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Expression of E-cadherin in prostatic carcinoma: prognostic significance.

A thesis submitted as partial fulfillment of the requirement for Master Degree in Pathology.

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(2011-2012)

بسم الله الرحمن الرحيم

"قَالوا سُبحانك لا عِلم لنَا إلاّ ما علّمتنا إنّك أنت العَليم الحَكيم"

صدق الله العظيم

سورة البقرة آية(32)

DEDICATION

TO MY BELOVED HUSBAND AND CHILDREN THIS WORK IS DEDICATED

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ACKNOWLEDGMENT:

I would like to thanks GOD for every effort done in this work and I wish to express my gratitude to my supervisors Dr.Adam Elzagheid and Prof. Omran Al-Fituri for their understanding, continuous encouragement and never failing throughout the study. My appreciation is also extends to Dr. Fatma Emaetig (head of the Department), Dr. Fatma Ben Khaial and my entire colleague for their profitable suggestion and advices. The excellent technical help of the staff of histopathology Department is greatly appreciated. Last but not least, without the moral support of my husband, my mother and my mother-in-low nothing would have been accomplished.

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ABBREVIATIONS:

ACS	American cancer society
AJCC	American Joint Committee on cancer
AR	Androgen receptors
ASR	Age-standardized rates
AST	Androgen suppression therapy
ATP	Adenosine tri phosphates
BCL2	B-cell lymphoma-leukemia
BPH	Benign prostatic hyperplasia
CAMs	Cell adhesion molecules
DCE	Dynamic contrast enhanced
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
EN2	Engrailed-2
FAK	Focal adhesion kinase
FC	Flow cytometry
FNA	Fine needle aspiration
GnRH	Gonadotrophic releasing hormone
GS	Gleason score
HIFU	High intensity focused ultra sound

IGF-1	Insulin like growth factor
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
m-	Mitochondrial
MIC-1	Macrophage inhibitory cytokine-1
MRI	Magnetic resonance image
MVD	Micro vessels density
NSAIDs	Non steroidal anti inflammatory drugs
P53	Tumor suppressor gene
PAP	Prostatic acid phosphatase
PBP	Prostate binding protein
Pca	Prostatic carcinoma
PCA3	Prostate cancer gene 3
PET	Positron emission tomography
PIN	Prostatic intraepithelial neoplasia
PLND	Pelvic lymph-node dissection
PSA	Prostate specific antigen
PSMA	Prostate specific membrane antigen
QALYs	Quality adjusted life years
RNA	Ribonucleic acid
RP	Radical prostatectomy

RRP	Radical retropubic prostatectomy
S phase	Synthesis phase of the cell cycle
SEER	Surveillance epidemiology and end result
SPF	S-phase fraction
SVI	Seminal vesicle invasion
TNM	Tumor-Nodes-Metastases
TRUS	Transrectal ultrasound
TURP	Transurethral resection of the prostate
US	Ultra sound
WHO	World Health Organization
XIAP	X-Linked inhibitor of apoptosis
XMRV	Xenotropic mulv-related virus

CHAPTER 1

1.1. ABSTRACT

Background and aim: Improvements in the diagnosis of prostate cancer (Pca) have now resulted in screening programs that reveal many more organ confined lesions, with a concomitant increase in the number of radical surgeries. Whether this truly improves overall survival is not clear because of the unpredictable biological potential of these tumors. Not every prostate cancer will progress; on the other hand, metastases still develop in a considerable group of patients with localized disease that are treated radically. The value of grade and clinical stage of the disease is controversial, although the pathological stage seems to be one of the best prognostic factors available to date. Clearly, methods that allow more accurate prediction of the clinical behavior are urgently needed. In this respect, the identification of molecular prognostic markers has now gained considerable attention, So that few of these have yet established clinical value. One of these markers, the epithelial cell adhesion molecule, E-cadherin, is of particular interest since it can function as an invasion suppressor gene. The aim of the current study to assessing the expression of E-cadherin in prostatic carcinoma and determine its relation to different clinicopathological features. **Patients and method:** Paraffin blocks of 50 Libyan cases of Pca were retrieved. Immunohistochemistry was performed. Membranous staining was evaluated separately. E-cadherin immunoexpression was categorized for statistical analysis. Statistical tests were used to determine the association of E-cadherin with clinicopathological characteristics; age, tumor stage, Gleason score, tumor grade, perineural invasion. **Result:** E-cadherin immunostaining results showed membranous E-cadherin expression in Pca. There was no significant correlation between E-cadherin expression and tumor stage and perineural invasion (P> 0.05). How ever, E-cadherin expression was significantly associated with old age (P 0.026), Gleason score (P 0.003), and tumor grade (P 0.004). **Conclusion:** The results implicate the usefulness of E-cadherin expression as prognostic tools of prostatic carcinoma.

Key words: Pca, E-cadherin expression, IHC, prognosis

1.2. INTRODUCTION

Carcinoma of prostate (Pca) is the most common visceral cancer in males, ranking as the second most common cause of cancer related death in men older than 50 years of age after carcinoma of the lung (Alfitori, 2007). In Libya the incidence of Pca is 7% of all male malignancies and comes fourth (El Mistiri, 2010). It is predominantly disease of older males, with peak incidences between the ages of 65 and 75 years. Latent cancers of prostate are even more common than those that are clinically appeared with an overall frequency of more than 50% in men older than 80 years of age (Alfitori, 2007; Robbin et al., 2007). The tumour arises anywhere in the prostate, but often in the periphery of the gland and especially on the posterior surface (Anderson, 1980).

Although the cause of carcinoma of prostate remains unknown, clinical experimental observations suggest that hormone, genes and environmental all have role in its pathogenesis. Symptomatic carcinoma of the prostate is more common and occurs at an earliest age in American blacks than in whites, Asians or Hispanics, Whether such racial difference occur as a consequence of genetic influence, environmental factors or some combination of the two remains unknown (Robbin et al., 2007).

There is a significant relation between lifestyle including food consumption and cancer prevention. (Wiseman, 2008).

The presence of Pca 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).

An important part of evaluating Pca is determining the stage, or how far the cancer has spread. Knowing the stage helps define prognosis and is useful when selecting therapies. The most common system is the four stages Tumor/Nodes/Metastases (TNM) system. Its components include the size of the tumor, the number of involved lymph nodes, and the presence of any other metastases (BMJ Group, 2009). A number of histological grading schemes have been proposed for carcinoma of the prostate. They are based on feature such as the degree of glandular differentiation. The architecture of the neoplastic glands, nuclear anaplasia and mitotic activity (Robbin & cotran, 2004).

A commonly used method for grading is the Gleason system that has proved to correlate reasonably well with both the anatomic stage of Pca and the progress and this system based on the degree of glandular differentiation and growth pattern of the tumor in relation to the stroma, these features are assessed by low power examination of prostatic tissue (Robbin et al., 2007; Al fituri, 2007).

In patients who undergo treatment, the most important clinical prognostic indicators of disease outcome are stage, pre-therapy PSA level, and Gleason score. In general, the higher the grade and the stage, the poorer the prognosis (Djulbegovic et al., 2003).

The control of cellular adhesion and motility is one of the crucial mechanisms responsible for tumor initiation and progression. The genes involved are also contributors to malignancy along with genes responsible for cell proliferation and survival. This research work proposed to summarize the current knowledge on one of the most important tumor suppressor gene, as E-cadherin (Berx et al., 1995; Guilford, 1999).

E-cadherin is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherens junctions (Gumbiner, 1996).

As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMs), the cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding. Classical cadherins, E and N-cadherins being the best characterized play important roles in the formation of tissues during gastrulation, neurulation and organogenesis (Barth et al., 1997).

E-cadherin has probably been studied in most detail. It is essential for the formation and maintenance of epithelia, was first identified in chicken, and was originally called L-CAM (Gallin et al., 1987). The mouse counterpart of this protein, uvomorulin (Ringwald et al., 1991), has 80% identity in both nucleotide and amino acid sequences to the human counterpart (Mansouri et al., 1998).

Besides its role in normal cells, this highly conserved gene can play a major role in malignant cell transformation, and especially in tumor development and progression. The suppression of E-cadherin expression is regarded as one of the main molecular events

responsible for dysfunction in cell-cell adhesion. Most tumors have abnormal cellular architecture, and loss of tissue integrity can lead to local invasion. Thus, loss of function of E-cadherin tumor suppressor protein correlates with increased invasiveness and metastasis of tumors, resulting in it being referred to as the "suppressor of invasion" gene (Vleminckx et al., 1991). This study tries to explain the usefullness and accuracy of evaluting Ecadherin expression by using immunohistochemistry as prognostic tools of Pca.

1.3. AIMS OF THE STUDY:

1. To evaluate the expression of E-cadherin in Libyan prostatic cancer patients.
2. To study the relationship between traditional prognostic parameters such as age, stage, Gleson score, tumor grade, perineural invasion in correlation with E-cadherin expression of tumor cell.

CHAPTER 2

2. REVIEW OF LITERATURES

2.1. Development

The prostatic part of the urethra develops from the pelvic (middle) part of the urogenital sinus (endodermal origin). Endodermal outgrowths arise from the prostatic part of the urethra and grow into the surrounding mesenchyme. The glandular epithelium of the prostate differentiates from these endodermal cells, and the associated mesenchyme differentiates into the dense stroma and the smooth muscle of the prostate (Moore &Persaud, 2008).

The prostate glands represent the modified wall of the proximal portion of the male urethra and arise by the 9th week of embryonic life in the development of the reproductive system. Condensation of mesenchyme, urethra and Wolffian ducts gives rise to the adult prostate gland, a composite organ made up of several glandular and non-glandular components tightly fused (Moore &Persaud, 2008).

2.2. Structure

A healthy human prostate is slightly larger than a walnut. In actuality, it is approximately the size of akiwifruit. The mean weight of the "normal" prostate in adult males is about 11 grams, usually ranging between 7 and 16 grams (Leissner KH, Tisell LE 1979).

The prostate gland, mainly consisting of a fibromuscular glandular part and the stroma, has the shape of a pyramid and lies on the pelvic musculofascial floor, being surrounded by thin layer of connective tissue (McNeal, 1972; McNeal, 1988; Dixon et al., 1999; Raychaudhuri & Cahill, 2008). The gland has a base and an apex, anterior and posterior surfaces and two infero-lateral surfaces. The base is connected to the bladder neck and the apex is surrounded inferiorly by the external sphincter, all forming together the proximal urethra, the main continence mechanism in the male. The prostate is separated posteriorly from the rectum by the anterior layer of Dennonvillier´s fascia and is fixed anteriorly to the pubic bone with the puboprostatic ligaments, being held in the dorsal vein plexus between these structures (Dixon et al. 1999). A thin layer of connective tissue forms the”true” capsule in the periphery of the prostate, outside of which the pelvic fascia forms the ”false” capsule (Dixon et al., 1999).

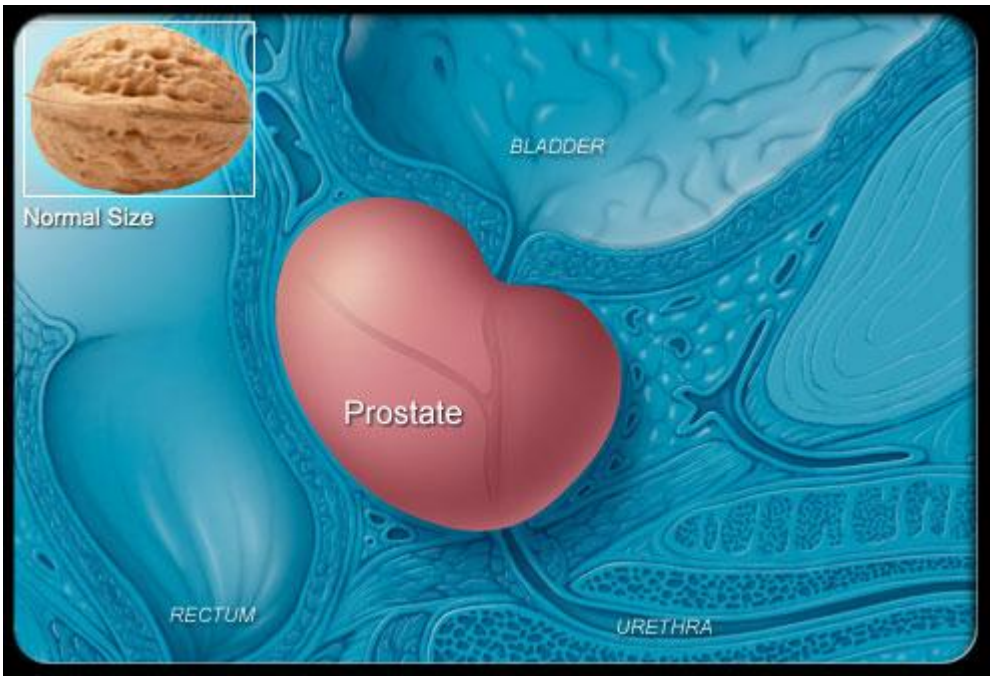

Figure 2.2. A. The prostate and other nearby organs. ©1996-2013 MedicineNet

Figure 2.2.B. The prostate and other nearby organs. ©1996-2013 MedicineNet

The main arterial supply to the prostate gland is from the prostatic branches of the inferior vesical artery, and it is also supplied by small branches from the middle rectal and pudendal vessels. The veins are situated mainly between the"true" and"false"

capsules. The lymphatic vessels from the prostate gland drain into internal iliac lymph nodes (Dixon et al., 1999). The prostatic urethra is about 3 cm long, and two ejaculatory ducts (one or two orifices) open in the colliculus seminalis (or verumontanum) near the external sphincter.

Histologically, the prostate gland can be divided into three parts. The peripherial zone forms about 70% of glandular part, and its ducts open into the distal prostatic urethra. The central zone forms about 25% of the glandular prostate, the ducts of which open mainly into the middle prostatic urethra. The transitional zone (about 5%) consists of two small lobes, and the ducts open almost into the sphincteric part of the urethra. The entire duct-acinar system with the exception of the main lateral ejaculatory ducts is lined by columnar secretory cells, which are separated from the prostatic stroma by a layer of basal cells belonging to the basement membrane (McNeal, 1972; Blacklock, 1974; Dixon et al. 1999).

The human prostate gland receives dual autonomic innervation from both parasympathetic (cholinergic) and sympathetic (noradrenergic) nerves in the prostatic nerve plexus, a part of the pelvic autonomic plexus that lies adjacent to the prostate gland. The pelvic plexus receives its parasympathetic input from the sacral segments of the spinal cord (S2-4) and sympathetic fibres from the hypogastric presacral nerves (T10–L2). The autonomic nerves arising from the pelvic plexus escort the vascular supply.

Both cholinergic and noradrenergic fibres innervate the prostate stroma, and cholinergic nerves innervate the smooth muscle of the capsule and the space around the blood vessels and are responsible for the secretory function of the epithelial part. The sympathetic nerves control the prostatic musculature, and their excitation closes the bladder neck during ejaculation of the seminal fluid into the urethra (Dixon et al., 1999).

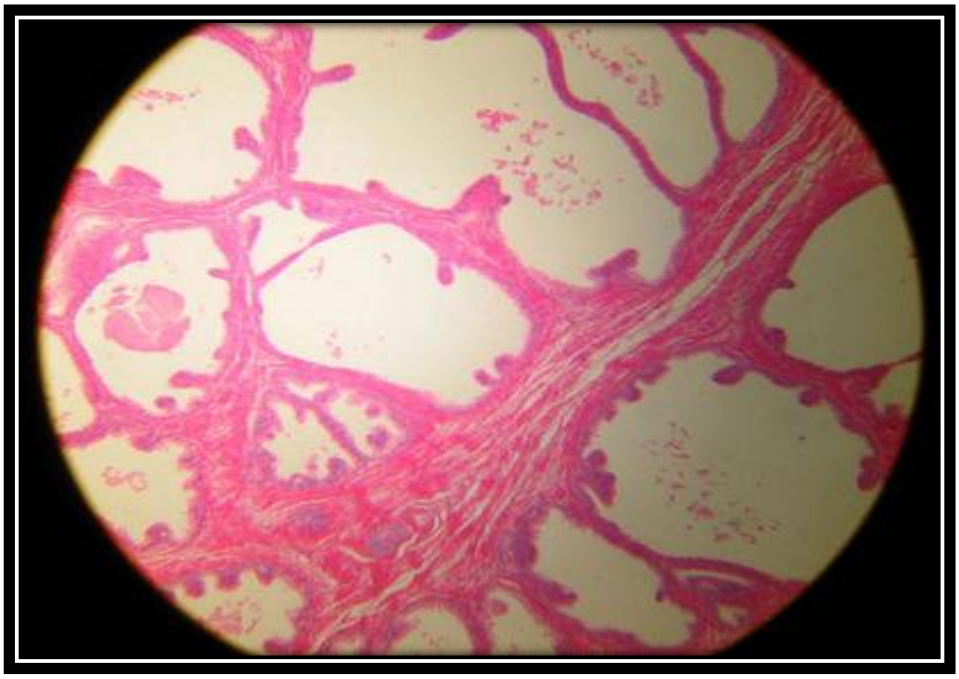

Figure 2.2.C. Normal histology of prostate http://www.histology

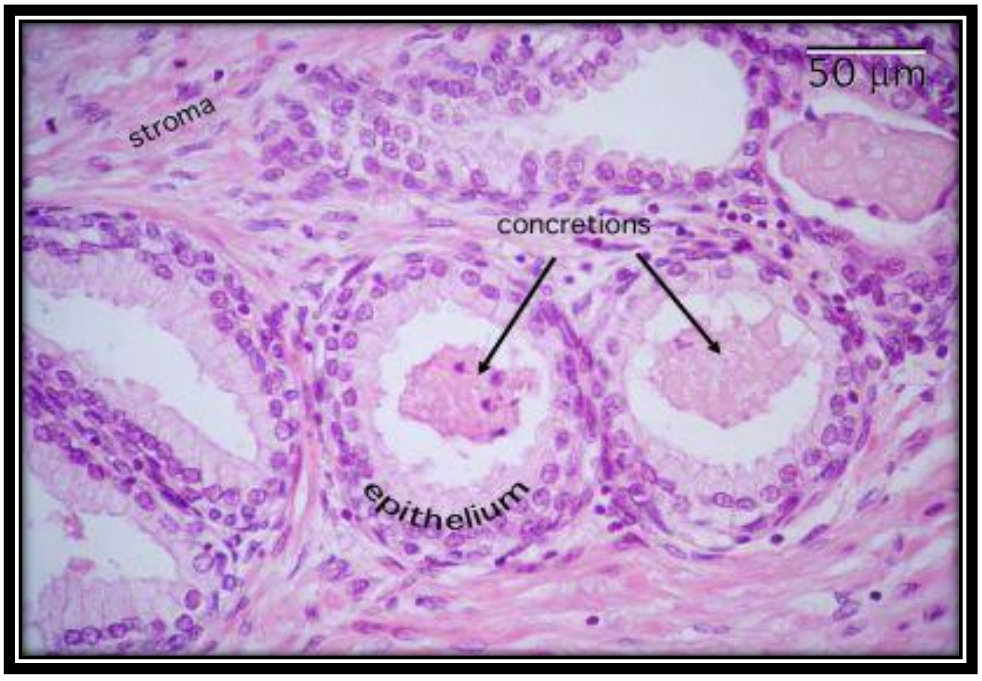

Figure 2.2.D. Normal histology of prostate http://www.histology

2.3. Function and secretions

The prostate gland provides about 20 percent of the seminal fluid volume. Its secretions are thin, watery, and alkaline, which accounts for the slight alkalinity of the semen. Calcium, cholesterol, and citric acid are among the prostate secretions, and it is also the source of the protein hydrolytic enzymes. The remaining gland provide other substances necessary for sperm survival for example, seminal fluid is well buffered with both phosphate and bicarbonate so that the PH remains slightly alkaline .This alkalinity ensures high sperm viability when sperm are deposited in the acid secretions of the vagina (Ewald, 1982). 57 major protein groups, of which 27 are non-serum proteins (i.e. presumably exuded by the epithelial cells), have been identified. Major prostatic-specific proteins are prostatic acid phosphatase (PAP), prostate specific antigen (PSA) and prostate binding protein (PBP), which are expressed at pubertal and adult ages. Proteolysis is the major function of prostate secretion, being rich in exopeptidase and endopeptidase. The most extensively studied protease is PSA, also known as seminin, seminal protease or chymotrypsin-like protease (Neal et al., 1992; Dixon et al., 1999).

2.4. Prostate cancer

Pca tends to develop in men over the age of fifty and although it is one of the most prevalent types of cancer in men, many never have symptoms, undergo no therapy, and eventually die of other causes (Siegel et al., 2011).

