cells beneath the secretory lining, as well as interspersed endocrine-paracrine cells. Frequent deviations from normal histology include post-inflammatory atrophy, basal cell hyperplasia, benign nodular hyperplasia, atypical adenomatous hyperplasia, and duct-acinar dysplasia. These lesions may at times be confused with carcinoma, especially in biopsy material (McNeal, 1988).

## 2.2 Pathology of Prostate:

### 2.2.1 Prostatic Hyperplasia:

Nodular prostatic hyperplasia (also termed benign prostatic hyperplasia or BPH) is a common condition as men age. Perhaps a fourth of men have some degree of hyperplasia by the fifth decade of life. By the eighth decade, over 90% of males will have

prostatic hyperplasia (Bushman, 2009).The mechanism for hyperplasia may be related to accumulation of dihydrotestosterone in the prostate, which then binds to nuclear hormone receptors which then trigger growth (Andriole et al., 2004). The normal prostate weighs 20 to 30 gm, but most prostates with nodular hyperplasia can weigh from 50 to 100 gm. Hyperplasia begins in the region of the veru-montanum, in the inner zone of the prostate, and extends to involve lateral lobes. This enlargement impinges upon the prostatic urethra, leading to the difficulty on urination with hesitancy that is typical for this condition. Dysuria, dribbling, and nocturia are also frequent. The urinary tract obstruction leads to urinary retention and risk for infection. In severe, prolonged cases, hydroureter with hydronephrosis and renal failure can ensue (Wasserman, 2006).

Microscopically, nodular prostatic hyperplasia consists of nodules of glands and intervening stroma. Most of the hyperplasia is contributed by glandular proliferation, but the stroma is also increased, and in rare cases may predominate. The glands may be more variably sized; with larger glands have more prominent papillary enfolding. Nodular hyperplasia is not a precursor to carcinoma (Homma et al., 1996).

### 2.2.2 Prostatic Intraepithelial Neoplasia:

Prostatic intraepithelial Neoplasia (PIN), which is dysplasia of the epithelium lining prostate glands, is a probable precursor of prostatic carcinoma. The appearance of PIN may precede carcinoma by 10 or more years. It can be divided into low grade

and high grade PIN. Low grade PIN may be found even in men in middle age. PIN does not routinely increase the serum prostate specific antigen (PSA). PIN usually involves an acinus or a small cluster of acini, but it can be more extensive on occasion. The acini are usually medium-sized to large, with rounded borders. The partial involvement ofan acinus is a helpful feature to distinguish PIN from adenocarcinoma. PIN is characterized histologically by progressive basal cell layer disruption, loss of markers of secretary differentiation, nuclear and nucleolar abnormalities, increasing proliferative potential, increasing micro vessel density, variation in DNA content, and allelic loss. Unlike adenocarcinoma, with which it may coexist, glands with PIN retain an intact or fragmented basal cell layer (Ayala and Ro, 2007).

Low grade PIN has epithelial cells that are crowded and irregularly spaced, with nuclei that are hyper chromatic and pleomorphic, with small nucleoli. High grade PIN has even more hyperchromatism and pleomorphism, the cells are more crowded and heaped up, and nucleoli can be prominent. Immunohistochemical staining with antibody to low molecular weight keratin can help to identify the fragmented basal cell layer. Anti-androgenic drug therapy may cause regression of PIN (Ayala and Ro, 2007).

### 2.2.3 Prostatic Adenocarcinoma:

Adenocarcinoma of the prostate is common. It is the most common non-skin malignancy in elderly men. It is rare before the age of 50, but autopsy studies have found prostatic

adenocarcinoma in 80% of men more than 80 years old. Many of these carcinomas are small and clinically insignificant. However, some are not, and prostatic adenocarcinoma is second only to lung carcinoma as a cause for tumor-related deaths among males (Bostwick et al., 2004).