This is because cancer of the prostate is, in most cases, slow-growing, symptom-free, and since men with the condition are older they often die of causes unrelated to the Pca, such as heart/circulatory disease, pneumonia, other unconnected cancers, or old age. On the other hand, the more aggressive Pca account for more cancer-related mortality than any other cancer except lung cancer (Siegel et al., 2011).

Many factors, including genetics and diet, have been implicated in the development of Pca. The presence of Pca 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).

2.4.1. History

Although the prostate was first described by Venetian anatomist Niccolò Massa in 1536, and illustrated by Flemish anatomist Andreas Vesalius in 1538, prostate cancer was not identified until 1853(Adams, 1853).

Pca was initially considered a rare disease, probably because of shorter life expectancies and poorer detection methods in the 19th century. The first treatments of Pca were surgeries to relieve urinary obstruction (Lytton, 2001).

Removal of the entire gland (radical perineal prostatectomy) was first performed in 1904 by Hugh H. Young at Johns Hopkins Hospital (Young, 1905). Surgical removal of the testes (orchiectomy) to treat Pca was first performed in the 1890s, but with limited success. Transurethral resection of the prostate (TURP) replaced radical prostatectomy for symptomatic relief of obstruction in the middle of the 20th century because it could better preserve penile erectile function. Radical retropubic prostatectomy was developed in 1983 by Patrick Walsh (Walsh et al., 1983). This surgical approach allowed for removal of the prostate and lymph nodes with maintenance of penile function.

In 1941, Charles B. Huggins published studies in which he used estrogen to oppose testosterone production in men with metastatic Pca.

This discovery of "chemical castration" won Huggins the 1966 Nobel Prize in Medicine (Huggins & Hodges, 1941).

The role of the hormone GnRH in reproduction was determined by Andrzej W. Schally and Roger Guillemin, who both won the 1977 Nobel Prize in Medicine for this work. Receptor agonists, such as leuprolide and goserelin, were subsequently developed and used to treat prostate cancer. (Schally et al., 1971; Tolis et al., 1982).

Radiation therapy for Pca was first developed in the early 20th century and initially consisted of intraprostatic radium implants. External beam radiation became more popular as stronger radiation sources became available in the middle of the 20th century. Brachytherapy with implanted seeds was first described in 1983(Denmeade & Isaacs, 2002).

Systemic chemotherapy for Pca was first studied in the 1970s. The initial regimen of cyclophosphamide and 5-fluorouracil was quickly joined by multiple regimens using a host of other systemic chemotherapy drugs (Scott et al., 1975).

On 30 July 2010 Owen Witte M.D. et al. of UCLA published a series of studies in Science during which they had introduced viruses known to cause cancerous mutation in prostate cells: AKT, ERG, and AR into isolated samples of basal and luminal cells and grafted the treated tissue into mice. After 16 weeks, none of the luminal samples had undergone malignant mutation, while the basal samples had mutated into prostate-like tubules which had then developed malignancy and formed cancerous tumors, which appeared identical to human samples under magnification. This led to the conclusion that the prostate basal cell may be the most likely "site of origin" of Pca (Witte et al., 2010).

2.4.2. Epidemiology

2.4.2.1. Incidence and mortality

The frequency of Pca in the world is widely variable (Matsuda & Saika, 2009). According to the ' American Cancer Society , there are strong differences in the geographic and ethnic frequency, in fact, Pca is less common among Asian men, more common among black men, the incidence among men in Europe is intermediate compared to the previous two populations (Hoffman et al., 2001).

Pca is the second most frequently diagnosed cancer of men (899 000 new cases, 13.6% of the total) and the fifth most common cancer overall. Nearly three-quarters of the registered cases occur in developed countries (644 000 cases). Incidence rates of Pca vary by more than 25-fold worldwide, the highest rates are in Australia/New Zealand (104.2 per 100,000), Western and Northern Europe, Northern America, largely because the practice of prostate specific antigen (PSA) testing and subsequent biopsy has become widespread in those regions. Incidence rates are relatively high in certain developing regions such as the Caribbean, South America and sub-Saharan Africa. The lowest age-standardised incidence rate is estimated in South-Central Asia (4.1 per 100,000).

With an estimated 258 000 deaths in 2008, Pca is the sixth leading cause of death from cancer in men (6.1% of the total). Because PSA testing has a much greater effect

on incidence than on mortality, there is less variation in mortality rates worldwide (10-fold) than is observed for incidence (25-fold), and the number of deaths from Pca is almost the same in developed and developing regions. Mortality rates are generally high in predominantly black populations (Caribbean, 26.3 per 100,000 and sub-Saharan Africa, ASRs 18-19 per 100,000), very low in Asia (ASR 2.5 per 100,000 in Eastern Asia for example) and intermediate in Europe and Oceania (Ferlay et al., 2010).

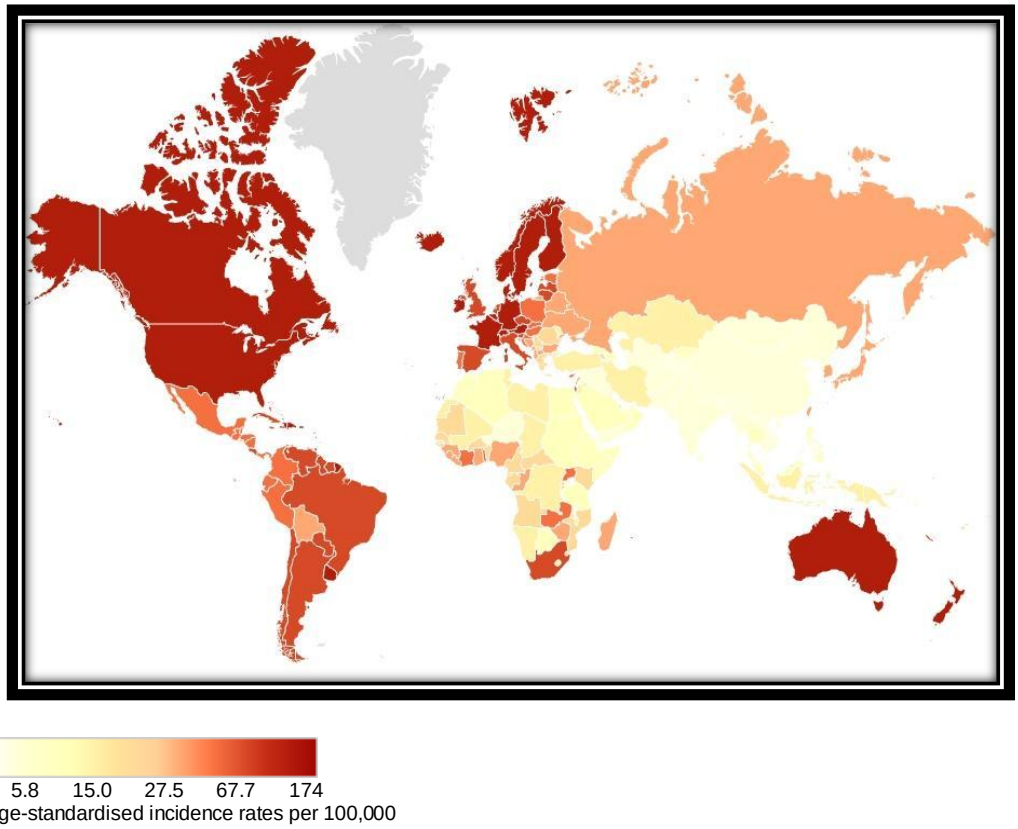

Figure 2.4.2.1A Estimated Prostate Cancer Incidence Worldwide in 2008 (www@iarc.fr).

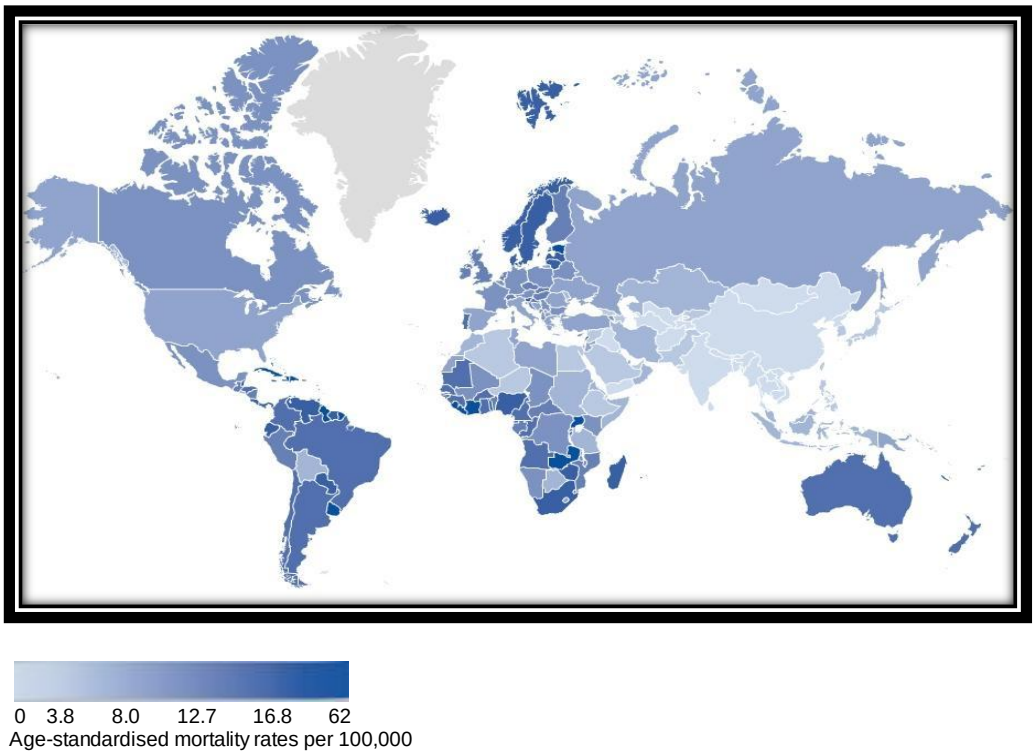

Figure 2.4.2.1B Estimated Prostate Cancer Mortality Worldwide in2008 (www@iarc.fr).

2.4.2.2. Risk factors

Age: Pca is one of the most common cancers diagnosed in men over 40 years of age in the United States (Dunn et al., 2011). Age is the primary risk factor for Pca as over 75% of cases are diagnosed in men over the age of 65 (Spickett et al., 2010) , and rarely seen in men younger than 40.

Family History of Prostate Cancer: It is possible to inherit dysfunctional genes that lead to the development of a familial form of a particular cancer type. Individuals with a family history of Pca are therefore at an increased risk of developing the disease. The degree of risk depends upon the type of relative affected. For example, risk is higher if an immediate family member has been diagnosed with Pca (Cox et al., 2006). The more closely related an individual is to someone with Pca, the more likely they will share the same genes that predisposed the affected individual. Risk increases with the number of relatives affected. Twin studies conducted in Scandinavia suggest that forty percent of the risk factors can be attributed to hereditary (Lichtenstein et al., 2000). Studies have linked several Pca susceptibility genes to different locations within the genome (Verhage et al.,2003). The mechanisms by which these genes lead to cancer, however, are still not well understood.

Race: African American men have the greatest incidence of Pca in the United States. Cancer survival rates are greatly affected by the stage at which a cancer is detected. Compared to both European American and Hispanic men in the United States, African American males are also more likely to be diagnosed at a more advanced stage. Reasons for these differences among ethnic groups are still unclear. Such differences may be due to a combination of genetic, environmental, and/or social factors (Consedine et al., 2006).

Dietary Factors: It is very difficult to identify dietary items that cause a particular cancer. Studies indicate that many dietary factors may influence Pca risk. It has been suggested that a diet rich in dairy and meat products may be associated with an higher risk, while a diet rich in fish and tomato-based products may be associated with lower risk of Pca (Wolk et al., 2005).

Medication exposure: There are also some links between Pca and medications, medical procedures, and medical conditions (Jacobs et al., 2005). Use of the cholesterol lowering drugs known as the statins may also decrease prostate cancer risk (Shannon et al., 2005). Daily use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin can reduce the risk (Jacobs et al., 2005).

Viral: Researchers on 2006 associated a previously unknown retrovirus, Xenotropic MuLV-related virus or XMRV, with human prostate tumors. Subsequent reports on the virus have been contradictory (Urisman et al., 2006). A group of US researchers found XMRV protein expression in human prostate tumors (Schlaberg et al., 2009), while German scientists failed to find XMRV-specific antibodies or XMRV-specific nucleic acid sequences in Pca samples (Hohn et al., 2009). An Australian study 2012 examining both cancerous and healthy prostate tissue found an association between Infection with Human Papillomavirus and Epstein - Barr virus and an increased risk of Pca. The role of the viruses in causing the cancer is not yet known (Whitaker et al., 2013).

2.4.3. Pathophysiology

The prostate gland of humans and many other animals has the major function of accumulating and secreting extraordinarily high levels of citrate. This specialized metabolic process of "net citrate production" is the result of unique metabolic capabilities of the secretory epithelial cells. Most importantly, in Pca the capability for net citrate production is lost. In addition to citrate, the normal and BPH (benign prostatic hyperplasia) prostate also accumulates the highest levels of zinc in the body. As with citrate, in Pca the ability for high zinc accumulation is diminished. These and other correlations between zinc and citrate in the prostate have been indicative of an important role of zinc in the regulation of citrate metabolism in normal and malignant prostate epithelial cells. The link between zinc and citrate metabolism has now been established. The intramitochondrial accumulation of high zinc levels inhibits mitochondrial (m-) aconitase activity, which inhibits citrate oxidation. This essentially truncates the Krebs cycle and markedly decreases the cellular energy (ATP) production normally coupled to citrate oxidation. It is also clear that zinc accumulation in citrate-producing prostate epithelial cells is regulated by testosterone and by prolactin. These

relationships form the basis for a new concept of the role of zinc and citrate-related energy metabolism in prostate malignancy. The inability of malignant prostate cells to accumulate high zinc levels results in increased citrate oxidation and the coupled ATP production essential for the progression of malignancy. The concept offers new approaches to the treatment of Pca (Costello et al., 1998).

RUNX2 is a transcription factor that prevents cancer cells from undergoing apoptosis thereby contributing to the development of Pca (Leav et al., 2010).

The PI3k/Akt signaling cascade works with the transforming growth factor beta/SMAD signaling cascade to ensure Pca cell survival and protection against apoptosis (Zha et al., 2009). X-linked inhibitor of apoptosis (XIAP) is hypothesized to promote Pca cell survival and growth and is a target of research because if this inhibitor can be shut down then the apoptosis cascade can carry on its function in preventing cancer cell proliferation (Watanabe et al., 2009).

Macrophage inhibitory cytokine-1 (MIC-1) stimulates the focal adhesion kinase (FAK) signaling pathway which leads to prostate cancer cell growth and survival (Senapati et al., 2010).

The androgen receptor helps Pca cells to survive and is a target for many anti cancer research studies; so far, inhibiting the androgen receptor has only proven to be effective in mouse studies (Narizhneva et al., 2009).

Prostate specific membrane antigen (PSMA) stimulates the development of Pca by increasing folate levels for the cancer cells to use to survive and grow; PSMA increases available folates for use by hydrolyzing glutamated folates (Narizhnev et al., 2010).

2.4.4. Signs and symptoms

Early Pca usually causes no symptoms. Sometimes, prostate cancer often similar to those of diseases such as benign prostatic hyperplasia. These include frequent urination, nocturia (increased urination at night), difficulty starting and maintaining a steady stream of urine, hematuria (blood in the urine), and dysuria (painful urination) (Miller et al., 2003).

Pca is associated with urinary dysfunction as the prostate gland surrounds the prostatic urethra. Changes within the gland, therefore, directly affect urinary function.

Because the vas deferens deposits seminal fluid into the prostatic urethra, and secretions from the prostate gland itself are included in semen content, Pca may also cause problems with sexual function and performance, such as difficulty achieving erection or painful ejaculation (Miller et al., 2003).

Advanced prostate cancer can spread to other parts of the body, possibly causing additional symptoms (van der Cruijsen et al., 2005).

The most common symptom is bone pain, often in the vertebrae (bones of the spine), pelvis, or ribs. Spread of cancer into other bones such as the femur is usually to the proximal part of the bone. Pca in the spine can also compress the spinal cord, causing leg weakness and urinary and fecal incontinence (van der Cruijsen et al., 2005).

2.4.5. Pathological Anatomy

In the last decade, improved diagnostic methods has allowed an increasingly early detection of masses confined to the parenchyma, making it difficult for the pathologist to recognize the macroscopic tumor in the surgical specimen through the only time inspection , and very often in fact, small tumors are detected by palpation as hard wooden masses within the gland. To the cutting surface, the tumor is white in color, with spiculated margins and penetrating the surrounding parenchyma. The natural evolution of the tumor involves the local expansion in the context of the gland and the subsequent infiltration of the seminal vesicles , the bladder neck and of prostatic urethra , an event that results from obstructive events with dysuria , urinary frequency and strangury , sometimes hemospermia (Porena, 2003).

In the later stages there is also infiltration of the pelvic floor and the rectum resulting in tenesmus . The lymphatic vessels are most frequently involved in order of frequency (Harrison, 2006).

2.4.6. Microscopic appearance

The neoplastic glands are typically smaller than normal; massive hyperplasia , with loss of the papillae and the basal layer outside. A nucleus large, rich nucleoli , surrounded by cytoplasm clear or sometimes intensely eosinophilic (with characteristic dark color) (Robbins & Cotran, 2008).

Although these islands are easily identifiable tumor, histological diagnosis is often made difficult by the lack of pleomorphism and mitotic figures are atypical.

This feature, together with the characterization difficult compared to benign hyperplastic tissue, can complicate the detection of neoplastic tissue in frustules biopsy obtained with cutting needle (Robbins & Cotran, 2008). Normal staining with hematoxylin-eosin showing glands small, crowded cells with loss of papillae are therefore only suggestive of Pca, the diagnosis is confirmed if you have one or more of these characteristics, Large nuclei and amorphous, dark cytoplasm, prominent nucleoli, Absence of basal cells in the context of basal glands (sought with immunological markers) & Perineural invasion (Robbins & Cotran, 2008). Further histological entity of great importance are the prostatic intraepithelial neoplasia high grade or PIN , glandular lesions characterized by large formations with papillary protrusions (first element of distinction with malignant neoplasms), intra-acinar proliferation non-invasive, anaplastic nuclear and prominent nucleoli (Robbins & Cotran, 2008). Another important feature to be searched in the PIN is the presence of basal cells, absent in carcinoma (McNeal, 1969; Busch et al., 1998). The PIN is an important lesion that must be followed in time for the possible malignant transformation (Konz etal., 2001) ty to give rise to metastases , which covers most commonly the bones , the lymph nodes , the rectum and thebladder (Häggman et al., 1997).

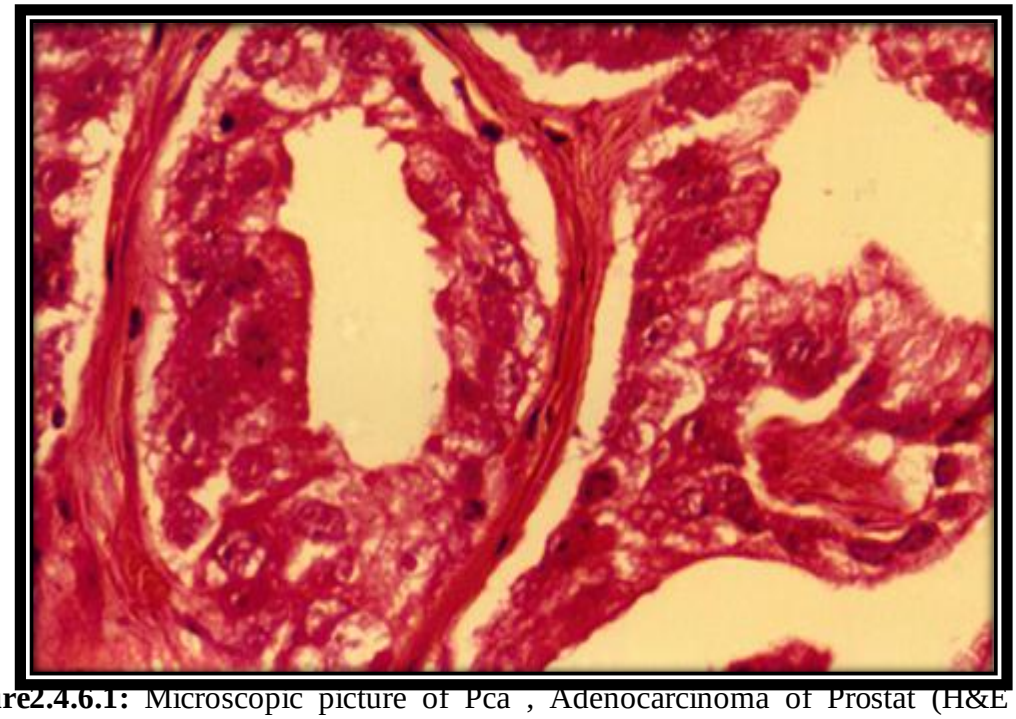

Figure2.4.6.1: Microscopic picture of Pca , Adenocarcinoma of Prostat (H&E ×40). Histopathology, Department. ,Benghazi University Malignant gland lined by malignant cellc with prominant nucleoli in their nuclei.

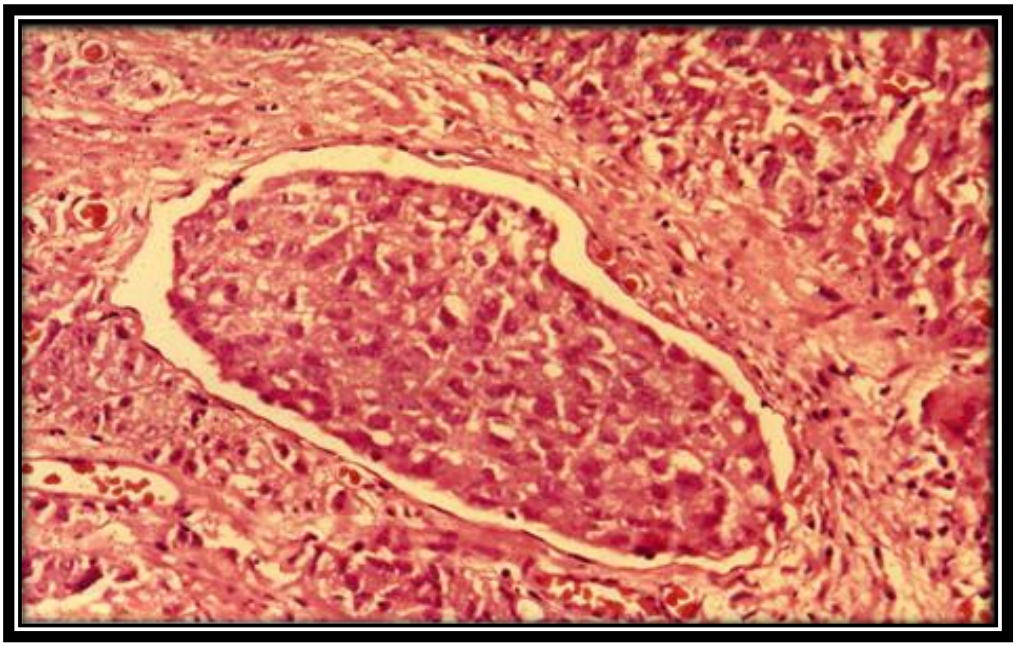

Figure2.4.6.2: Microscopic picture of Pca, Lymphovascular invation by tumor (H&E×40) Histopathology, Department. ,Benghazi University

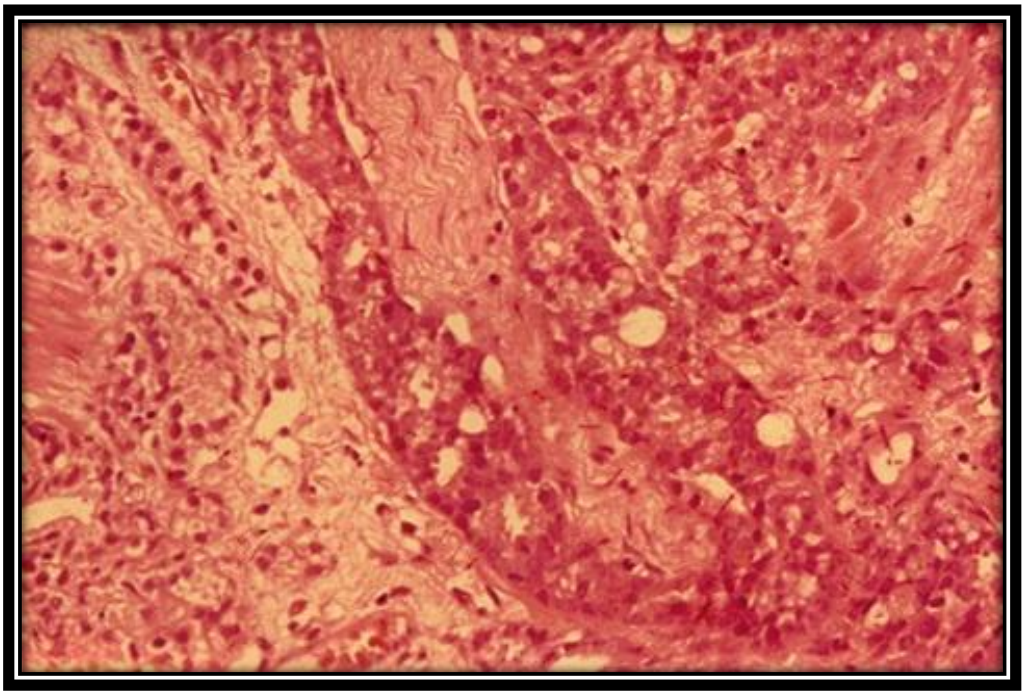

Figure2.4.6.3: Microscopic picture of Pca, Perineural invation (H&E×40). Histopathology, Department. ,Benghazi University

2.4.7. Diagnostic Profile

The cancer screening is a method of discovering tumors diagnosed. Screening tests can encourage the use of more specific tests, such as biopsy . The choice of diagnostic screening in the case of Pca includes the ' rectal examination and determination of PSA. In general, the screening begins after the age of 50, but may be made prior in men who have high risk factors of Pca (Grubb, 2005; Giusti, 2010).

Digital rectal examination: Is a procedure in which the examiner inserts a gloved, lubricated finger into the rectum of the patient, in order to evaluate the size, shape and texture of the prostate: zones irregular, hard or bozzolute must be subjected to further assessments, because they could be indicative of cancer (Chodak et al., 1989).

The rectal exam is able to evaluate only the rear part of the prostate, but fortunately 85% of the tumors originated in this part tends to give way to appreciate tumors already at an advanced stage (Chodak et al., 1989). It has never been demonstrated that, as the only screening test, the rectal examination is able to reduce the mortality rate (Krahn et al., 1994).

PSA: The dosage of the PSA measure the blood level of an enzyme produced by the prostate. PSA levels below 4 ng / ml (nanograms per milliliter) are generally considered normal, while levels above 4 ng / ml are considered abnormal (although in men over the age of 65 levels up to 6.5 ng / ml may be acceptable, depending on the parameters of each reference laboratory) (Laxman et al., 2008; Hessels et al., 2009).

PSA levels between 4 and 10 ng/ml indicates a risk of tumor higher than normal, but the risk itself does not appear directly proportional to the level (de la Taille, 2007).

When the PSA is above 10 ng / ml, the association with the tumor becomes stronger, however, that of the PSA is not a perfect test (Bussemakers et al., 1999).

Some men with Pca have no place in high levels of PSA, and the majority of men with an elevated PSA do not have cancer. Today it is possible to measure an additional marker for Pca, the PCA3 (prostate cancer gene 3), the overexpression of this gene (assessed by determination of mRNA in the urine) is closely associated with malignant transformation of prostate cells. The assay is particularly useful in patients already undergoing biopsy to predict the evolution of the tumor (Marks et al., 2007; Neves et al., 2008; Haese et al., 2008).

EN2: Engrailed-2 (EN2) is a transcription factor expressed in Pca cells but not in normal adult cells. We have shown that, unusually for a transcription factor, EN2 is secreted from cancer cells and can be subsequently found in the urine in men with Pca, making it a potential biomarker for this disease (Morgan et al., 2011; Pandha et al., 2012). Preliminary data has also shown that EN2 might be present on the surface of cancer cells and is thus a potential target for immunotherapy, either through direct targeting using an anti-EN2 antibody or by stimulating the immune system using a vaccine (Hjerrild et al., 2004).

Biopsy: When you suspect a Pca, or a screening test is indicative of an increased risk, it raises a more invasive. The only examination able to fully confirm the diagnosis is the biopsy , ie the removal of small fragments of tissue for examination under a microscope. However, before the biopsy, you can use less invasive methods to collect information about the prostate and the urinary tract: the cystoscopy shows the urinary tract from inside the bladder using a small endoscope inserted in the 'urethra , the ultrasound transrectal draws an image of the prostate through ultrasound emitted from a probe inserted into the rectum (Essink et al., 1998).

If you suspect a tumor biopsy is used. With it are obtained samples of tissue from the prostate through the rectum: a gun from biopsy needles inserted and then removes special hollow point (usually three to six for each side of the prostate) in less than a second. The tissue samples are then examined under a microscope to determine the presence of cancer, assess aspects histomorphological (grading according to the Gleason score system). In general, prostate biopsies are performed on outpatients. Fifty five percent of men report suffering during the procedure (Essink et al., 1998).

Prostate imaging: Ultrasound (US) and Magnetic Resonance Imaging (MRI) are the two main imaging methods used for Pca detection. Urologists use transrectal ultrasound during prostate biopsy and can sometimes see a hypoechoic area. But US has poor tissue resolution and thus, is generally not clinically used. In contrast, prostate MRI has superior soft tissue resolution. MRI is a type of imaging that uses magnetic fields to locate and characterize Pca. Multi-parametric prostate MRI consists of four types of MRI sequences called T2 weighted imaging, T1 weighted imaging, Diffusion Weighted Imaging, MR Spectrocopic Imaging and Dynamic-Contrast Enhanced Imaging (Bonekamp et al., 2011).

Genitourinary radiologists use multi-parametric MRI to locate and identify Pca. 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).

2.4.8. The Gleason Score

Tumor grading of Pca is a fundamental determinant of disease biology and prognosis. Tumor grading is defined as a property of cancer independent of tumor location found in either biopsy or radical prostatectomy specimens. Prognosis refers to the expected biologic aggressive potential of a patient's Pca to spread to other organs. The Gleason score, the most widespread method of prostate cancer tissue grading used today is the single most important prognostic factor in Pca (Gleason, 1966; Allsbrook et al., 1999).

It is one determinant of a patient's specific risk of dying due to prostate cancer (Baillar et al., 1966; Albertsen et al., 1998). Hence, once the diagnosis of prostate cancer is made on biopsy, tumor grading, especially the Gleason score, strongly influences decisions regarding options for therapy (D'Amico& Whittington, 1998).

The diagnostic quality of prostate biopsies is really a team effort between the urologist and the pathologist (an expert physician trained in the study of disease) working on a patient's case. A correct Pca diagnosis and Gleason score are possible only when the biopsy is both performed and interpreted correctly by the urologist and pathologist respectively (Gerry et al., 2001).

Incomplete biopsy sampling of the prostate is one reason why the predicted Gleason score on biopsy does not always correlate with the actual observed Gleason score of the prostate cancer in the gland itself (Gerry et al., 2001). Hence, the accuracy of Gleason scoring is dependent upon not only on the expertise of the pathologist reading the slides, but also on the completeness and adequacy of the prostate biopsy sampling strategy (Stamey, 1995). The Gleason scoring system is based on microscopic tumor patterns assessed by a pathologist while interpreting the biopsy specimen (Gerry et al., 2001).

When Pca is present in the biopsy, the Gleason score is based upon the degree of loss of the normal glandular tissue architecture (i.e. shape, size and differentiation of the glands) as originally described and developed by Dr. Donald Gleason in 1974 (Gleason & The Veteran, 1977).

The classic Gleason scoring diagram shows five basic tissue patterns that are technically referred to as tumor "grades". The subjective microscopic determination of this loss of normal glandular structure caused by the cancer is abstractly represented by a grade, a number ranging from 1 to 5, with 5 being the worst grade possible (Figure2.4.8.1). The Gleason score (GS) and the Gleason sum are one and the same. However, the Gleason grade and the Gleason score or sum is different. The biopsy Gleason score is a sum of the primary grade (representing the majority of tumor) and a secondary grade (assigned to the minority of the tumor), and is a number ranging from 2 to 10. The higher the Gleason score, the more aggressive the tumor is likely to act andthe worse the patient's prognosis (Gerry et al., 2001).

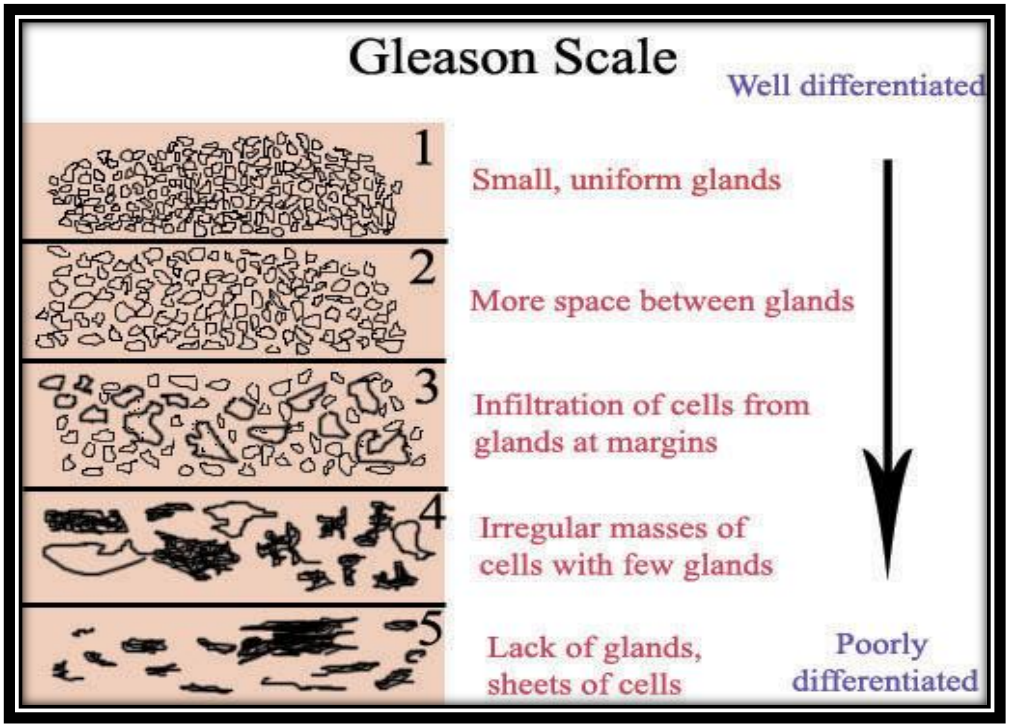

Figure 2.4.8.1: Gleason grading system. ©1996-2013 MedicineNet. The Primary Gleason grade has to be greater than 50% of the total pattern seen (i.e. the pattern of the majority of the cancer observed). The Secondary Gleason grade has to be less than 50%, but at least 5%, of the pattern of the total cancer observed. The sum of the primary and secondary Gleason grades is shown as the Gleason score or sum (i.e. primary grade + secondary grade = GS; i.e. 4+3 or 3+4 = GS 7) (Gerry et al., 2001).

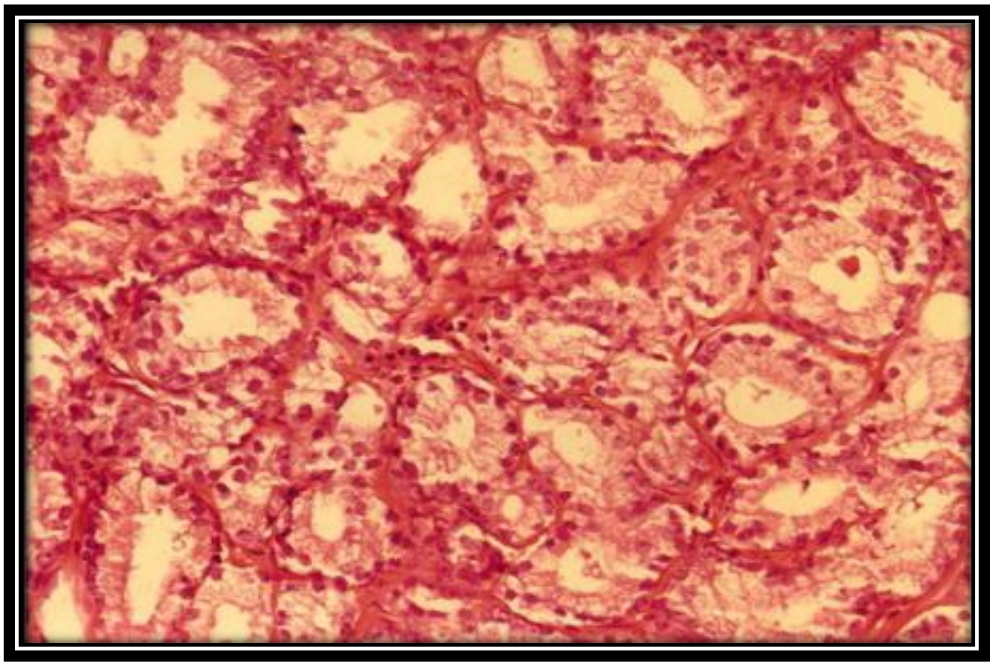

Figure2.4.8.2 A: Microscopic picture of Pca ,Grade 3 lower power (H&E×20).

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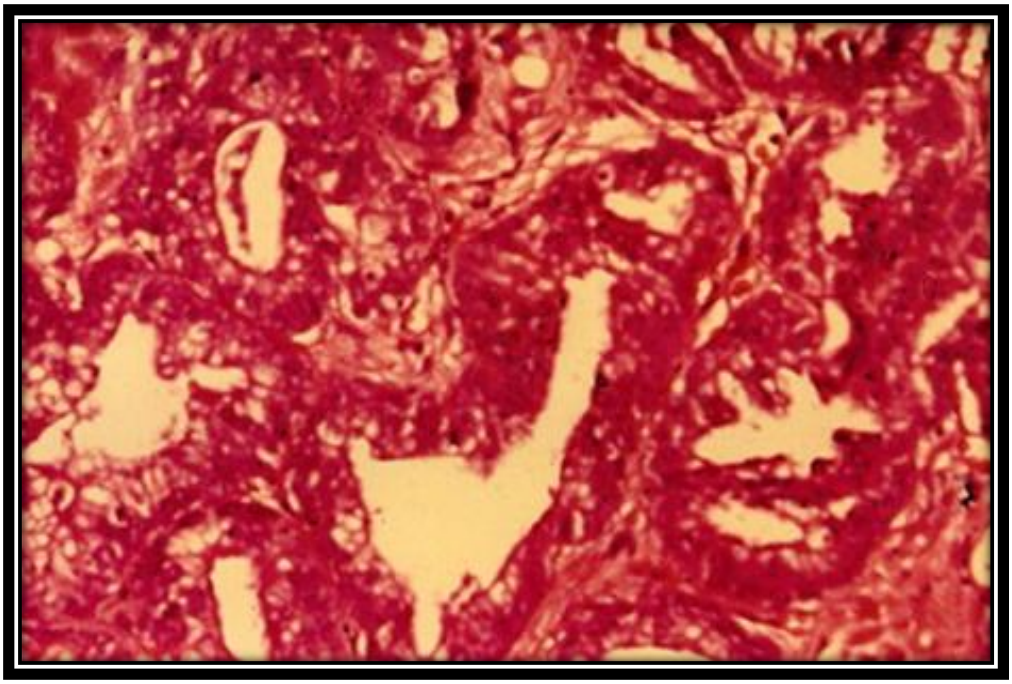

Figure2.4.8.2 B: Microscopic picture of Pca ,Grade 3 high power(H&E×40).