Prostatic adenocarcinomas are composed of small glands that are back-to-back, with little or no intervening stroma. Cytologic features of adenocarcinoma include enlarged round, hyper chromatic nuclei that have a single prominent nucleolus. Mitotic figures suggest carcinoma. Less differentiated carcinomas have fused glands called cribriform glands, as well as solid nests or sheets of tumor cells, and many tumors have two or more of these patterns. Prostatic adenocarcinomas almost always arise in the posterior outer zone of the prostate and are often multifocal (Pearson et al., 1996).

**2.3 Grading of Prostate Cancer:**

Prostatic adenocarcinomas are usually graded according to the Gleason grading system based on the pattern of growth. There are 5 grades (from 1 to 5) based upon the architectural patterns. Adenocarcinomas of the prostate are given two grades based on the most common and second most common architectural patterns. These two grades are added to get a final grade of 2 to 10. The stage is determined by the size and location of the cancer, whether it has invaded the prostatic capsule or seminal vesicle, and whether it has metastasized. The grade and the stage correlate well with each other and with the prognosis. The

prognosis of prostatic adenocarcinoma varies widely with tumor stage and grade. Cancers with a Gleason score of <6 are generally low grade and not aggressive. Advanced prostatic adenocarcinomas typically cause urinary obstruction, metastasize to regional (pelvic) lymph nodes and to the bones, causing blast metastases in most cases. Metastases to the lungs and liver are seen in a minority of cases (Gleason, 1992, Bostwick, 1996, Epstein, 2010).

**2.4 Risk Factor for Prostate Cancer:**

**Age:**

Prostate tumor is very rare in men younger than 40, but the chance of having prostate tumor rises rapidly after age 50. About 6 in 10 cases of prostate tumor are found in men over the age of 65 (American Cancer Society, 2013).

**Genetics**:

Genetic background may contribute to prostate cancer risk, as suggested by associations with race, family, and specific gene variants. Men who have a first-degree relative (father or brother) with prostate cancer have twice the risk of developing prostate cancer, and those with two first-degree relatives affected have a fivefold greater risk compared with men with no family history (Steinberg et al., 1990). In the United States, prostate cancer more commonly affects black men than white or Hispanic men, and is also more deadly in black men (Gallagher and Fleshner, 1998, Hoffman et al., 2001). In contrast, the incidence and mortality rates for Hispanic men are one third lower than for non-

Hispanic whites. Studies of twins in Scandinavia suggest that 40% of prostate cancer risk can be explained by inherited factors(Lichtenstein et al., 2000).

No single gene is responsible for prostate cancer; many different genes have been implicated. Mutations in BRCA1 and BRCA2, important risk factors for ovarian cancer and breast cancer in women, have also been implicated in prostate cancer (Struewing et al., 1997). Other linked genes include the hereditary prostate cancer gene 1 (HPC1), the androgen receptor, and the vitamin D receptor(Gallagher and Fleshner, 1998).

**Social class:**

There are a relationship between social class and prostate cancer. Lower social class is associated with increased mortality from prostate cancer, probably due to lack of knowledge and willingness to seek medical advice (Fitzpatrick et al., 1998).

**Diet:**

Nutrition may play a significant role in both the prevention and progression of prostate cancer. For example, soya proteins, vitamin E derivatives, the essential trace element selenium and reduced fat intake, especially animal fat, may have a protective effect against prostate cancer (Fair et al., 1997).

### 2.5 Diagnosis of Prostate Tumor:

#### 2.5.1 Digital Rectal Examination:

The digital rectal examination (DRE) was the test for early detection of prostate cancer (Optenberg et al., 1997).Most prostate tumors are located in the peripheral zone of the prostate

and may be detected by DRE when the volume isabout 0.2 mL or larger. The risk of a positive DRE turning out to be cancer is heavily dependent on the PSAvalue (Carvalhal et al., 1999, Catalona et al., 1994).

### 2.5.2 Prostate Imaging:

Ultrasound (US) and magnetic resonance imaging (MRI) are the two main imaging methods used for prostate cancer detection. Urologists use transrectal ultrasound during prostate biopsy and can sometimes see a hypoechoic area (tissues or structures that reflect relatively less of the ultrasound waves directed at them). But ultrasound has poor tissue resolution and thus, is generally not clinically used. Prostate MRI has better soft tissue resolution than ultrasound (Bonekamp et al., 2011).Currently, MRI is used to identify targets for prostate biopsy using fusion MRI with ultrasound (US) or MRI-guidance alone. In men who are candidates for active surveillance, fusion MR/US guided prostate biopsy detected 33% of cancers compared to 7% with standard ultrasound guided biopsy (Natarajan et al., 2011).