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These pictures show back to back proliferation of small to intermediated sized tumor acini with scant to moderate intervening stroma

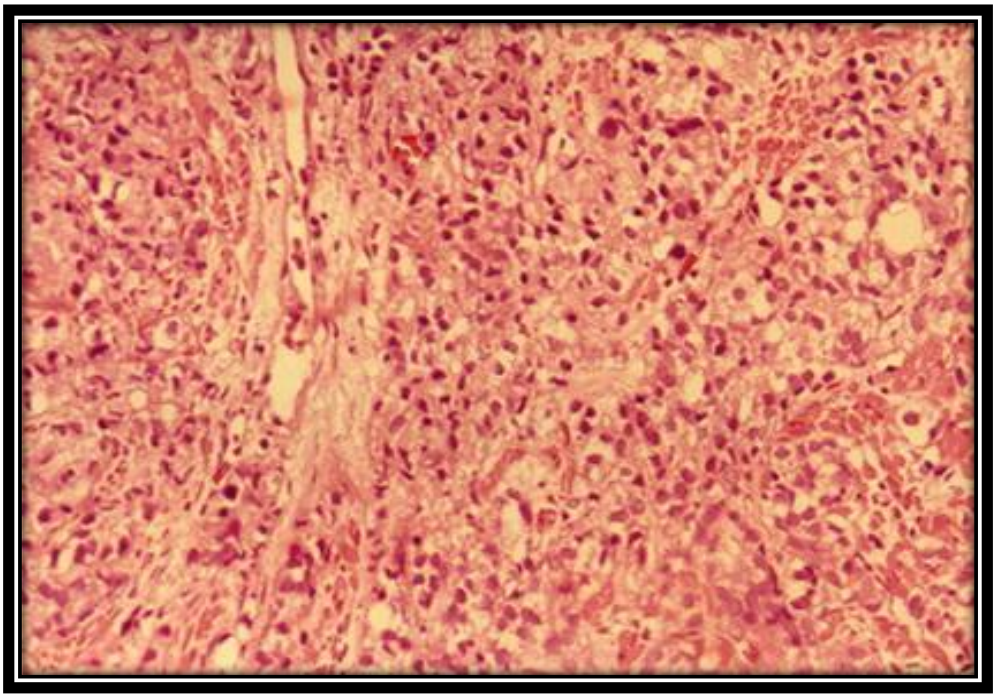

Figure2.4.8.3 A: Microscopic picture of Pca, Grade 4 lower power(H&E×20). Histopathology, Department. ,Benghazi University

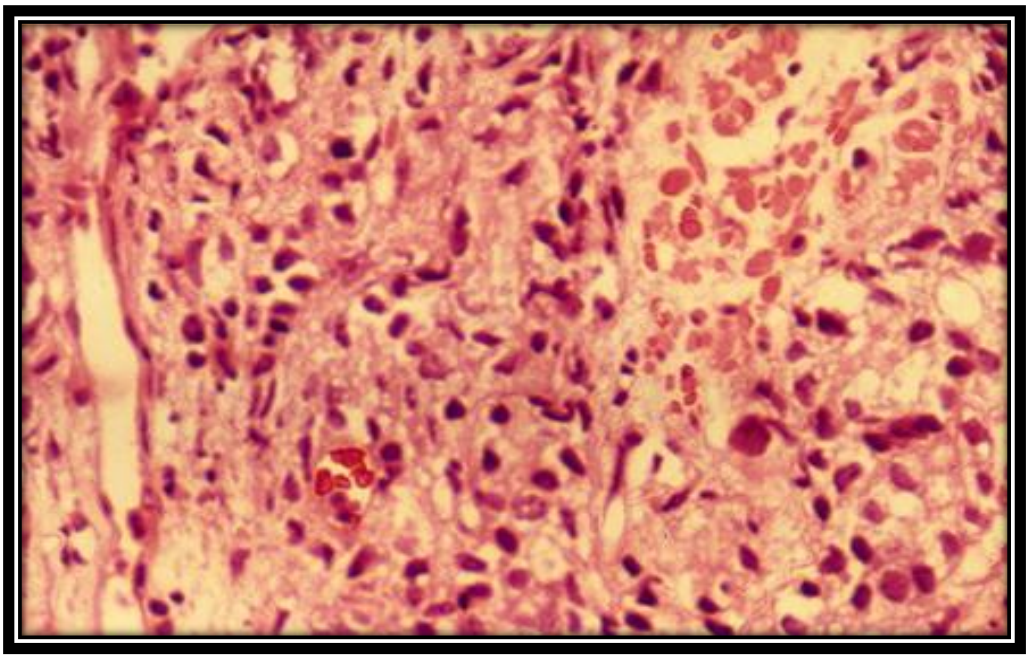

Figure2.4.8.3 B: Microscopic picture of Pca ,Grade 4 high pwer(H&E×40). Histopathology, Department. ,Benghazi University

These pictures show fused glands irregularly infiltrating fibrotic stroma.

2.4.9. Staging

Two systems are in common use for the staging of Pca. The Jewett system (stages A to D) was described in 1975 and has since been modified (Jewett, 1975).

In 1997, the American Joint Committee on Cancer (AJCC) and the International Union against Cancer adopted a revised `subcategories of T stage, such as a stage to describe patients diagnosed through PSA screening. This revised TNM system is clinically useful and more precisely stratifies newly diagnosed patients. The AJCC further revised the TNM classification system in 2002 and, most recently, in 2010 (Montie et al., 1995; Edge et al., 2010).

Definitions of TNM

The AJCC has designated staging by TNM classification to define Pca (Edge et al., 2010).

Table 2.1. System of staging Pca, Primary Tumor (T) [a]:

Clinical	
TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
T1	Clinically inapparent tumor neither palpable nor visible by imaging.
T1a	Tumor incidental histologic finding in ≤5% of tissue resected.
T1b	Tumor incidental histologic finding in >5% of tissue resected.
T1c*	Tumor identified by needle biopsy (e.g., because of elevated PSA).
T2**	Tumor confined within prostate.b
T2a	Tumor involves ≤one-half of one lobe.
T2b	Tumor involves >one-half of one lobe but not both lobes.
T2c	Tumor involves both lobes.
T3	Tumor extends through the prostate capsule.c
T3a	Extracapsular extension (unilateral or bilateral).
T3b	Tumor invades seminal vesicle(s).
T4	Tumor is fixed or invades adjacent structures other than seminalvesicles such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall.

(Edge et al., 2010)

*Tumor found in one or both lobes by needle biopsy, but not palpable or reliably visible by imaging, is classified as T1c.

**Invasion into the prostatic apex or into (but not beyond) the prostatic capsule is classified not as T3 but as T2 (Edge et al., 2010).

Table 2.2. System of staging Pca, Pathological (pT) [a, b]:

Clinical	
pT2	Organ confined.
pT2a	Unilateral, ≤one-half of one side.
pT2b	Unilateral, involving >one-half of side but not both sides.
pT2c	Bilateral disease.
pT3	Extraprostatic extension.
pT3a	Extraprostatic extension or microscopic invasion of bladder neck.
pT3b	Seminal vesicle invasion.
pT4	Invasion of rectum, levator muscles, and/or pelvic wall.

(Edge et al., 2010)

Table 2.3. System of staging Pca, Regional Lymph Nodes (N)[a]:

Clinical	
NX	Regional lymph nodes were not assessed.
N0	No regional lymph node metastasis.
N1	Metastases in regional lymph node(s).
Pathological	
pNX	Regional nodes not sampled.
pN0	No positive regional nodes.
pN1	Metastases in regional node(s).

(Edge et al., 2010**)**

Table 2.4. System of staging Pca, Distant Metastasis (M)[a,b]:

Clinical	
M0	No distant metastasis.
M1	Distant metastasis.
M1a	Nonregional lymph node(s).
M1b	Bone(s).
M1c	Other site(s) with or without bone disease.

(Edge et al., 2010**)**

When more than one site of metastasis is present, the most advanced category is used pM1c is most advanced (Edge et al., 2010).

Table 2.5. Anatomic Stages and Prognostic Groups[ab]:

Group	T	N	M	PSA*	Gleason
I	T1a–c	N0	M0	PSA <10	Gleason ≤6
	T2a	N0	M0	PSA <10	Gleason ≤6
	T1–2a	N0	M0	PSA X	Gleason X
IIA	T1a–c	N0	M0	PSA <20	Gleason 7
	T1a–c	N0	M0	PSA ≥10 <20	Gleason ≤6
	T2a	N0	M0	PSA ≥10 <20	Gleason ≤6
	T2a	N0	M0	PSA <20	Gleason 7
	T2b	N0	M0	PSA <20	Gleason ≤7
	T2b	N0	M0	PSA X	Gleason X
IIB	T2c	N0	M0	Any PSA	Any Gleason
	T1–2	N0	M0	PSA ≥20	Any Gleason
	T1–2	N0	M0	Any PSA	Gleason ≥8
III	T3a–b	N0	M0	Any PSA	Any Gleason
IV	T4	N0	M0	Any PSA	Any Gleason
	Any T	N1	M0	Any PSA	Any Gleason
	Any T	Any N	M1	Any PSA	Any Gleason

*PSA = prostate-specific antigen.

(Edge et al., 2010).

2.4.10. Prognostic factors

Prognostic factors in Pca are defined as "variables that can account for some of the heterogeneity associated with the expected course and outcome of a disease". Bailey defined prognosis as "a reasoned forecast concerning the course, pattern, progression, duration, and end of the disease" (Johan & Dirk, 2oo7). Prognostic factors are not only essential to understand the natural history and the course of the disease, but also to predict possible different outcomes of different treatments or perhaps no treatment at all. This is extremely important in a disease like prostate cancer where there is clear evidence that a substantial number of cases discovered by prostate specific antigen (PSA) testing are unlikely ever to become clinically significant, not to mention mortal (Draisma et al., 2003). Furthermore, prognostic factors are of paramount importance for correct interpretation of clinical trials and for the construction of future trials. Finally, according to World health organization (WHO) national screening committee criteria for useing a national screening programme, widely accepted prognostic factors must be defined before assessing screening (Johan & Dirk, 2oo7).

2.4.10.1. Clinico-pathological prognostic factors

Clinical prognostic factors are those that can be assessed through physical examination: blood tests, radiological evaluation, and microscopy of biopsy material (Buhmeida et al., 2006).

Age: The role of age of the patient as a significant prognostic factor in Pca engenders debates (Umas et al., 1992; Austin et al., 1993; Gronberg et al., 1994). Harold and colleauges in analysis of 567 patients undergoing external beam radiotherapy (EBRT) found that age more than 65yr was a significant predictor of distant metastasis and poor outcome at 5yr. Obek and colleauges suggested that young age might be an independent favorable prognostic factor for disease recurrence after radical prostatectomy (Herold et al., 1998; Obek et al., 1999). Freedl and and colleauges also found that young men had more favorable outcomes after radical prostatectomy than older men, which made younger patients suitable subjects in screening.

Tumor Type: The morphologic variants of Pca, although relatively uncommon, can be associated with different disease progression patterns (Young et al., 2000).

Standard acinar adenocarcinoma arises in the peripheral zones of the prostate gland and accounts for more than 90% of all newly diagnosed Pca (Young et al., 2000). Prostatic duct carcinomas originate from larger dilated central ducts of the gland, immune stain positively for PSA and prostatic acid phosphatase (PSAP), and usually are of low to intermediate aggressiveness (Cantrell et al., 1981; Young et al., 2000).

Endometrioid adenocarcinomas are included in the prostatic duct carcinoma group (Bostwick et al.,1985; Ro et al., 1988). Mucinous carcinomas also stain for both PSAP and PSA, rarely respond to hormonal therapy, and often cause bone metastases. (Epstein et al., 1985). Adenoid cystic carcinomas are nonreactive for both PSA and PSAP and may be associated with distant metastasis (Kuhajda et al., 1984; Young et al., 2000).

Squamous and adenosquamous carcinomas may develop in patients treated with radiation therapy or after conventional adenocarcinoma has been treated with estrogens (Saito et al., 1984; Devaney et al., 1991). Signet-ring cell carcinomas are negative for neutral and acid mucins, immune reactive for PSAP and PSA, and clinically aggressive (Ro et al., 1988; Alline et al., 1992). Small cell and neuroendocrine carcinomas are associated with a uniformly poor prognosis (Ro et al., 1987).

Prostatic transitional cell carcinomas arise from the periurethral glandular epithelium or from meta plastic prostatic epithelium, are negative for PSA and PSAP, and often develop into aggressive tumors that do not respond to hormonal therapy (Johnson et al., 1972; Young et al., 2000).

Lymphoepithelioma like carcinomas are poorly differentiated carcinomas with a syncytial growth pattern, prominent lymphocytic stroma, and adverse clinical behavior (Young et al., 2000). Carcinosarcomas and sarcomatoid carcinomas are rapidly progressive biphasic tumors featuring a sarcomatous component (Shannon et al., 1992; Lauwers et al., 1993; Young et al., 2000). Basal cell carcinomas and carcinoid tumors are additional rare prostatic neoplasms with adverse clinical outcomes.

Tumor Volume: Tumor volume is a significant predictor of pathologic stage, lymph node and distant metastasis, and overall disease outcome (Gleason et al., 1977; McNeal et al., 1990; Stamey et al., 1993). The number and length of involvement of multiple (sextant or octant) needle biopsy cores has been successful at predicting overall tumor volume, pathologic stage, and disease outcome (Ravery et al., 2000).

Tumor Grade: The microscopic grade of a prostate cancer correlates significantly with the local extent of the disease, incidence of lymph node and bone metastasis, response to various therapies, and overall disease outcome (Amanatullah et al., 2000; Tarone et al.,2000).

The two grade summation scoring system developed by Gleason (Gleason et al., 1977) has correlated with cell proliferation rate, aneuploid DNAcontent, oncogene activation, and tumor suppressor gene mutation (Amanatullah et al., 2000; Tarone et al.,2000) and is predictive of rapid PSA progression (Koch et al., 2000). However, although the Gleason score of prostatic adenocarcinoma is clearly one of the strongest predictors of biologic behavior and metastatic potential, in most studies, it does not seem to be capable of predicting disease outcome when used alone (Amanatullah et al., 2000; Tarone et al., 2000). The correlation of Gleason score for the needle biopsy specimen with the final score at radical prostatectomy is best for moderately and poorly differentiated adenocarcinomas (Bostwick et al., 1994). Discrepancies between the Gleason score for biopsy specimens and corresponding radical prostatectomy specimens are greatest when Gleason scores are low and the quantity of tumor in the biopsy specimens is limited (Mills et al., 1986).

In addition, it has been documented that grading accuracy for needle biopsy specimens might be higher when the grading pathologists are experienced subspecialists in urologic pathology (Steinberg et al., 1997).

This may be manifest in the higher incidence of grade changes, upward or downward at radical prostatectomy encountered when community hospital pathologists performed the original biopsy grading (Steinberg et al., 1997). Foci of Gleason patterns 4 and 5 seem to be predictive of adverse outcome even when present in only a minute or tertiary focus (Pan et al., 2000; D'Amico et al., 2000).

Tumor Stage: Extra prostatic extension is reportedly very common in prostatic adenocarcinoma, with an incidence as high as 90% in one series (Robinette et al., 1984; Montie et al., 1995). Patients with focal intracapsular penetration by the tumor are reported to have an intermediate prognostic risk between those with organ-confined disease and those with diffuse extra prostatic extension (Epstein et al., 1993). The presence of perineural invasion in the needle biopsy specimen has been reported to be a specific marker for capsular penetration of the tumor in a prostatectomy specimen

(Bastacky et al., 1993), although the over all prognostic significance of perineural invasion remains controversial (Rubin et al., 2000).Seminal vesicle involvement by Pca is associated with high tumor grade, large tumor volume, extra prostatic extension, lymph node metastasis, and poor prognosis (Ohori et al., 1993). Positive margins of resection significantly affect disease outcome and correlate with high preoperative serum PSA levels, high tumor grade, and an euploid DNA content (Epstein et al., 1993; Epstein et al., 1996; Cheng et al., 1999). Vascular space invasion also has been associated with disease progression (Herman et al., 2000). The presence of nodal metastases is highly associated with significant tumor progression, with an overall incidence averaging 40% (Fowler et al., 1981). Studies of micro metastasis detection using molecular methods have differed in their conclusions (Potter et al., 2000; Okegawa et al., 2000).

Morphometrics: A variety of morphometric techniques have been used on Pca specimens with the nuclear roundness factor measurement achieving the most significant potential clinical usefulness. Pca featuring almost perfectly round nuclei typically are well-differentiated and slow-growing cancers. Tumors with irregular nuclear contours and correspondingly low nuclear roundness have been associated with high tumor grade and a propensity for the development of distant metastasis and short end survival (Cadeddu et al., 1993; Hurwitz et al., 1999).

Study of morphometric features in Pca found that suboptimal circle fit and Feretdiameter ratio measurements could predict disease relapse after radiation therapy (Hurwitz et al., 1999).

Heterogeneity and multicentricity: Pca is characteristically multifocal with as many as five or six tumors occurring in a single prostate (Miller & Cygan, 1992). A great challenge for diagnostic pathologists was the characteristic heterogeneous appearance of prostatic carcinoma. The availability of radical prostatectomy specimens has provided the chance for examine the interrelationships of histological heterogeneity and multicentricity in same specimens (Murphy, 1994).

The influence of grade heterogeneity and tumor multifocality on the ability to predict the prognosis of patients with Pca is profound. The multifocal and heterogeneous nature of prostate makes it difficult to obtain representative biopsy samples from the tumours (Verhagen, 2002). Hammerer and associates considered the number of biopsies positive for cancer as quantitative measure of tumour multicentricity

(Hammerer & Huland, 1992). The data of Djavan et al suggested that multifocal Pca is associated with higher grade, stage, and recurrence rate than unifocal Pca (Djavan et al., 1999).

2.4.10.2. Biological prognostic factors

Nuclear Hormone Receptors

Although androgen receptor (AR) loss and clinical lack of benefit from anti androgen therapy have been associated with high-grade and high-stage Pca. AR activity has not independently predicted disease-related death (Newmark et al., 1992).

AR expression can be heterogeneous in Pca, which might reflect AR genetic instability and the future development of androgen-independent tumorgrowth (Sadi et al., 1993). Assays of AR activity have not been used to select patients for androgen ablation therapy before prostatectomy. Research interest in AR activity has focused on the relationship between expression of various growth factors and matrix metalloproteinases associated with prostate cancer progression and AR status (Collembell et al., 1993; Amanatullah et al., 2000). Although the development of androgen-independent tumor growth has been associated with various specific point mutations in the AR gene, disease outcome has not correlated with AR expression (Amanatullah et al., 2000). Further characterization of AR activity in prostate cancer seems warranted to better understand the events that produce the capability of androgen-independent growth for some aggressive tumors and the interaction of AR with other prognostic markers.

Cell Proliferation Markers

S-phase fraction: S-phase fraction (SPF) is the proportion of cells in the S-phase of the cell-cycle. High SPF is associated with rapid tumour proliferation, shorter overall survival and shorter time to local progression and metastasis in clinically localized Pca (Gerdes et al., 1989).

Ki-67: Ki-67 is one of the several cell-cycling-regulating proteins. It is a DNA-binding protein, which is expressed in all phases of cell cycle but undetectable in resting cells. Ki-67 index is higher for carcinomas than for hyper plastic glands (Sadi et al., 1993). With in carcinomas, ki-67 indices in patients with metastatic disease were significantly higher than in those without metastasis.

Cyclin-Dependent Kinase Inhibitors: In the G1 to S phase transition of the cell cycle, two families of cyclin-dependent kinase inhibitors have been known: the Cip/Kip and INK4 groups (Amanatullah et al., 2000). Both p21 and p27 proteins, members of the Cip/Kip family, have been studied as prognostic factors in Pca (Amanatullah et al., 2000). Maintenance of p21 immunoreactivity is associated with prolonged disease-free survival (Cheng et al., 2000). Loss of p27 expression has been associated with adverse disease outcome in a number of studies (Yang et al., 1998; Kuczyk et al., 1999; Amanatullah et al., 2000).

In addition, the homeobox protein skp-2 has been shown to be expressed inversely to p27 in Pca and might represent a drug target candidate for the disease (Drobnjak et al., 2003). The p16INK4 tumor suppressor gene is rarely mutated in Pca, but decreased protein expression has been associated with gene deletions and promoter hypermethylation (Halvorsen et al., 2000). Interestingly, increased p16 immune staining has been associated with the presence of prostate cancer, but this marker has not become a useful prognostic factor to date (Halvorsen et al., 2003). Over expression of p34cdc2 cyclin dependent kinase, involved in the S to G2M transition of the cell cycle, has been associated with aggressive high-grade disease featuring an increased incidence of biochemical failure after primary therapy (Kallakury et al., 1997).

DNA Ploidy Determination: The majority of retrospective studies have shown that an euploid DNA content in Pca independently predicts a poor prognosis for the disease (Montgomery et al., 1990; Ross et al., 1993). DNA ploidy measurements have been performed on needle biopsy specimens by using the tissue section image analysis technique (Ross et al., 1994).

An euploid DNA ploidy status determined on needle biopsy specimens has correlated successfully with the ploidy status of corresponding radical prostatectomy specimens and independently predicted disease outcome (Ross et al., 1994). DNA ploidy determination on needle biopsy specimens has been used to confirm biopsy grading (Ross et al., 1994). Although this role is reduced substantially when grading of the biopsy specimen is performed by experts (Brinker et al., 1999).

Tumor Vascularity and Microvessel Density: Tumor angiogenesis also has correlated with adverse outcome in Pca as measured by micro vessel counting studies (Weidner et al., 1994). Significantly higher micro vessel counts have been obtained in

areas of adenocarcinoma than in the benign tissues of radical prostatectomy specimens (Bigler et al., 1993). Pca seem to have the greatest concentration of Micro vessels in the centers of the tumoral areas, which might account for the infrequency of necrosis in Pca (Siegal et al., 1995). Increased micro vascularity has been found to correlate with the pathologic stage of the disease (Weidner et al., 1994; Brawer et al., 1994; Silberman et al., 1997).

Micro vessel density has been associated with the presence of metastasis (Weidne et al., 1994) and with a significant risk for disease progression after radical prostatectomy in some studies (Silberman et al., 1997; Strohmeyer et al., 2000; Bono et al., 2002) but has failed to achieve significance as an outcome predictor in others (Gettman et al., 1989).

These conflicting results might reflect methodological differences in micro vessel counting techniques. The application of micro vessel counts to prostate cancer needle biopsy specimens, in which the counts could be used prospectively to plan therapy, has not received sufficient study (Jeffrey et al., 2003).

Color Doppler flow has been shown to be an important aid to gray-scale sono graphy in the detection of Pca and has been correlated with tumor grade and stage (Gettman et al., 1989; Shigeno et al., 2003) However; little evidence has been presented to date linking sono graphic findings to micro vessel counts in the respective tissue samples. In fact, in one study, micro vessel density and tumor size were no different in specimens with normal or with increased color Doppler flow (Louvar et al., 1998).

p53: The assessment of p53 status in prostate cancer has included both molecular techniques (single-strand conformation polymorphism and direct sequencing) and immunohistochemical analysis (multiple methods and reagents).

Positive immunostaining for p53 has been associated with the detection of the more stable mutant protein and predicts the presence of p53 gene mutation with about 80% to 90%accuracy (Tullo et al., 2003). By using a variety of antibodies and immunehistochemical techniques, p53 protein expression has been reported frequently in Pca, with average immune reactivity ranging from 13% to 23% (Ross et al., 1997). A positive association between nuclear p53 immuno reactivity and aggressive biologic behavior of prostate cancer has been confirmed in multiple studies (Kallakury et al.,

1994; Oxley et al., 2002). Mutations of p53 seem to be frequent in metastatic Pca (Visakorpi et al., 1991).

Although immunohistochemical analysis can be an inaccurate predictor of p53 gene status, when molecular biologic techniques are used, it has been reported that 42% of Pca can harbor mutant p53 sequences (Chi et al., 1994). Mutations of the p53 locus in benign prostate tissue have been reported, suggesting that p53 mutations might occur early in the pathogenesis of Pca (Meyers et al., 1993; Kallakury et al., 1994). New studies of p53 status, including functional assays, must be performed on needle biopsy specimens to achieve prognostic value for prospective treatment planning in Pca.

PSA: The values of total PSA (t PSA), free (f PSA) and PSA complexed to a-antichymotrypsin (PSA-1 ACT) are all independent prognostic factors of Pca (Bjork et al., 1999). Serum PSA level is a strong prognostic determinant of outcome following radiotherapy for prostate cancer and appears to add prognostic information independent of tumour stage and grade (Zagars et al., 1992).

Also, after radical prostatectomy a rising serum PSA level almost always precedes clinical recurrence of the cancer (Pound et al., 1999). The clinical significance of pre-treatment serum PSA value studied by Kariyama and colleagues revealed that serum PSA can be used to predict the stage and prognosis of Pca (Kariyama et al., 1996). In particular, pre-operative serum PSA levels are highly predictive of tumour burden and risk of recurrence after radical prostatectomy (Hull et al., 2002).

Apoptosis and bcl-2: Expression of bcl-2 has been studied in Pca, initially by immunohistochemical techniques, and found to react with primary and metastatic Pca specimens obtained from patients with tumors refractory to hormonal therapy (Bruckheimer et al., 2000). Immunoreactivity for bcl-2 is most intense in basal cells rather than secretory cells (Krajewski et al., 1995) and may be limited to normal prostatic and seminal vesicle epithelium and to rare cases of poorly differentiated but not well-differentiated prostatic carcinomas (Shabaik et al., 1995). In Pca, over expression of bcl-2 protein is not associated with rearrangements in the 2.8-kilobase major breakpoint region or with accumulation of p53 protein (Kallakury et al., 1996).

Several studies have linked the over expression of the bcl-2 anti apoptosis protein with decreased expression of the proapoptotic protein bax and adverse outcome in Pca associated with resistance to cytotoxic chemotherapy in patients with hormone-

refractory disease (Borre et al., 1998; Amanatullah et al., 2000; Pollack et al., 2003). Other studies, however, have not found prognostic significance for bcl-2 expression (Cesinaro et al., 2000; Zellweger et al., 2003).

Tumor Invasion–Associated Proteases: Cathepsin D, a lysosomal protease and autocrine mitogen, has been associated with prognosis in breast cancer (Pujol et al., 1993). In Pca, increased tumor cathepsin D immunoreactivity has been correlated with pathologic stage (Makar et al., 1994) and with tumor grade and DNA content (Ross et al., 1995). Increased serum levels of soluble urokinase plasminogen activator receptor have been linked to progressive Pca (McCabe et al., 2000). Finally, localization of tumor collagenases (Isaacs et al., 1997) and matrix metalloproteinases has been linked to the development and progression of Pca (Lokeshwar et al., 1999; Ross et al., 2003).

Cytokines: In hormone-refractory Pca, up-regulation of inflammation-associated interleukin (IL)-4, IL-6, and IL-10 has been described (Wise et al., 2000). Serum IL-6 levels have been linked to adverse outcome (Nakashima et al., 2000). Tissue-based cytokine measurements have not been associated with Pca prognosis.

Insulin- like Growth Factors: The association between IGF-I and Pca risk is well established. However, there is no evidence that the measurement of IGF-I enhances the specificity of Pca detection beyond that achievable by serum prostate-specific antigen (PSA) levels. Until now, there is no consensus on the possible association between IGFBP-3 and Pca risk. Although not well established, it seems that high insulin levels are particularly associated with risk of aggressive prostatic tumours (Lima et al., 2009).

On an othere study, the insulin-like growth factor (IGF) axis plays a role in growth and progression of Pca. High circulating IGF-1 levels have been associated with an increased risk of Pca. Results for IGF binding protein 3 (IGFBP-3) are inconclusive. Some studies have indicated that the positive association with IGF-1 is observed only for low-grade Pca (Gleason sum <7) (Katharina et al., 2011).

Adhesion molecules: Cell adhesion molecules (CAMs) are cell surface glycoproteins that are important in cell-cell and cell- matrix interaction and play an important role in cell growth and differentiation (Bendardaf et al., 2005). Cell adhesion molecules control cell behavior by mediating contact between cells or between cells and

extracellular matrix which are essential for maintaining tissue integrity (Jang et al., 2011).

Epithelial differentiation is critically dependent on maintenance of intact inter cellular junctions by cell-cell adhesion molecules. Impairment of these junctions facilitates the invasion of epithelial cells, thus favoring the progression of carcinoma. There are several cell- cell adhesion molecules, including cadherins (E-,P-,N-cadherins),catenins (α, β , and γ catenins),and the CD44 family (standard et al., 2000; Lin et al., 2004; Fernandes et al., 2004).

CD44: The CD44 antigen is a cell-surface glycoprotein involved in cell–cell interactions, cell adhesion and migration. In humans, the CD44 antigen is encoded by the CD44 gene on Chromosome 11 (Spring et al., 1988). It is expressed in a large number of mammalian cell types. The standard isoform, designated CD44s, comprising exons 1–5 and 16–20 is expressed in most cell types. CD44 splice variants containing variable exons are designated CD44v. Some epithelial cells also express a larger isoform (CD44E), which includes exons v8–10 (Goodison et al., 1999). variations in CD44 are reported as cell surface markers for some breast and Pc (Li et al., 2007). and has been seen as an indicator of increased survival time in epithelial ovarian cancer patients (Sillanpää et al., 2003). Endometrial cells in women with endometriosis demonstrate increased expression of splice variants of CD44, and increased adherence to peritoneal cells (Griffith et al., 2010). CD44 variant isoforms are also relevant to the progression of head and neck squamous cell carcinoma (Assimakopoulos et al., 2002; Wang et al., 2010).

Catenin: Catenins are a family of proteins found in complexes with cadherin cell adhesion molecules of animal cells. The first two catenins that were identified (Peyriéras et al., 1985) became known as alpha-catenin and beta-catenin. Alpha-catenin can bind to beta-catenin and can also bind actin. Beta-catenin binds the cytoplasmic domain of some cadherins. Additional catenins such as gamma-catenin and delta-catenin have been identified. The name "catenin" was originally selected ('catena' means 'chain' in Latin) because it was suspected that catenins might link cadherins to the cytoskeleton (Ozawa et al., 1989).

Function: Several types of catenins work with N-cadherins to play an important role in learning and memory. Cell-cell adhesion complexes are required for

simple epithelia in higher organisms to maintain structure, function and polarity (Reynolds et al., 2011). These complexes, which help regulate cell growth in addition to creating and maintaining epithelial layers, are known as adherens junctions and they typically includes at least cadherin, β-catenin, and α-catenin (Reynolds et al., 2011). Catenins play roles in cellular organization and polarity long before the development and incorporation of Wnt signaling pathways and cadherins (Dickinson et al., 2011).

Interaction with cadherins: F9 embryonal carcinoma cells are similar to the P19 cells and normally have cell to cell adhesion mediated by E-cadherin with beta-catenin bound to the cytoplasmic domain of E-cadherin. F9 cells were genetically engineered to lack beta-catenin, resulting in increased association of plakoglobin with E-cadherin (Fukunaga et al., 2005). In F9 cells lacking both beta-catenin and plakoglobin, very little E-cadherin and alpha-catenin accumulated at the cell surface (Fukunaga et al., 2005). Mice lacking beta-catenin have defective embryos. Mice engineered to specifically have vascular endothelium cells deficient in beta-catenin showed disrupted adhesion between vascular endothelial cells (Cattelino et al., 2003). Mice lacking plakoglobin have cell adhesion defects in many tissues, although beta-catenin substitutes for plakoglobin at many cellular junctions (Bierkamp et al., 1999). Keratinocytes engineered to not express alpha-catenin have disrupted cell adhesion (Vasioukhin et al., 2001) and activated NF-κB (Kobielak et al., 2006). A tumor cell line with defective delta-catenin, low levels of E-cadherin and poor cell-to-cell adhesion could be restored to normal epithelial morphology and increased E-cadherin levels by expression of normal levels of functional delta-catenin (Vasioukhin et al., 2001).

E-cadherin: Cadherins (named for "calcium-dependent adhesion") are a class of type-1 transmembrane proteins. They play important roles in cell adhesion, ensuring that cells within tissues are bound together. They are dependent on calcium (Ca^{2+}) ions to function, hence their name (Hulpiau & van, 2009).

The cadherin superfamily includes cadherins, protocadherins, desmogleins, and desmocollins, and more (Hulpiau & van 2009; Angst et al., 2001). In structure, they share cadherin repeats, which are the extracellular Ca^{2+}-binding domains. There are multiple classes of cadherin molecule, each designated with a prefix (in general, noting

the type of tissue with which it is associated). It has been observed that cells containing a specific cadherin subtype tend to cluster together to the exclusion of other types, both in cell culture and during development (Bello et al., 2012). For example, cells containing N-cadherin tend to cluster with other N-cadherin-expressing cells. However, it has been noted that the mixing speed in the cell culture experiments can have an effect on the extent of homotypic specificity (Duguay et al., 2003). In addition, several groups have observed heterotypic binding affinity (i.e., binding of different types of cadherin together) in various assays (Volk et al., 1987; Niessen et al., 2002). One current model proposes that cells distinguish cadherin subtypes based on kinetic specificity rather than thermodynamic specificity, as different types of cadherin homotypic bonds have different lifetimes (Bayas et al., 2005).

E-cadherin gene and protein structure

The human epithelial (E)-cadherin gene CDH1 maps to chromosome 16q22.1. (Berx et al., 1995) isolated the full-length gene by using recombinant lambda phage, cosmid and P1 phage clones. The gene they cloned encompasses 16 exons and spans a region of ~100 kb. The exons range from 115 to 2245 bp. Further analysis of the gene showed 15 introns ranging from 120 bp (intron 4) to 65 kb (intron 2). The intron-exon boundaries are highly conserved in comparison with other "classical cadherins", and in intron 1 a 5' high-density CpG island was identified that may have a role in transcription regulation (Berx et al., 1995). This island covers the region from exon 1 to exon 2 of the human E-cadherin gene, while other exons lacked such features, including the biggest (exon 16 of 2245 bp) (Nives & slaus, 2003).

The chromosomal location of CDH1 on 16q22.1 was later confirmed by fluorescent in situ hybridization (FISH) analysis. It is interesting that the human P-cadherin gene was located only 32 kb upstream from E-cadherin (Bussemakers et al., 1994), and also the M-cadherin gene was positioned on chromosome 16q24.1-qter (Kaupmann et al., 1992), which further suggests clustering of cadherin genes originating probably from gene duplication, while possible co-evolution might be explained by gene conversion (Berx et al., 1995).

All classical cadherin genes analyzed so far have 16 exons separated by 15 introns. CDH1 encodes a 120 kDa glycoprotein with a large extracellular domain, a single transmembrane segment and a short cytoplasmic domain, which interacts with

the actin cytoskeleton through linker molecules, alpha- beta- and gamma-catenins (Humphries & Newham, 1998). On the cytoplasmic side of the membrane, a bundle of actin filaments is linked to the E-cadherin molecules via a protein complex. Alpha-catenin and either beta- or gamma-catenins are included in this complex. Beta- and gamma-catenins share significant homology and bind to a specific domain at the E-cadherin C-terminus. Alpha-catenin links the bound beta- or gamma-catenin to the actin cytoskeleton (Nives & slaus, 2003). Alpha-catenin has structural similarities with vinculin, one of the key components of fibroblast membrane attachment sites of microfilaments, beta-catenin shows homology to Armadillo of Drosophila melanogaster and gamma-catenin is identical to plakoglobin, a protein found in desmosomes (Rietmacher et al., 1995). The C-terminal cytoplasmic domain of ~150 residues is highly conserved in sequence, and has been shown experimentally to regulate the cell-cell binding function of the extracellular domain of E-cadherin, possibly through interaction with the cytoskeleton. The juxtamembrane region of the cadherin cytoplasmic tail has been identified as a functionally active region supporting cadherin clustering and adhesive strength; one of the interacting proteins involved in clustering and cell adhesion is p120ctn (Yap et al., 1998).

The structure of the extracellular domain of classical E-cadherin contains five tandem repeats of a 100-residue-amino-acid-motif, and the biggest part of N-terminal of these repeats contains the sites with adhesive activity. This part of the molecule also has binding sites for calcium ions situated in the pockets between the repeats. The amino acid sequences that form the Ca2+ binding pockets are highly conserved between different members of the cadherin family and between different species. Cell-cell adhesion is mediated through homotypic interactions of E-cadherin extracellular domains in a process of lateral dimerization. Parallel dimers are able to interdigitate with dimers from neighbouring cells forming the points of adhesion. Those findings introduce a cadherin-cadherin interface at the cellular surface. Cadherins were thought to be involved only in homophilic interactions; however, E-cadherin has now been shown to be a ligand for two integrins, alphaEbeta7 and alpha2beta1. The first interaction might serve to retain intraepithelial lymphocytes in mucosal tissue, while the second may contribute to the organization of epithelial multilayers (Huber & Weis, 2001).

Cadherins have been identified in a large variety of species, including mammals, Xenopus, Drosophila, Caenorhabditis elegans. Many new cadherin families have been isolated and the cadherin group of genes has grown very fast in the last decade. Proteins of several isolated molecules show a great deal of homology with the "members classical" cadherins (Takeichi, 1993).

Other cadherin like molecules share with the classical cadherins putative Ca2+ binding motifs in repeated extracellular domains but diverge considerably in various regions, particularly in the cytoplasmic domains. Even more deviations were observed for new cadherins, such as K-cadherin (Xiang et al., 1995) and LI-cadherin (Berndorff et al., 1994), while the ret protooncogene product also shows some similarity to cadherin (Schneider, 1992; Iwamoto et al., 1993). Although the extracellular domain has several similar repeats with putative Ca2+ binding motifs, this transmembrane tyrosine kinase is considered to be unrelated to the cadherins. Since the ret gene lacks matching of the splice sites to the CDH1 gene, this might be the explanation. It is possible that this kinase has acquired its cadherin-like motifs by convergent evolution (Berx et al., 1995).

Subunit structure

Homodimer; disulfide-linked. Component of an E-cadherin/ catenin adhesion complex composed of at least E-cadherin/CDH1, beta-catenin/CTNNB1 or gamma-catenin/JUP, and potentially alpha-catenin/CTNNA1; the complex is located to adherens junctions. The stable association of CTNNA1 is controversial as CTNNA1 was shown not to bind to F-actin when assembled in the complex. Alternatively, the CTNNA1-containing complex may be linked to F-actin by other proteins such as LIMA1. Interaction with PSEN1, cleaves CDH1 resulting in the disassociation of cadherin-based adherens junctions (CAJs). Interacts with AJAP1, CTNND1 and DLGAP5. By similarity Interacts with TBC1D2. Interacts with LIMA1. Interacts with CAV1. Interacts with the TRPV4 and CTNNB1 complex .By similarity Interacts with PIP5K1C. Interacts with RAB8B. By similarity Interacts with DDR1; this stabilizes

CDH1 at the cell surface and inhibits its internalization (Bharti, 2004; Serban et al., 2005; Abe et al., 2008; Eswaramoorthy et al., 2010; Frasa et al., 2010).

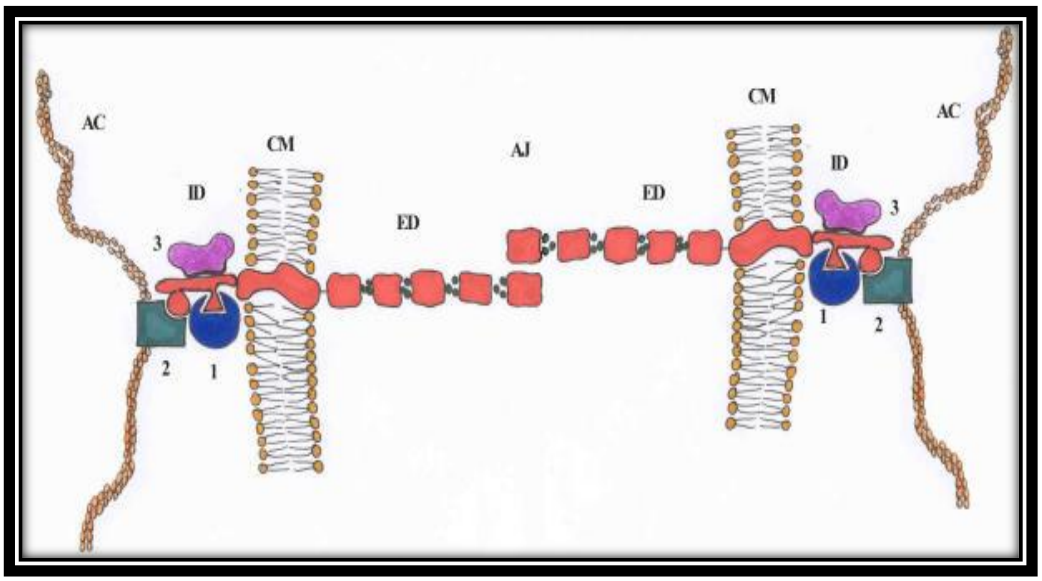

Figure 2.4.10.2.1 Shematic illustration of E-cadherin in adherens junction.

E-cadherin homodimer on the cytoplasmic membranes of adjacent cells is shown. The juxtamembrane region with the interacting molecules is also shown. CM – cytoplasmic membrane; AJ – adherens junction; ED – extracellular domain; ID – intracellular domain; AC – actin cytoskeleton; 1-beta-catenin; 2-alpha-catenin; 3-p120.

(Pećina-Šlaus,2003)

Subcellular location: Cell junction. Cell membrane; Single-pass type I membrane protein. Endosome. Golgi apparatus › trans-Golgi network. Note: Colocalizes with DLGAP5 at sites of cell-cell contact in intestinal epithelial cells. Anchored to actin microfilaments through association with alpha-, beta- and gamma-catenin. Sequential proteolysis induced by apoptosis or calcium influx, results in translocation from sites of cell-cell contact to the cytoplasm. Colocalizes with RAB11A endosomes during its transport from the Golgi apparatus to the plasma membrane (Ito et al., 1999; Laprise et al., 2004; Lock et al., 2005; Eswaramoorthy et al., 2010).

Types: Cadherins can be classified into four groups: classical, desmosomal, protocadherins, and unconventional (Stefan &Walter, 2008).