### 2.5.3Tumor Markers:

Tissue samples can be stained for the presence of PSA and other tumor markers in order to determine the origin of malignant cells that have metastasized (Leav et al., 2010). Prostate specific antigen (PSA) is a substance made by cells in the prostate gland (both normal cells and cancer cells). PSA is mostly found in semen, but a small amount is also found in the blood. Most healthy men have levels under 4 nanograms per milliliter (ng/mL)

of blood. The chance of having prostate cancer goes up as the PSA level goes up (American Cancer Society 2013).The expression of BCL-2, Ki-67 and ERK5 by immunohistochemistry may be a significant predictor of patient outcome for men with prostate cancer (Yao et al., 2010).

### 2.5.4 Biopsy:

A biopsy is a procedure in which a sample of body tissue is removed and then looked at under a microscope. A core needle biopsyis the main method used to diagnose prostate cancer. It is usually done by urologist, using transrectal ultrasound to see the prostate gland. When the needle is pulled out, it removes a small cylinder (core) of prostate tissue. This is repeated from 8 to18 times, but most urologists will take about 12 samples (American Cancer Society, 2013).

## 2.6 Prognosis of Prostate Tumor:

Prognosis of prostate cancer depends on many factors. For non-metastatic disease, age, clinical stage, tumor grade, serum PSA level and co-morbidity are the most important prognostic factors. If discovered early enough, prostate cancer can be cured in about 90% of cases (Denmeade and Isaacs, 1997).

## 2.7 Hepsin gene:

Serine protease hepsin is an enzyme that in humans is encoded by the HPN gene. Hepsin is a cell surface serine protease (Leytus et al., 1988).Hepsin is a type II integral membrane serine protease that has been found to be one of the most upregulated genes in prostate cancer. The physiological function of hepsin

remains unknown, but within the carcinogenesis pathway of the prostate it appears to play a role in cancer cell migration/invasion rather than cell proliferation (Wu and Parry, 2007). The human hepsin gene was localized to chromosome 19 at qll-13.2. The messenger RNA of hepsin is 1.85 kilobases in size and present in most tissues, with the highest level in liver. Hepsin is synthesized as a single polypeptide chain, and its mature form of 51 kilo Dalton was found in various mammalian cells including HepG2 cells and baby hamster kidney cells. It is present in the plasma-membrane in a molecular orientation of type I1 membrane-associated proteins, with its catalytic subunit (carboxyl-terminal half) at the cell surface and its amino terminus facing the cytosol. Hepsin is found neither in cytosol nor in culture media. Theresearch results suggest that hepsin has an important role(s) in cell growth and function (Tsuji et al., 1991).

**2.8 Previous studies:**

The mechanisms by which hepsin were affected prostate cancer progression were not fully understood, thus a pathway approach to discovering a combination of HPNrelated gene variants contributing to a phenotype such as aggressive prostate cancer may be challenging. Disorganization of the basement membrane via enzymatic activity of hepsin may be through activation of urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator and the subsequent initiation of the plasminogen/plasmin proteolytic pathway (Moran et al., 2006). Interestingly, transgenic mice models where overexpression of

hepsin in prostate tissue showed disorganization of the basement membrane also showed weaker staining of laminin-332 (Klezovitch et al., 2004).

# Chapter Three

# 3.Materials and Methods

## 3.1 Study Design:

This is a descriptive retrospective hospital based case study.

## 3.2 Study Area:

The study was carried out in Gezira state, National Cancer Institute, (NCI) University of Gezira, during the period from April 2012 to July 2013.

## 3.3Study Population and Sample Size:

One hundred blood samples were randomly collected from patients previously diagnosed as prostate tumors, of whom 50 patients were diagnosed as benign prostate hyperplasia as control group and 50 patients were diagnosed as prostate cancer as case group.

## 3.4 Sample Collection:

2.5 ml blood in EDTA tube was collected from each patient.

## 3.5 Sample Techniques:

### 3.5.1 DNA Purification from Blood (Spin Protocol):

Procedure:

20 µl QIAGEN protease (or proteinase K) waspipetted into the bottom of a 1.5ml micro centrifuge tube, then 200 µl samples were added to the micro centrifuge tube, after that used up to 200 µl whole blood in 200 µl PBS**.**

200 µlbuffer ALwasadded to the sample. Mixed by pulse-vortexing for 15s, and Incubated at 56°C for 10 min. Briefly the 1.5 ml micro centrifuge tube wascentrifuged to remove drops from the inside

of the lid, 200 µl ethanol (96–100%) wasadded to the sample, mixed again by pulse-vortexing for 15 s. After mixing, briefly the 1.5 ml micro centrifuge tube wascentrifuged to remove drops from the inside of the lid.

Carefully the mixturewas appliedto the QIAamp Mini spin column(in a 2 ml collection tube) without wetting the rim. Thecap was closed, and centrifuged at 6000 x g(8000 rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2 ml collection tube (provided) and discarded the tube containing the filtrate.

Carefully the QIAamp Mini spin column was opened and 500 µl buffer AW1 wasadded without wetting the rim. The cap wasclosed and centrifuged at 6000 x g(8000 rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2 ml collection tube (provided) and discarded the collection tube containing the filtrate.

Carefully the QIAamp Mini spin columnwas opened and 500 µl buffer AW2 was added without wetting the rim. Thecap was closed and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min.

Recommended: The QIAamp Mini spin column was placed in a new 2 ml collection tube (not provided) and discarded the old collection tube with the filtrate and centrifuged at full speed for 1 min.

TheQIAamp Mini spin column was placed in a clean 1.5 ml micro centrifuge tube (not provided), and discarded the collection tube

containing the filtrate. Carefully the QIAamp Mini spin column was opened and 200 µl buffer AE or distilled water was added. Incubated at room temperature (15–25°C) for 1 min, and then centrifuged at 6000 x g(8000 rpm) for 1 min.

### 3.5.2 PCR Technique:

Procedure:

22µl was taken from premix reagent in each tube (new PCR tube 0.2 tube), then5µl of DNA sample was added, after that negative control was made (added 5µl of H2O) and positive control was made by DNA ladder (different reading of bands) then PCR programmed was running.

PCR programmed: (according to protocol of conventional PCR)

Pre-denaturation: temp 94, time 10min, denaturation: temp 94, time 1min, annealing: temp 52, time 30 sec, extension: temp 72, time 1min.

Final extension: temp 72, time 10min, run the results in agarose gel by using electrophoresis machine and photo the results by digital camera.

### 3.6 Result Interpretation:

The presence of hepsin gene in prostate tumor was reviewed by experienced technologist according to protocol of PCR technique using negative control and ladder as positive control. Blood samples were selected because the tissue samples were not possible to collect and this test was very expensive and needs long time.

### 3.7 Statistical Analysis:

The obtained results analyzed using SPSS statistical program, frequency, mean and Pearson Chi-square test were used.