Classical

Different members of the cadherin family are found in different locations (Stefan & Walter, 2008).

CDH1 - E-cadherin (epithelial): E-cadherins are found in epithelial tissue

CDH2 - N-cadherin (neural): N-cadherins are found in neurons

CDH12 - cadherin 12, type 2 (N-cadherin 2)

CDH3 - P-cadherin (placental): P-cadherins are found in the placenta.

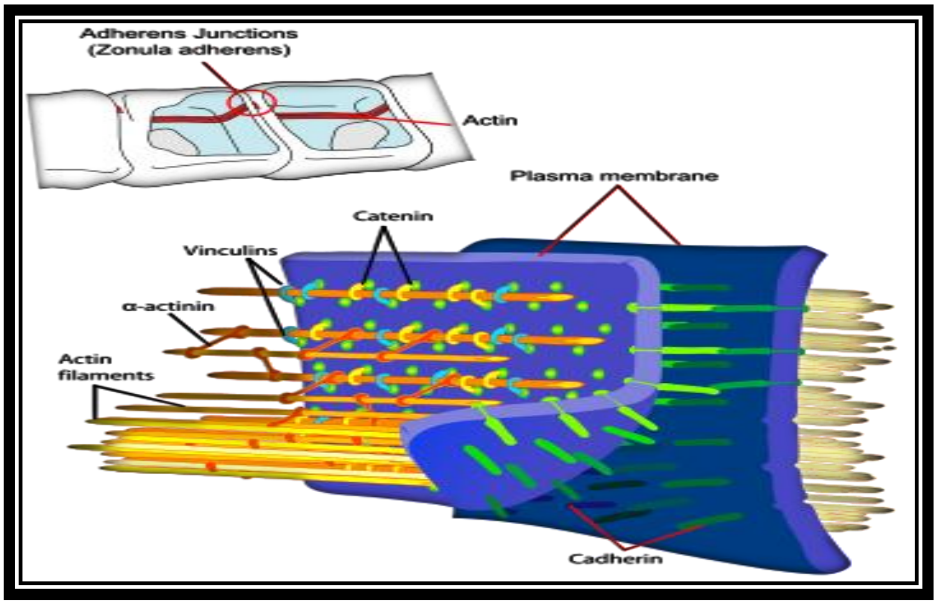

Figure 2.4.10.2.2. Interactions of structural proteins at cadherin-based adherens junction.

The exact means by which cadherins are linked to actin filaments is still under investigation (Hulpiau & van, 2009).

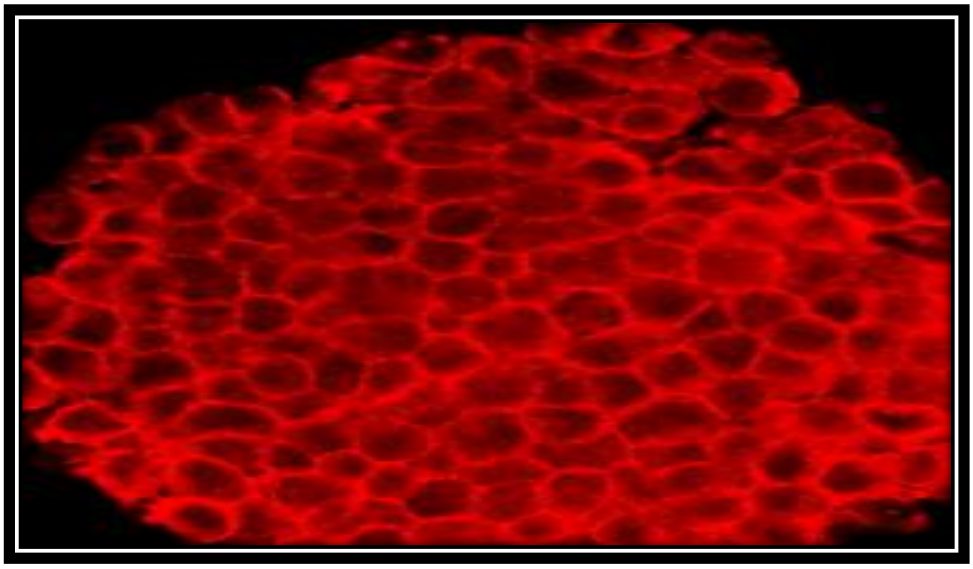

Figure 2.4.10.2.3. Beta-catenin at cell-to-cell contacts of P19 embryonal carcinoma cells . http://en.wikipedia.org/wiki/Catenin

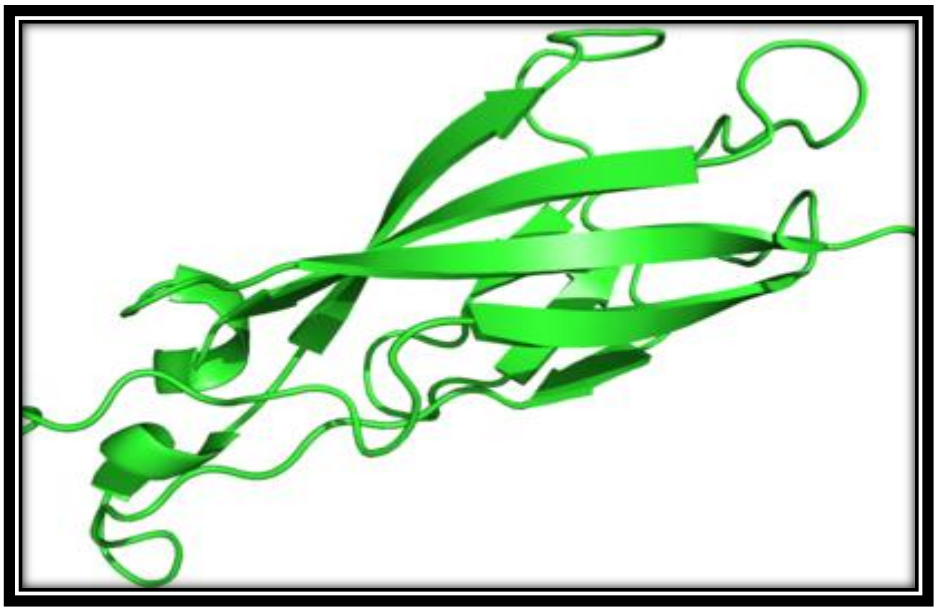

Figure 2.4.10.2.4. Ribbon representation of a repeating unit in the extracellular E-cadherin ectodomain of the mouse (Mus Musculus) (PDB 3Q2V et al., 2011)

Function

Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells.

Has a potent invasive suppressor role. It is a ligand for integrin alpha-E/beta7- (Agiostratidou et al., 2006).

E-Cad/CTF2 promotes non-amyloidogenic degradation of Abeta precursors. Has a strong inhibitory effect on APP C99 and C83 production (Agiostratidou et al., 2006).

Role of E-cadherin in normal cells, wnt signalling, adherens junctions

Expression of E-cadherin in embryonic development is very early, at the two-cell stage (Larue et al., 1994; Rietmacher et al., 1995). Epithelial differentiation and polarization (processes fundamental to cell differentiation) occur early in ontogeny in the morula stage, when the embryo compacts and each cell polarizes along its apicobasal axis to generate an epithelial-like phenotype. E-cadherin plays an important role in the adhesion of the blastomeres, and early embryo's ability to compact (Fleming et al., 1992). E-cadherin is expressed in the membrane even before compaction of the morula occurs, is distributed in a non-polar manner, and does not exhibit adhesive function (Hyafil et al., 1980; Vestweber &Kemler, 1984). The mechanism that renders E-cadherin functional is unknown, but it does include phosphorylation of the protein (Sefton et al., 1992). Controlled epithelial-mesenchymal conversion is the most important exhibit of E-cadherin's function in development (Thiery, 2002). Loss of epithelial adhesion and polarity causing mesenchymal cell morphology occurs during mesoderm formation. Rietmacher and co-workers (Rietmacher et al., 1995) introduced a targeted mutation in mouse embryonal stem cells and generated a mouse without E-cadherin sequences essential for Ca^{2+}binding and for adhesive function. Heterozygous mutant animals were normal and fertile. In vitro, they were able to form normal blastocysts with normal blastocoels that consequently expanded. On the other hand, the homozygous E-cadherin -/- embryos showed severe abnormalities before implantation. This included failure to maintain a polarized and compacted state and also failure to form a trophectoderm epithelium; they distort at the blastocyst stage, making the

mutation lethal. The initial compaction that was observed in -/- embrios is probably due to the presence of E-cadherin proteins from maternal sources (Sefton et al., 1992).

Investigation of zebrafish E-cadherin expression during early embryogenesis confirmed observed expression in blastomeres, but also led to the detection of a protein expressed in the anterior mesoderm during gastrulation and developing epithelial structures (Babb et al., 2001). In the developing nervous system, CDH1 was detected at the pharyngula stage in the midbrain-hindbrain boundary and was preceded by wnt 1 expression (Babb et al., 2001).

As far as normal adult epithelial tissue structure and integrity is concerned, E-cadherin is also involved in its maintenance and homeostasis. As already mentioned, its function lies primarily in the formation of adherens junctions. Cadherin mediated adhesion is a dynamic process that is regulated by several signal transduction pathways. There is also evidence that cadherins are not only targets for signaling pathways that regulate adhesion, but may themselves send signals that regulate basic cellular processes, such as migration, proliferation, apoptosis and cell differentiation (Hulsken et al., 1994; Barth et al., 1997; Morin et al., 1997).

The image of individual adhesion molecule performing its function, or linear downstream signalling cascade is somewhat abandoned scheme. Instead of separating and dividing into distinct fields, the cellular mechanisms of signalling and adhesion are nowadays thought to be closely connected mechanisms where components have double (or more) functions and interconnect in a signalling-structural network. The clearest example is recently discovered interaction of E-cadherin with epithelial growth factor receptor (EGFR) (Pece & Gutkind, 2000). The recently discovered wnt/wingless pathway, of mouse and Drosophila respectively is one of the most interesting kind of signal transduction, in which key components have multiple functions in adhesion and signalling. Information on Wnt signalling can be found on the wnt gene site (Nives & Slaus, 2003). In vertebrate cells, it is named after Wnt proteins, a family of highly conserved secreted signaling molecules that regulate cell-to-cell interactions during embryogenesis. Insights into the mechanisms of Wnt action have emerged from several systems: genetics in Drosophila and Caenorhabditis elegans; biochemistry in cell culture; and ectopic gene expression in Xenopus embryos. Many Wnt genes in the mouse have been mutated, leading to very specific developmental defects. As currently

understood, Wnt proteins bind to receptors of the Frizzled family on the cell surface. Through several cytoplasmic relay components, the signal is transduced to beta-catenin, which then enters the nucleus and forms a complex with TCF to activate transcription of Wnt target genes (Morin et al., 1997). It has been well documented that wnt genes, together with other components of wnt signalling pathway, are implicated in cancer (Peifer & Polakis, 2000).

Another tantalizing compartment of the wnt signalling pathway lies in downstream transcriptional activation. In response to WNT signalling, cytoplasmic beta-catenin is stabilized, accumulates in the cytoplasm and enters the nucleus, where it finds a partner, a member of the DNA binding protein family LEF/TCF (T cell factor-lymphoid enhancer factor). Together they activate new gene expression programs. One of the target genes for beta-catenin/TCF encodes c-MYC protein (Guilford, 1999), explaining why constitutive activation of the wnt pathway can lead to cancer.

E-Cadherin Function Is Suppressed during Carcinogenesis

A large number of studies have revealed that E-cadherin function is frequently inactivated during the development of human carcinomas, including those of the breast, colon, prostate, stomach, liver, esophagus, skin, kidney, and lung (Birchmeier & Behrens, 1994; Bracke et al., 1996). Abrogation of E-cadherin function may occur by any of several mechanisms, but it frequently involves deletion or mutation of the CDH1 gene (Birchmeier & Behrens 1994; Bracke et al., 1996). Remarkably, germline mutations in CDH1 have been identified in cases of familial gastric cancers,indicating that aberrations in this gene are sufficient to predispose to the development of malignant cancer (Guilford et al., 1998). Furthermore, changes in the expression of proteins that are part of the E-cadherin–adhesion complex have also been found to impair E-cadherin–mediated cell-cell adhesion. For example, down-regulation of alpha- catenin or beta-catenin expression (De Leeuw et al., 1997), as well as the expression of mutant forms of either acatenin (Bullions et al., 1997) or b-catenin (Oyama et al., 1994) sometimes coincides with malignant transformation. Moreover, mutations of the b-catenin gene are frequently found in colon cancers (Sparks et al. 1998).

However, it remains to be determined, for each type of cancer, how changes in the expression of either a-catenin or b-catenin affect E-cadherin function. In addition to

deletions and mutations, several other mechanisms have been identified that directly affect Ecadherin expression and function. For example, chromatin rearrangement, hypermethylation, and loss of transcription-factor binding frequently coincide with suppression of E-cadherin–promoter activity in invasive carcinoma cells (Yoshiura et al., 1995; Hennig et al., 1996). Notably, in some tumor types, inactivation of the E-cadherin gene by hypermethylation appears to be a major mechanism—for example, in papillary thyroid carcinoma, where hypermethylation of the E-cadherin promoter has been found in 83% of the cases examined (Graff et al., 1998). Other epigenetic events that are required for E-cadherin function during normal developmental processes may also be involved in the misregulation of E-cadherin during tumorigenesis. For example, E-cadherin function can be affected by the Rho family of GTPases (Braga et al., 1997; Jou & Nelson 1998), growth factor–receptor signaling (Hoschuetzky et al., 1994; Shibamoto et al., 1994), integrin-linked kinase (Novak et al., 1998), and matrix metalloproteases (Lochter et al., 1997). Because decreased E cadherin function generally correlates with dedifferentiation, infiltrative tumor growth, and metastasis, E-cadherin has been proposed as a. marker to indicate poor prognosis (Birchmeier and Behrens, 1994; Bracke et al., 1996). Although E-cadherin is not the only cadherin that is expressed in epithelial cells, few others have been implicated in cancer. One exception is H-cadherin, which has been found to be lost during the development of breast cancer (Lee, 1996).

E-cadherin as prognostic factor

E-cadherin expression has been proposed for predicting prognosis in Pca. A study of E-cadherin levels by immunohistochemistry in nonmalignant and malignant specimens of human prostatic tissue revealed that almost 50% of tumours examined had reduced levels of this protein, and in some tumours E-cadherin was absent when compared to non-malignant prostate, which uniformly stained strongly positive (Umbas et al., 1992). To determine the potential prognostic significance of the findings, Pca specimens from 89 patients were evaluated immunohistochemically using specific antibodies raised against E-cadherin (Umbas et al., 1994).

The results were related to histological grade, tumour stage, presence of metastasis, and survival. Patients showing low immunohistochemical expression of E-cadherin have on average shorter survival than patients with high immunohistochemical expression. Because mutational inactivation of alpha-catenin can be the cause of the impaired E-cadherin function, Umbas et al. studied the relationship between E-cadherin and alpha-catenin expression. The results suggest that loss of alpha-catenin expression could be one of the mechanisms responsible for the loss of E-cadherin mediated cell-cell adhesion in human prostate cancer and might in some cases provide prognostic information (Umbas et al., 1997).

The same was concluded by Aaltomaa et al who studied the expression of alpha-catenin in locally advanced prostate cancer. They found that alpha-catenin had prognostic significance in the early phases of cancer progression (Aaltomaa et al., 1999). Low alpha-catenin expression was related to worse prognosis than high alpha-catenin expression. De-Marzo et al correlated the down-regulation of E-cadherin and pathologic stage at radical prostatectomy (Buhmeida et al., 2006).

In univariate analysis they found that reduced levels of E-cadherin correlated with advanced Gleason score ($p = 0.003$) and advanced pathologic stage ($p = 0.008$) (De Marzo et al., 1999). In multivariate analysis, E-cadherin, preoperative PSA, and Gleason score all contributed independently to the prediction of high stage disease ($p < 0.001$). They concluded that a prospective study on E-cadherin is warranted. The study should evaluate E-cadherin as a potential biomarker of disease progression in patients with clinically organ-confined Pca who undergo radical prostatectomy. Moderate or strong expression of a transcriptional repressor EZH2 (enhancer of zestor homolog2)

coupled with at most moderate expression of E-cadherin was the biomarker combination that was most strongly associated with recurrence of Pca (Rhodes et al., 2003). In the clinical situation low E-cadherin immunostaining suggested clinical recurrence (Buhmeida et al., 2006).

2.4.11.1. Immunohistochemistry

The term immunostaining was originally used to refer to the immunohistochemical staining of tissue sections, as first described by Albert Coons in 1941 (Coons et al., 1941). IHC is a useful research tool and used to localize specific antigens in tissue sections with labeled antibodies based on antigen-antibody interactions. The immune reactive products can be visualized by a marker including fluorescent dye, enzyme in general; radioactive element or colloidal gold also can be used. Not only used in research laboratories, IHC is also used in clinical diagnosis; it has been one of the routine diagnostic approaches in daily clinic activities. Prior to the IHC generation, histochemistry was used frequently in clinical diagnosis and laboratory research. However, the histochemistry has been gradually replaced by IHC based on its apparently advantage, the specific antigen-antibody reaction. Theoretically, IHC can identify any existing protein antigen in tissue and thus make it become a critical technique in laboratory research and clinical diagnostic method (Xiao et al., 2010).

The Immunohistochemistry Technique

The immunohistochemistry technique is used in the search for cell or tissue antigens ranging from amino acids and proteins to infectious agents and specific cellular populations. The technique comprises two phases: (1) slide preparation (specimen fixation and tissue processing) and stages evolved for the reaction (in order: antigen retrieval, non-specific site block, endogenous peroxidase block, primary antibody incubation, and the employment of systems of detection, revealing and counterstaining and also slide mounting and storage); (2) interpretation and quantification of the obtained expression (Leandro et al., 2010).

Applications and importance

The immunohistochemical reactions can be used in different situations within research or pathological anatomy laboratories. The most important are: 1)

histogenetic diagnosis of morphologically non-differentiated neoplasias ; 2) subtyping of neoplasias (such as lymphomas, for example); 3) characterization of primary site of malignant neoplasias; 4) research for prognostic factors and therapeutic indications of some diseases; 5) discrimination of benign versus the malign nature of certain cell proliferations ; identification of structures, organisms and materials secreted by cells (Leandro et al., 2010).

Advantage of the Immunohistochemistry Technique

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues .This has made it a widely-used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures (O'Malley & Pinder, 2006).

Limitations, difficulties and problems

Although a relatively simple technique, immunohistochemistry has some particularities and its outcome depends on many factors. The usefulness and contribution of immunohistochemistry in solving problems in pathological anatomy is directly proportionate to the experience of the hands that perform the reactions and also the eyes that interpret the results (Jaffer et al., 2004). Therefore, even though very simple in concept, immunostaining methods requires rigor of execution and may present significant bias. Hence, its outcomes must be interpreted with caution. A wide variety of protocols for standardizing the immunohistochemistry technique are being proposed to minimize undesirable effects (Leandro et al., 2010). The Committee of Quality Control in Immunohistochemistry of the French Pathology Society published a report in 1997 demonstrating that two of the main causes of diagnosis mistakes in immunohistochemistry are the non-employment of antigen retrieval techniques and the use of amplifying methods with low power. Other renowned international quality programs are the electronic database Immunoquery *("*Immunohistochemistry Literature Database Query System*")* and the UK NEQAS quality program *(*"United Kingdom National External Quality Assessment Scheme for Immunocytochemistry" (Lewis, 1995).

The acquisition, handling, fixation, specimen delivery to the laboratory and antigen retrieval are all critical factors. Fresh specimens that are inadvertently submitted to long periods of fixation may significant lose antigenicity (Yaziji &Barry,2006). The specimen fixation in formaldehyde and its consequent inclusion in paraffin are the internationally most used histological processing procedures. Some specialists propose that this procedure should be the standard for comparing diagnostic outcomes among immunohistochemistry reactions. However, formaldehyde fixation results in a variably reversible loss of immunoreactivity by its masking or damaging some antibody binding sites. Although such epitopes may be demasked by several epitope retrieval methods, the immunohistochemical detection system must still be sensitive enough to produce a strong signal. For some epitopes, the duration of the formaldehyde fixation is critical. With some antibodies, depending on the resistance of its target epitope to autolytic change, delay in fixation may cause loss of immunoreactivity (Wasielewski et al., 1998).

Other fixatives often used in pathology include alcohol and alcohol-based fixatives such as acetone. Alves et al studied the fixation in ethanol and formalin for trypsin digestion in immunohistochemical detection of cytokeratins and vimentin in a case of ovarian cystadenofibrocarcinoma. They found superior reactivity for both markers in achieved ethanol-fixed sections, even in samples stocked up to 60 days. Cytokeratin reaction in formalin-fixed sections was better when trypsin was used. However, this digestion was deleterious to vimentin detection. This was an import work to alert surgeons and oncologists on the relevance of fixation of specimens suspicious for neoplasia, since different epitopes may require different fixatives and the inadequate choice in the operative room may impart difficulties when immunohistochemistry is necessary (Leandro et al., 2010). It is important to emphasize that in tissue processing, inclusion in paraffin at high temperatures (in general, over 60 °C) may compromise the specimen antigenicity. Another important point addresses the preparation of slides. The block slices must preferentially present a thickness ranging between 3 and 7 μm and must be deposited on slides previously prepared with some kind of adhesive (the most used are silane and polylysine). Slices less than 3 μm thick could result in very weak immunostaining while those thicker than 7 μm may lead to loss of tissue on the glass slide or may hamper analysis of the resultant immunostaining (Yaziji &Barry,2006). The amount of material to be analyzed is being discussed, especially

now that pathologists are expected to reach a precise diagnosis with small samples. In the majority of situations a block is sufficient, preferentially when it contains a fragment of the tumor-surrounding parenchyma interface (prepared in the macroscopic examination), distally to hemorrhagic or extensively necrotic areas, as well as a fragment representative of the tissue distal to the neoplasm. Whenever possible, tissue that was previously submitted for frozen examination must be avoided (Leandro et al., 2010).

Regarding antigen retrieval, the simplification of procedures, costs and technical error risk reduction are important factors. Irradiation techniques with microwaves or by humid heat in pressure or vapor pan, with exposition times adapted to offer the same pattern of staining in a group of case-controls has been suggested (Alves et al.,1999).

The use of detection systems (secondary antibodies) is also considered valuable in error reduction. Among high discharge amplification systems, the avidin-biotin-peroxidase complex (ABC) and the labeled streptavidin-biotin complex (LSAB) are the most important. Specific situations require adaptations and even the use of alternative detection methods(Giorno, 1984).

The selection of an adequate method is one of the great technical responsibilities faced in an immunohistochemistry laboratory. The advance in the technique, with systems of epitope retrieval through heat (HIER) and amplification methods, as well as the reactions performed in a single stage (EPOS) and the method of catalyzed product deposition (CARD), have introduced a paradox in immunohistochemistry. On the one hand numerous cases hitherto unsolved because of negativity in many panels, became positive and began to permit precise diagnosis. On the other hand, antibodies that were expressed characteristically in certain neoplasias began to react non-specifically in other situations (Alves et al.,1999).. Concerned about the so called “anarchy” then introduced, Swanson proposed that no method should be universally applicable, the choice should be based on the technique that, in the experience of the laboratory or of the school followed by researchers, best solves the diagnostic question (Alves et al.,1999).

Due to their flexibility and relatively low cost, the most used protocols currently (such as the ABC method, for example) are indirect and therefore require many stages of incubation. High sensitivity could be obtained with the application of immunological principles, enzymatic amplification reactions and/or the employment of avidin-biotin complex, however the various steps required must be rigorously followed in order to

avoid non-desirable interactions. It is fundamental that, on technical planning, all reagents follow the sequence rigorously established, where the employment of work flow charts for such stages are very useful in avoiding false results. Making notes of all reaction stages and pattern of each antibody are equally important and are suggested in patterning technique programs (Alves et al.,1999).

The ability of the specialized technician who performs the reactions is a guarantee against the introduction of crossed immunological reactions with endogenous immunoglobulins during the test preparations, or with different sequence experiments of immunostaining with many colors(Leandro et al., 2010). The selection of antibody panels is one of the most important aspects for optimal applicability of immunohistochemistry(Alves et al.,1999). The knowledge of each reagents' characteristics, especially those of antibodies, requires new titration in each new batch or clone, selecting the dilution that offers the greatest "true/background positivity" contrast(Yaziji &Barry,2006).

The primary antibodies can be divided into two categories: poly or monoclonal. The polyclonal group are those obtained from animal immunization (example: rabbit, goat, monkey, rat, mouse, ewe etc) and results in antibodies that are capable of recognizing many epitopes of the same antigen, generating higher detection sensitivity. The monoclonal type, however, are developed from hybrids and provide antibodies against only one antigen epitope, yielding more specific results (Nadji, 1986).

Regarding the validation of findings and their interpretation, it is necessary to observe the reactivity patterns of the negative and positive, internal and external controls. The external controls (histological slices of specific tissues for each antibody) must be included in each panel, prepared from the samples fixed under the same conditions as the test cases and submitted to the same stages of the reaction. Attention must also be paid to the reactivity of structures present on the slide of the case being studied that may be used as internal positive controls, such as the reactivity of vessels for vimentin, muscle and endothelial markers, or breast ducts adjacent to the neoplasm for estrogen and progesterone receptors. Similarly, structures knowingly negative for a marker offer an excellent internal negative control, since they were submitted to the same treatment as the test-tissue, for example the erythrocytes within blood vessels—a great endogenous source of peroxidase(Leandro et al., 2010).

CHAPTER 3

3. PATIENTS AND METHODS

Clinicopathological features and follow up data

The records of all newly diagnosed prostate cancer cases between January 2005 to April 2012 based on availability of representative paraffin blocks were retrieve from the files of the Histopathology Department, Benghazi University, 50 libyan male patients were diagnosed with Pca. For each patient, the following information were obtained: age, diagnosis, grading, then the patient files and hospital information system were reviewed to get more information about the type of initial surgical procedure, the PSA level at initial presentation, date of initial diagnosis, staging and type of treatment.

One pathologist confirmed all histological diagnoses and the following histopathological features were recorded included tumour type, tumor stage, Gleason score, tumour grade (well, moderately or poorly differentiated), perineural invasion, metastasis, and status of patients.

E-cadherin Immunostaining

Fifty samples were available for successful staining by immunohistochemistry. Formalin-fixed, paraffin-embedded prostate tumour tissues were obtained from all 50 samples. Sections were cut serially at 5μm for immunohistochemical (IHC) analysis. IHC analysis was done using the automatic system (BenchMark XT, Ventana Medical Syatem, Inc. Tucson, Arizona, and USA). This fully automated processing of code-labeled slides included baking of the slides, solvent free deparaffinization, antigen retrieval in a cell conditioning buffer CCI (Mild:36 minutes conditioning, and standard:60 minutes conditioning), incubation with Rabbit monoclonal anti- E-cadherin antibody, 7.0 ml ready-to-use from Spring Bioscience (clone: ECH-6, Catalog no. M3641, 6920 Koll Center Parkway, CA 94566, USA), for 32 min, at 37C. Applicatoin of I-View™ DAB Detection Kit (Lot no. B05860AZ), which, includes: I-View DAB HRP, I-View DAB Inhibitor, I-View DAB Biotin, I-view DAB H2O2, and I- view DAB copper. Counterstaining with haematomtoxylin II (C00758) was done for 4 minutes, and post-counterstaining with blueing reagent (B11129) was done for 4 minutes as well. After staining, the sections was dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of membranous E-cadherin staining

E-cadherin staining was evaluated using regular light microscope at the magnification of x40, blinded by the information on tumour grade, stage or clinical outcome. Membranous staining was evaluated. For cell membrance staining, four categories were used, (+++, ++, +, 0), 0) no expression, no detectable staining in < 10% of the membrance 1) weak but detectable discontinuous staining present in 10-39% of the membrance 2) moderate, clearly positive discontinuous staining present in 40-90% of the membrance and 3) intense continuous staining of the membrance create a honeycomb pattern (Elzagheid et al., 2002; Elzagheid et al., 2006). The membrance index (MI) was calculated with both the intensity of staining and fraction of positively-stained cells taken into account using the following formula:

$$\mathbf{I = 0* f0 +1* f1 + 2* f2 + 3* f3}$$

Where I; is the staining index, f0-f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index scores could vary between 0 and 3 (Lipponen Collan, 1992; Buhmeida A et al., 2008).

Statistical analysis

Statistical analyses were performed using the IBM SPSS statistics (IBM Company, NY, and USA) and STATA (StataCrop., Texas, USA) software packages (IBM PASW statistics for windows, version 18.0.3 and STATAA/SE 11.1). Frequency tables were analysed using the Chi-square test, with likelihood ratio (LR) or Fischer's exact test being used to assess the significance of the correlation between the categorical variables. Odds Ratios and their 95% Confidence Intervals (95%CI) were calculated where appropriate, using the exact method. Difference in the means of continuous variables was analysed using non-parametric tests (Mamm-Whitney or Kruskal-Wallis) and multiple independent samples, respectively. Analysis of variance (ANOVA) was only used deriving the mean values (and their 95% CI) of each individual stratum.

In all tests, the values $p < 0.05$ were regarded statistically significant. Results were represented in tables and figures using Microsoft Office Excell.

Table3.1. Clinico-pathological characteristics of 50 cases of the study.

Character		No. of Patients (%)	
Age (yrs)	≤ 73 years	24	(48%)
	>73 years	26	(52%)
Staging	II ,III	13	(26%)
	IV	37	(74%)
Gleason scor	G6	2	(4 %)
	G7	17	(34%)
	G8	11	(22%)
	G9	13	(26%)
	G10	7	(14%)
Grading	Moderated	19	(38%)
	Poor	31	(62%)
Perineural invasion			
	Yes	11	(22%)
	N0	39	(78%)
Metastasis	M0	12	(24%)
	M1	37	(74%)
	Unknown	1	(2%)
Histopathological type			
	Ductal adenocarcinoma	1	(2 %)
	Mucinous adenocarcinoma	49	(98%)
Patient status	Alive	18	(36%)
	Dead	21	(42%)
	Unknown	11	(22%)

CHAPTER 4

4. Results

4.1.E-cadherin expression pattern in prostatic cancer

In the 50 primary prostate cancers, heterogeneous staining patterns were observed in malignant glands with alternating patterns of strong and weak or negative staining in adjacent glands and the expression of this marker was membranous in the primary prostate cancer cells. The staining patterns of E-cadherin in primary prostatic carcinoma are illustrated in figure 4.1A&B, 4.2, 4. 3 and 4.4A &B.

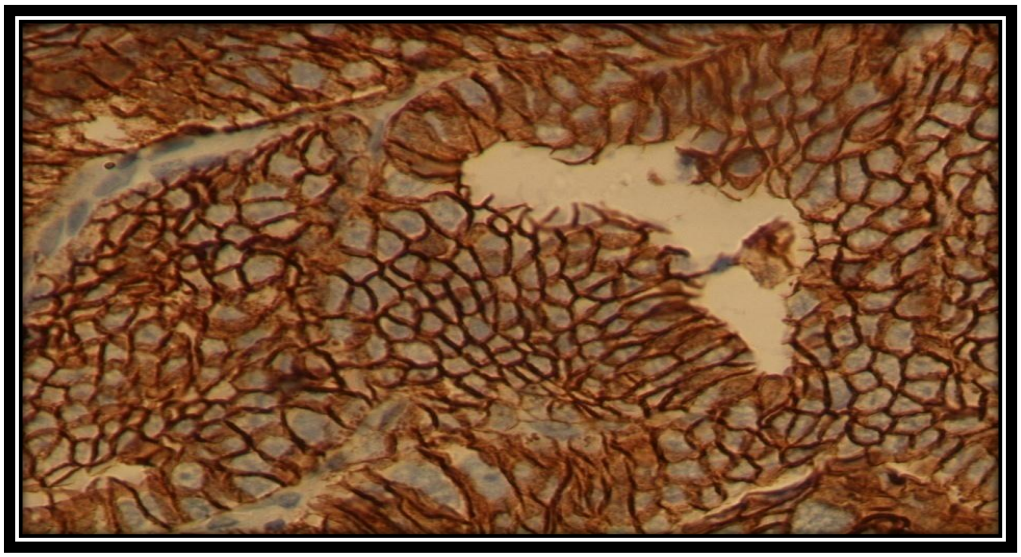

Figure 4.1. A Strong membranous E-cadherin expression in Pca cell(×40). Histopathology, Department. ,Benghazi University.

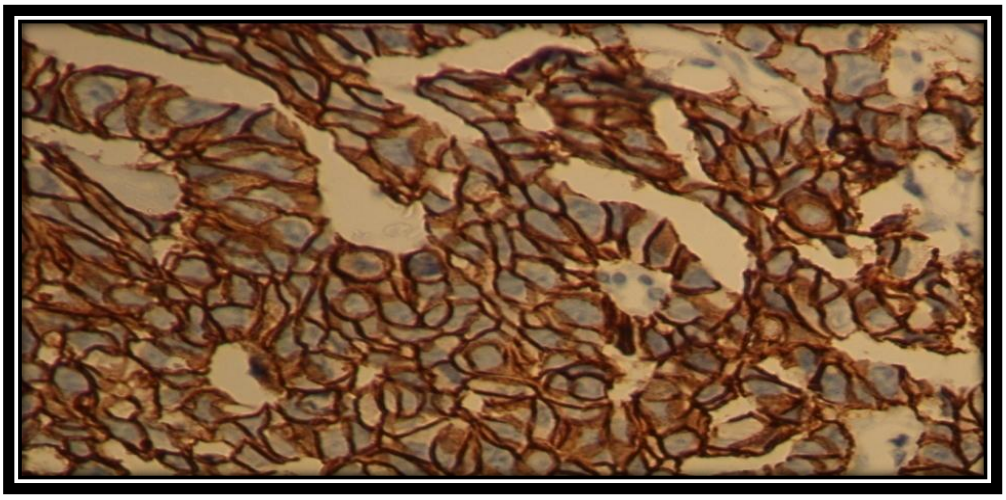

Figure 4.1. B Strong membranous E-cadherin expression in Pca cell(×40). Histopathology, Department. ,Benghazi University.

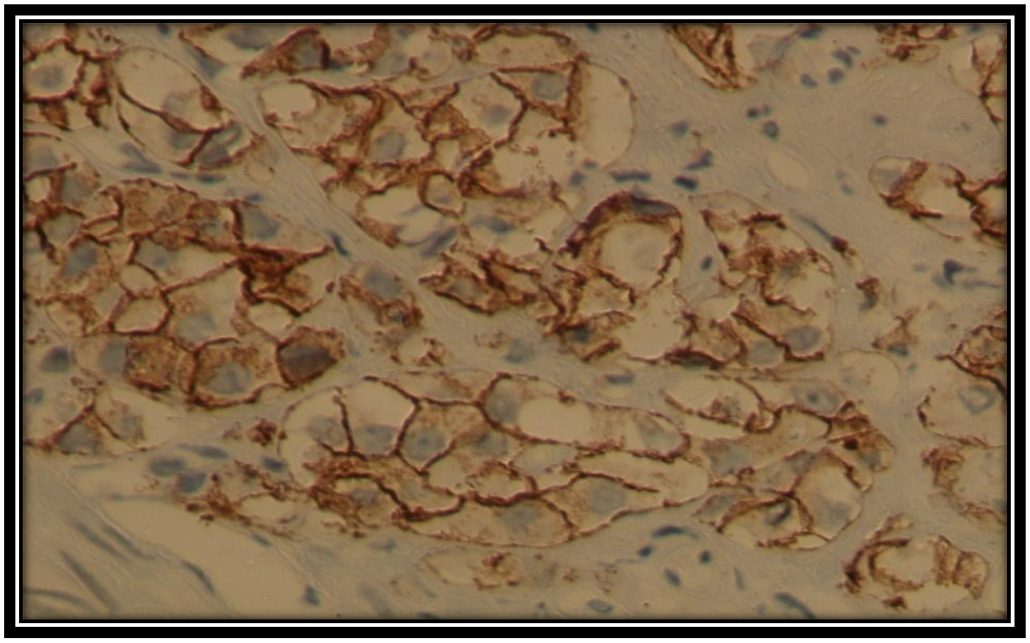

Figure 4.2. Moderate membranous E-cadherin expression in Pca cell(×40). Histopathology, Department. ,Benghazi University.

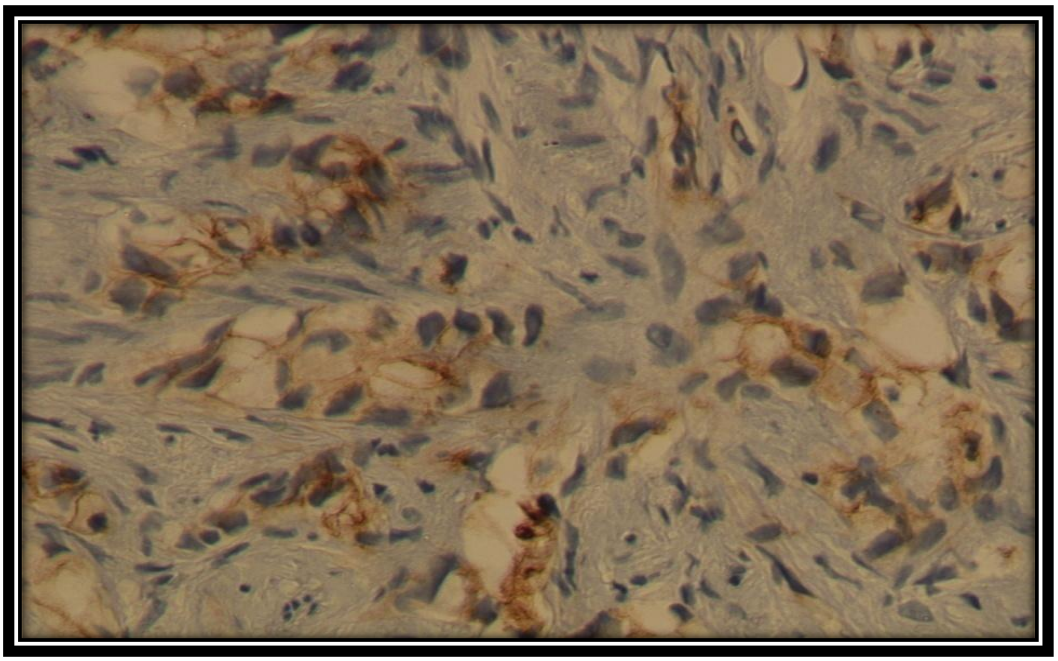

Figure 4.3. Weak membranous E-cadherin expression in Pca cell(×40). Histopathology, Department. ,Benghazi University.

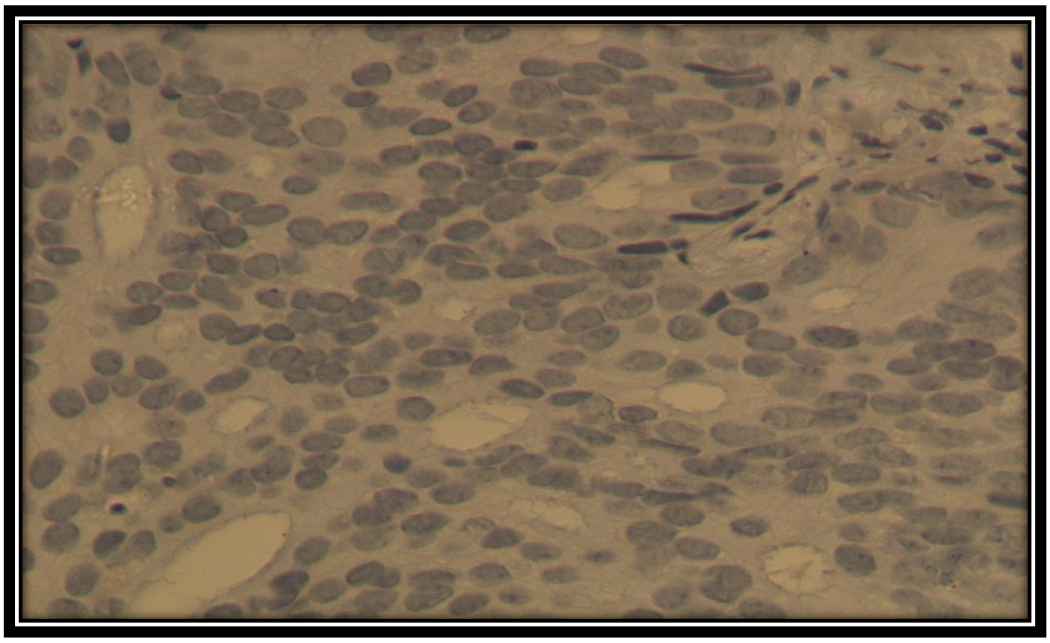

Figure 4.4.A Negative E-cadherin expression in Pca(×40). Histopathology, Department. ,Benghazi University.