### 3.8 Ethical Consideration:

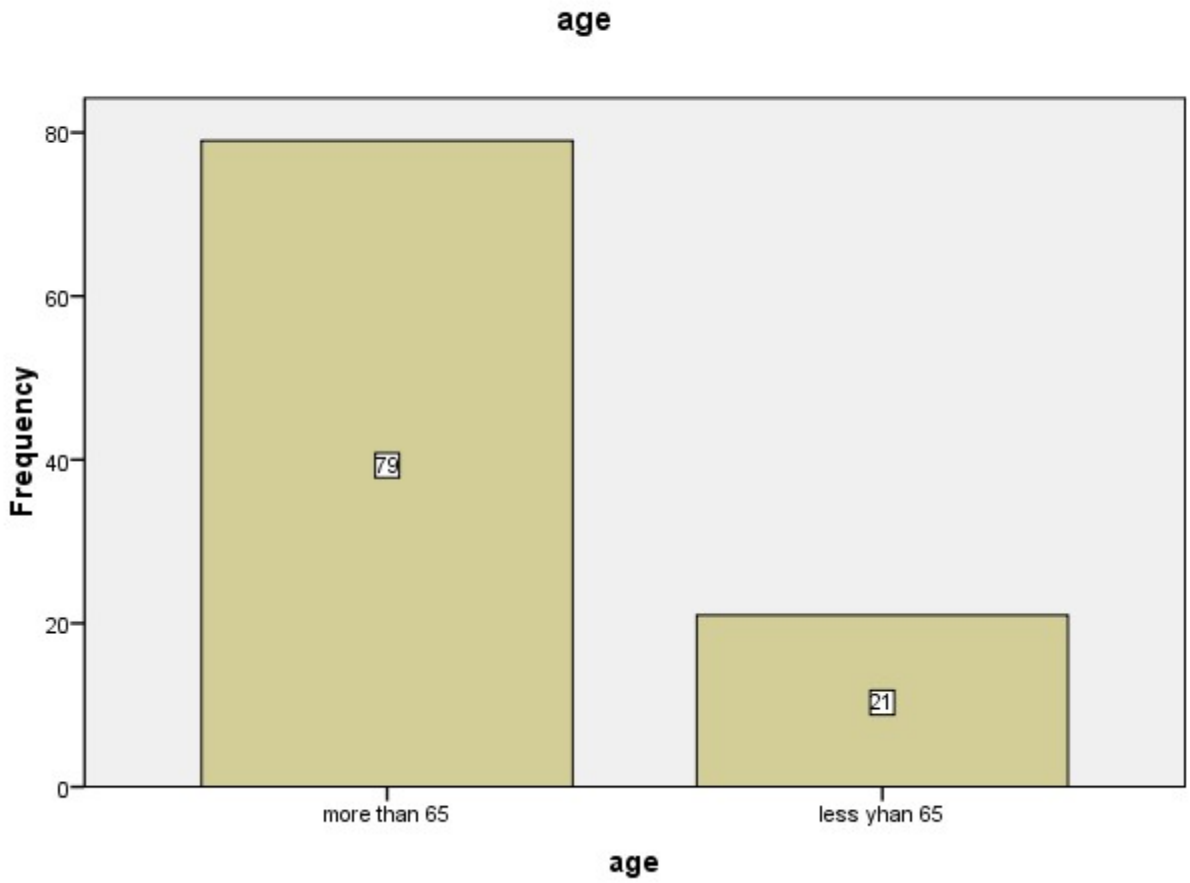
The ethical rules were applied during all the steps of this study and were approved, all patients were informed about the aim of study, all patients were agreed to be participants in this study after filling agreement form.

# Chapter Four

## Results

The study included 100 patients divided into 50 patients reported as prostate cancer as study group and 50 patients reported as benign prostate hyperplasia as control group .There age ranged from (50 - 90) with mean age70.2 ± 8.9.This study was confirmed that most of cases are found in patients with age more over than 65 years . The result showed that 10 cases were positive hepsin gene and 40 cases were negative, all the 50 control cases indicated negative hepsin gene ,with significant relation between presence of hepsin gene and tumor behavior (P=0.001) as showed in table (1) .

The result showed that 47 cases (prostate cancer) and 32 cases (benign prostate hyperplasia) were more over than 65 years, while 3 cases (prostate cancer) and 18 cases were less than 65 years, with significant relation between the age group and tumor behavior (P=0.000) as showed in table (2).