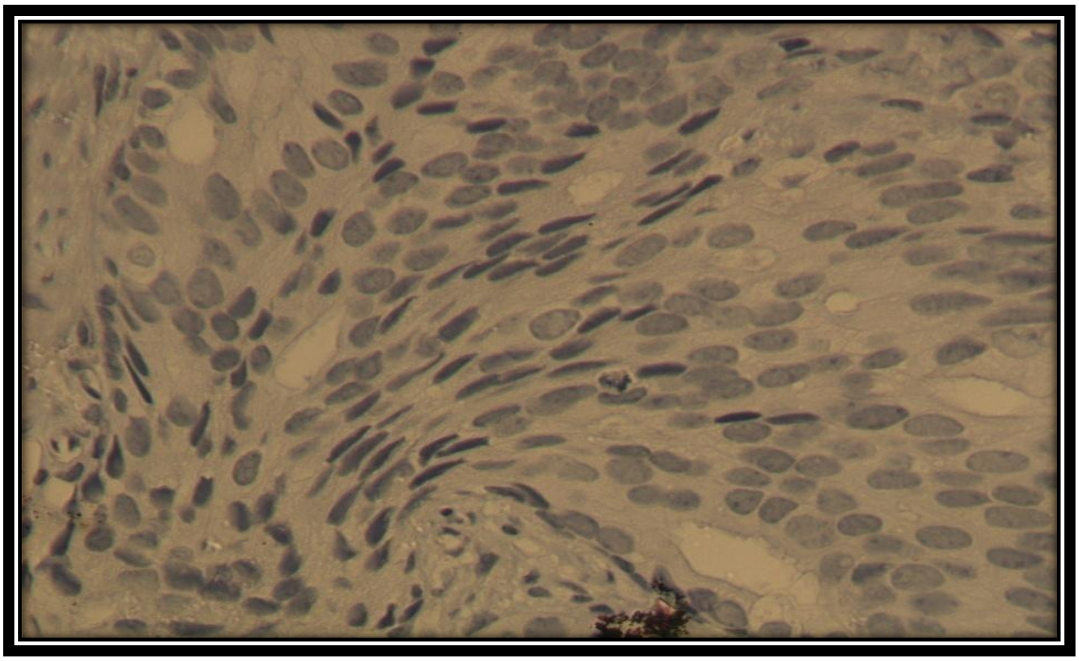

Figure 4.4. B Negative E-cadherin expression in Pca(×40). Histopathology, Department. ,Benghazi University.

4.2. Correlation of E-cadherin expression with clinicopathological features:

The distribution of E-cadherin in tumor samples in relation to clinicopathological features is presented in table 4.2.1, using different cut points (mean, 0,1,2,3, 2- teir score (0, 1Vs 2, 3) and (0 Vs 1, 2, 3).

Table 4.2.1. Expression of membranous E-cadherin in Libyan Pca patients as related to clinicopathological data.

Clinicopathological Features		Mean of E-cadherin Expression		P-Value	E-cadherin final index score				p-Value
		< mean	>mean		0	1	2	3	
Age	**≤ 73**	10(45.5%)	11(39.3%)	0.661	4(30.8%)	6(85.7%)	4(23.5%)	7(53.8%)	**0.026**
	>73	12(54.5%)	17(60.7%)		19(69.2%)	1 (14.3%)	13(76.5 %)	6(46.2%)	
Stage	**I**	3(13.6%)	1(3.7%)	0.431	2(15.4%)	1(14.3%)	0(0%)	1(7.7%)	0.509
	II	3(13.6%)	5(18.5%)		3(23.1%)	0(0%)	2(12.5%)	3(23.1%)	
	IV	16(72.2%)	21(77.8%)		8(61.5%)	6(85.7%)	14(87.5 %)	9(69.2%)	
Gleason score	**2= intermediate**	11(52.4%)	8(28.6%)	0.139	9(75%)	2(28.6%)	4(23.5%)	4(30.8%)	**0.03**
	3= high	10(47.6%)	20(71.4%)		3(25%)	5(71.4%)	13(76.5 %)	9(69.2%)	
Grade	**2= moderated**	10(52.6%)	9(32.1%)	0.16	8(80%)	2(28.6%)	4(23.5%)	5(38.5%)	**0.03**
	3= poor	9(47.4%)	19(67.9%)		2(20%)	5(71.4)	13(76.5 %)	8(61.5%)	
Perineural invasion	**0= N0**	13(68.4%)	22(81.5%)	0.307	8(72.7%)	4(66.7%)	14(87.5 %)	9(69.2%)	0.606
	1= yes	6(31.6%)	5(18.5%)		3(27.3%)	2(33.3%)	2(12.5%)	4(30.8%)	

Cont……. Significant and border- line results are in **BOLD**

Clinicopathological Features		E-cadherin expression score 0,1 vs 2,3		p-Value	E-cadherin expression score 0 vs 1, 2,3		p-Value
		0,1	2,3		0	1,2,3	
Age	**≤ 73**	10(50%)	11(36.7%)	0.349	4(30.8%)	17(45.9%)	0.34
	>73	10(50%)	19(63.3%)		9(69.2%)	9(54.1%)	
Stage	**I**	3(15%)	1(3.4%)	0.349	2(15.4%)	2(5.6%)	0.353
	II	3(15%)	5(17.2%)		3(23.1%)	5(13.9%)	
	IV	14(70%)	23(79.3%)		8(61.5%)	29(80.6%)	
Gleason score	**2= intermediate**	11(57.9%)	8(26.7%)	**0.029**	9(75%)	10(27%)	**0.003**
	3= high	8(42.1%)	22(73.3%)		3(25%)	27(73%)	
Grade	**2= moderated**	10(58.8)	9(30%)	**0.053**	8(80%)	11(29.7%)	**0.004**
	3= poor	7(41.2%)	21(70%)		2(20%)	26(70.3%)	
Perineural invation	**0= N0**	12(70%)	23(79.3%)	0.503	8(72.7%)	27(77.1%)	0.765
	1= yes	5(29.4%)	6(20.6%)		3(27.3%)	8(22.9%)	

CHAPTER 5

5. DISCUSSION

Cancer of the prostate (Pca) is currently the second most common cause of cancer death in men. In developed countries Pca accounts for 15% of male cancers compared with 4% of male cancers in developing countries (Heidenreich et al., 2011).

Progressive elucidation of the molecular mechanisms contributing to prostate cancer cell growth, survival, and metastasis may lead to better treatments for established prostate cancer (Angelo et al., 2004).

Progressive accumulation of somatic mutations in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways through which signals traffic via molecular networks. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells (Nives & slaus, 2003).

Malignant carcinoma cells are characterized in general by poor intercellular adhesion, loss of the differentiated epithelial morphology and increased cellular motility. Down regulation or a complete shutdown of E-cadherin expression, mutation of the E-cadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are observed in carcinoma cells. In human tumors, the loss of E-cadherin-mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the carcinoma cells (Rietmacher, et al., 1995). Thus, atumor invasion/suppressor role has been assigned to this gene (Efstathiou et al., 1999).

Immunohistochemical studies have suggested that E-cadherin may be a useful prognostic marker in prostate cancer (Liang et al., 1996). The study try to evaluate E-cadherin as a potential biomarker of disease progression in patients with clinically organ-confined prostate cancer who undergo radical prostatectomy (Buhmeida et al., 2006).

To determine the potential prognostic significance of the findings, prostate cancer specimens from 50 patients were evaluated immunohistochemically using specific antibodies raised against E-cadherin. The results were related to age, Gleason score, histological grade, tumour stage, and perineural invasion.

The current study illisterated some clinicopathological characterstics of Pca patients and revealed that the mean age of the patients was 73 years, about 24 (48%) of them were less than or equal to 73 year and 26 (52%) of them was older than 73 years (table 3.1).

Regarding TNM staging of Pca, it is found that majorty of patients 37 (74%) had tumor at stage IV while others, 13 (26%) have had tumor at stage II, III (Table 3.1).

For Gleason scoring system of the tumor, the study find out that 2 (4%) of patients had Gleason score 6, 17 (34%) for G7 which consider moderately diffrentiated, and 11(22%) forG8, 13 (26%) for G9 and 10(7%) for G10 which considered as poorly differentiated, that is mean more than two third of patients tumors were poorly differentiated ,this in agreement with results reported by De Marzo et al., 1999 ,Rhodes etal., 2003 and Bohmeida etal., 2006 (Table 3.1).

This result infleuences the grades of Pca in the patients where more than 60% showed poor diffrentiated tumor 31 (62%) compare to only 19 (38%) moderately differentiated (Table 3.1).

This work ellicetaed that most of Pca patients 39 (78%) had no perineural invation while only in 11(22%) of them, the invation was present (Table 3.1).

Regarding distant metastasis of the Pca, the study revealed that 37(74%) presented with distant metastasis M1 in compareson to 12(24%) did not present with metastasis M0.Out of 50 patients, data about metastasis was missed or not available in 1 (2%) of them (Table 3.1).

Histopathologically, only 1(2%) of Pca was fonud to be of ductal carcinoma type but the vast majority of the tumor was mucinous adenocarcinoma type 49 (98%) (Table 3.1).

Lastly, about the status of the pteints, 21(42%) of them were died and 18(36%) were alive and unfortionatly no data were available of 11(22%) (Table 3.1).

The staining patterns of E-cadherin in primary prostatic carcinoma of this sample was worked out in Histopathology Department and illustrated in figures 4.1A&B, 4.2, 4. 3 and 4.4A &B where a heterogeneous staining patterns were observed in malignant glands with alternating patterns of strong and weak or negative staining in adjacent glands and the expression of this marker was membranous in the primary prostate cancer cells.

In the present study, the distribution of the expression of membranous E-cadherin in tumor samples in relation to clinicopathological features using different cut points (mean, 0,1,2,3, 2- teir score (0, 1Vs 2, 3) and (0 Vs 1, 2, 3) (table 4.2.1).

Mean E-cadherin expression (1.27) was determined for each of the clinically localized prostate cancer cases. Membranous staining was recorded as low (aberrant) or high (normal). Aberrant E-cadherin expression showed a statistical trend toward an association with age of patient at time of diagnosis (p =0.026), higher Gleason score (P =0.003), and grade (P =0.004).

In the current study there is significant statistical correlation between membranous E-cadherin expression and the age of patients were (P= 0.026). However study done by (Elzagheid et al., 2012), who reported that the expression of E-cadherin in colorectal cancer was associated with age at diagnosis.

The present study revealed that the expression of membranous E-cadherin was significantly associated with age at diagnosis in that tumors of yonger patients $\leq$ 73 years than tumours of the older patients >73years (P = 0.026).

E-cadherin expression were also found to be significantly associated with higher Gleason score (P =0.03, 0.029 and 0.003), that is mean the tumors with higher Gleason score express E-cadherin more.

In addition, E-cadherin expression was also showed significant correlation with higher tumor grades (p= 0.03, 0.05and 0.004).

According to this results membranous E-cadherin expression was correlated with Gleason score and tumor grade were (P= 0.003, P= 0.004) respectively(table 4.2.1) and these results are in agreement with those of (Ozekinci et al., 2010; Kazuhiro et al., 2011) as well as other studies which reported that aberrant E-cadherin staining correlated with loss of differenation (Gleason score) by (umbas et al., 1992, 1994; Richmond et al., 1997; De marzo et al., 1999; Rubin et al., 2001; Kallakury et al., 2001; Jaggi et al., 2005; Algaba et al., 2006; Musial et al., 2007).

However Köksal reported that aberrant staining of E-cadherin not correlated with Gleason score (Köksal et al., 2002). Ray et al., 2006 also demonstrated that E-cadherin staining was not associated with Gleason score.

The discrepancy between this results and other may be attributed to significant differences in the methodologies employed for samples selection, immunostaining techniques, statistical analysis, and the number of studied patients may play arole, also E-cadherin evaluation under the microscopic is subjective and interobserver error may be happen.

Moreover, No statistically significant associations was found between E-cadherin expression in tumors and TNM staging of the tumor and Perineural invasion ($p=0.349$ and 0.307) respectively (table 4.2.1).

It is observed that no significant statistical association between membranous E-cadherin expression and tumor stage ($p>0.349$) and this result is in agreement with Jaggi who reported that E-cadherin membranous expression were not significantly associated with final pathological stage where P value more than 0.05 (Jaggi et al., 2005). In other hand, the results of this study is not in agreement with the study done by (Bhaskar et al., 2001; Kallakury et al., 2001), who reported that membranous E-cadherin expression correlated with pathological stage.

It is also reported that, there is no correlation between membranous E-cadherin expression and perineural invasion, ($p >0.307$) (table 4.2.1).

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

- Prostatic carcinoma constitutes a significant proportion of the global burden of cancer morbidity and mortality.

- Wide variation exists internationally for prostate cancer rates due to differences in detection practices, treatment, and lifestyle and genetic factor

- Prostatic carcinoma is cancer of old age.

- Astrong correlation has been confirmed between E-cadherin expression and age, Gleason score, tumor grade.

- Results suggest that E-cadherin expression play a significant role in the prognosis of prostatic carcinoma in Libyan patients as used in combination with other prognostic markers.

- Further research with large number of patients, including follow up and survival follow up are need to hellp in the choice of the better therpy for individual patient.

CHAPTER 7

7. REFERRENCES

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CHAPTER 8

8. ARABIC SUMMARY

الملخص العربي:

البروستات هي عباره عن غدة بحجم وشكل الجوزة تقريبا ومكان غدة البروستات هو مقابل المستقيم تحت المثانه ، وهي تحيط بالحالبين ويقوم الحالب بنقل البول من المثانه إلى الخارج عبر القضيب وهناك أيضا زوج من الغدد تسمى الحويصلات المنويه (Seminal vesicles) محشوره بين المستقيم و المثانه ومربوطه بالبروستات ا وتقوم الحويصلات المنويه بتوفير المواد المغذيه للنطاف.

سرطان البروستاتا:

يأتي سرطان البروستاتا في المرتبة الثانية في أكثر أنواع السرطانات شيوع اً بين الرجال في الولايات المتحدة الأمريكية وفي واقع الأمر، فإن رجلاً من بين كل ستة رجال يتم تشخيص إصابته بسرطان البروستاتا كما أن الرجال الذين يخضعون لعوامل خطر محددة هم أكثر عرضة من غيرهم للإصابة بالمرض.

تشير البيانات المنشورة عالمياً إلى أن التشخيص المبكر والعلاج الدقيق هما من أكثر العوامل أهمية في علاج سرطان البروستاتا حيث تزيد نسبة التغلب على المرض والشفاء منه على 90%

سرطان البروستاتا وعوامل الخطر:

العمر: يمثل العمر عامل الخطر الرئيسي في سرطان البروستاتا، فالإصابة بالمرض نادرة في الرجال تحت سن الخامسة الثلاثين وتزداد فرصة الإصابة بسرطان البروستاتا بشدة كلما تقدم العمر ومعظم الرجال المصابين بسرطان البروستاتا في هذا البحث هم فوق73 سنة

التاريخ العائلي: يزداد خطر إصابة الرجل بسرطان البروستاتا إذا كان والده أو أخوه مصاباً بالمرض

العِرق: سرطان البروستاتا أكثر شيوعاً في الأمريكيين من أصل أفريقي عن الرجال البيض، بمن فيهم البيض من أصل أسباني كما أنه أقل شيوع اً في الأمريكيين من أصل أسيوي والأمريكيين الأصليين.

تغيرات معينه في البروستات ا : الرجال الذين توجد لديهم خلايا تسمى التنشؤات العنقيه بالخلايا الداخل ظهاريه بالبروستات ا مرتفعة الدرجه ربما يزيد لديهم خطر الإصابه بسرطان البروستاتا وتبدو هذه الخلايا بداخل البروستاتا غير طبيعيه تحت المجهر .

النظام الغذائي: تشير بعض الدراسات إلى أن الرجال الذين يعتمدون في نظامهم الغذائي على أطعمة غنية بالدهن الحيواني أو اللحوم يزيد لديهم خطر الإصابة بسرطان البروستاتا و الرجال الذين يعتمدون على الأنظمة الغذائية الغنية بالفواكه والخضروات يقل لديهم خطر الإصابة بالمرض.

أعراض سرطان البروستاتا:

أ . تكرار التبول، خاصة أثناء الليل

ب . ضعف إنسياب البول

ج . إنسياب البول الذي يبدأ ثم يتوقف أو الصعوبة في البداية والتوقف

د . ألم أو حرقة أثناء التبول

ه . دم في البول والسائل المنوي

و . ألم في أسفل الظهر والوركين والفخذين

ز . صعوبة في الوصول للقذف

الوقايه والكشف عن سرطان البروستات :

يتعين على كل رجل فوق سن 40 سنة أن يجري فحص جس المستقيم بالأصبع (DRE) كجزء من فحصه الطبي السنوي المنتظم إضافه الى ذلك تنصح الجمعيه الأمريكيه للسرطان كل رجل فوق سن الخمسين أن يجري تحليل المستضد النوعي البروستاتي (PSA) في الدم و PSA هو بروتين يتم تصنيعه في خلايا غدة البروستات ويتم في هذا التحليل قياس مستوى PSA في الدم حيث تسحب عينة دم ثم تقاس كمية البروتين PSA في المختبر وعندما تتضخم غدة البروستات تميل مستوياتPSA في الدم إلى الإرتفاع ويمكن أن ترتفع إما بسبب السرطان أو بسبب أمراض غير سرطانيه أخرى وفي حال وجود شكوك في النتائج يجب إجراء فحوصات إضافيه.

خزعة البروستاتا: تعتبر الخزعه عبر المستقيم والموجهه بالأمواج فوق الصوتيه هي الطريقه المعياريه لكشف سرطان البروستاتا وهذه الخطوه تحتاج إلى تخذير موضعي وهذا النموذج يحافظ على البنيه الغديه ويؤمن الدقه في التصنيف حسب معيار جليسون .

التصوير بالأمواج فوق الصوتيه عبر المستقيم (TRUS): يستخدم بشكل كبير لتصنيف مراحل سرطان البروستاتا والتي تظهر نموذجيا على شكل مناطق ناقصة الصدى بالإضافه إلى أن

الخزعه الموجهه بالأمواج فوق الصوتيه تعتبر الطريقه الأدق لتقييم الآفات المشكوك بها من تلك الموجهه بالأصبع .

مراحل سرطان البروستاتا:

يستخدم لوصف مدى أنتشار السرطان ولتحديد العلاج

المرحله الأولى : سرطان صغير ومازال محصور فى البروستاتا

المرحله الثانيه : سرطان أكثر تقدما ولكنه محصور فى البروستاتا

المرحله الثالثه : إنتشار السرطان إلى الحويصلات المنويه خارج البروستاتا

المرحله الرابعه: إنتشار السرطان إلى الغدد الليمفاويه والمثانه والمستقيم والعظام والرئتين.

ملخص البحث:

دراسة العوامل السريرية و المرضية المختلفة التي تتعلق بالإصابة بسرطان البروستات ا و مدى أهميتها كعوامل تنبؤية وتأثيرها على علاج المرضى ومدى إمتثالهم للشفاء منه.

هذه الدراسة تعتمد على دراسة مدى قدرة الأنسجة الورمية على أخذ الصبغة المناعية E-CAHDRIN، و علاقة هذه الصبغه بالعوامل التنبؤية التقليدية.

المرضى و طرق الدراسة:

تعتمد هذه الدراسة على عينات شمعية من سرطان البروستاتا مأخوذة مسبقا من 50 مريض ليبي بالمنطقة الشرقية و موجودة بقسم علم الأمراض بجامعة بنغازي و التي تم تشخيصها في الفترة من سنة 2005 وحتى 2012 و متابعة ملفاتهم الطبية بقسم الأورام بمركز بنغازي الطبي .
جهزت هذه العينات على شرائح خاصة وتم صبغها (بصبغة مناعية خاصة E-CADHERIN) ، ومن ثم تقييم مدى إستجابة النسيج للصبغة و إختبار النتائج إحصائياً.

نتائج البحث:

يشكل سرطان البروستاتا جزء مهم وكبير من وفيات السرطان العالمية ويوجد مدى واسع لمعدلات المرض عالمياً بسبب الإختلاف في طرق الإكتشاف والعلاج ونمط الحياة والعوامل الجينية الوراثية ،هذا ويرتبط سرطان البروستاتا غالباً بالفئات العمرية الكبيرة من الرجال .

وقد بينت نتائج هذة الدراسة إرتباطاً إحصائياً قوياً بين الصبغة المناعية الخاصة

E-CAHDRIN و معيار جليسون ودرجة الورم وعليه فإن E-CADHERIN يمكن إعتباره عامل مهم ومساعد للتنبؤ في مجال سرطان البروستات ا لدى المرضى الليبين بالإظافة للمؤشرات التنبؤية الأخرى.

ولهذا يقترح البحث إجراء المزيد من مثل هذة البحوث على عينات أكبر عدداً مشتملة على متابعات المرضى ومدى تطور الحالة لديهم مستقبلا ليساعد ذلك في إختيار أفظل سبل العلاج لكل مريض.