**Figure(1): Frequency of age group among study population**

**Table (1):The relation between the expressionof hepsin gene and tumor behavior**

| | Tumor behavior | | Total |
|---|---|---|---|
| | Cancer | BPH | |

**Figure(1): Frequency of age group among study population**

| | | |
|---|---|---|
| Tumor behavior | Positive | |
| Cancer | | |
| BPH | | |
| Total | | |

(P=0.001)

| **Table (2): The relation between the age group and the** | | | |
|---|---|---|---|
| More than 65 years | 47 | 32 | 79 |
| Less than 65 years | 3 | 18 | 21 |
| Total | 50 | 50 | 100 |

(P=0.000)

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**Photography (1): DNA product of prostate cancer samples positive with hepsin gene by PCR technique, run in gel electrophoresis; 1(negative control), 18(positive control), 10-11-12-13-24-33-37-38 were positive samples and all others samples were negative.**

## Chapter Five

## Discussion

This study found that most of study cases of prostate tumor were above 65 years.These findings supported the study by Chao. Their study reported that the risk of prostate cancer increases with age, and the mean age of patients was 63.3 years old(Chao et al., 2009).Albert and Clark, reported similar results. Their study recorded that prostate cancer screening, if conducted at all, may be discontinued at approximately 75 years of age in otherwise healthy men(Albert and Clark, 2008). This study found association between expression of hepsin gene and prostate cancer

supported by Pal, who reported hepsin expression patterns observed in malignant prostate cells (Pal et al., 2006). Hepsin has been shown to be over-expressed in 90% of prostate tumors, however the mechanism by which this up-regulation occurs is not clear (Magee et al., 2001),(Stephan et al., 2004). HPN is a cell surface protein and is absent or at low levels in BPH. Additionally, HPN expression in other cancers, including ovarian, breast, and renal, has been documented (Roemer et al., 2004)(Tozlu et al., 2006).

In Carsten et al study hepsin over expression in cancerous compared with noncancerous tissue was found in 81 of the 90 patient samples (90%, P=0.001). Hepsin is not tissue restricted. It is abundantly expressed in the prostate(Tsuji et al., 1991). Tanimoto study used quantitative RT-PCR, which is superior to the qualitative techniques for detection of hepsin gene, which associated with prostate cancer(Tanimoto et al., 1997).The search for potential new prostate cancer tumor markers has been accelerated by using gene expression profiling with cDNA microarrays. One study analyzed 4,712 genes and showed that hepsin was the only gene over expressed in malignant versusnonmalignant prostate samples (Magee et al., 2001). Interestingly hepsin was also found to be expressed at higher levels in prostate intraepithelial neoplasia lesions versus benign prostatic hyperplasia (BPH) tissue, indicating a correlation of hepsin over expression with neoplastic transformation in the prostate. The structure and its homology with other serine

proteases strongly imply a possible role for hepsin not only for promoting tumor growth, but also for cancer therapy(Magee et al., 2001). Welsh et al reported the high specific expression of hepsin gene in prostate tumor tissue(Welsh et al., 2001). Dhanasekaran reported that hepsin was also highly over expressed in high grade prostate intraepithelial neoplasia lesions, followed by primary prostate cancer, hormone refractory prostate cancer and benign prostate tissue to the lowest degree(Dhanasekaran et al., 2001).

The study of Bernards and Weinberg suggest that combinationof early oncogenic alterations in the primary tumordetermines its metastatic potential (Bernards and Weinberg, 2002). It was found that hepsin has no impact on cell proliferationand, therefore, it does not act as a classic oncogene. In contrast, increase in hepsin expression leads to disorganizationof the basement membrane and promotes primary prostatecancer progression and metastasis. These hepsin functions areconsistent with the classic model of metastatic process. On theother hand, the fact that hepsin becomes upregulated very earlyin the human prostate cancer at the stage of PIN-like lesions ismore in line with the alternative model of metastasis. The basement membrane is a specialized extracellular matrixstructure that separates the epithelial and stromal cell compartments. Loss of the basement membrane is a mandatory step that occurs during local invasion early in the metastatic process (Abate-Shen and Shen, 2000); (Robinson et al., 2004).

# Chapter Six

# Conclusion and Recommendation

## Conclusion:

This study concludes thatthe expression of hepsin gene is associated with prostate cancer, and most of study cases of prostate tumor were above 65 years.

## Recommendation:

On the base of this study we recommend: Further studies should be done with large sample size to detect the mutant hepsin gene in prostate tumor, and used it in prostate cancer therapy.