Significance of S100A4 Expression In Prostate Adenocarcinoma.

Thesis

Submitted for fulfillment of Master degree in Pathology

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<td>AgNOR</td>
<td>Nucleolar organiser regions</td>
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<td>AMACR</td>
<td>Alpha-methylacyl-CoA racemase</td>
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<td>APC</td>
<td>Adenomatous polyposis coli</td>
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<td>AR</td>
<td>Androgen receptor</td>
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<td>ASAP</td>
<td>Atypical small acinar proliferation</td>
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<td>BCL2</td>
<td>B-cell lymphoma 2</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BNIP3</td>
<td>Protein-interacting protein 3</td>
</tr>
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<td>BPH</td>
<td>Benign prostatic hyperplasia</td>
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<td>BRCA2</td>
<td>Breast cancer 2</td>
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<td>CAP</td>
<td>College of American Pathologists</td>
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<td>DHT</td>
<td>Dihydrotestosterone</td>
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<tr>
<td>EDCs</td>
<td>Endocrine disrupting chemicals</td>
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<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
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<tr>
<td>EMA</td>
<td>Epithelial membrane antigen</td>
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<td>EMT</td>
<td>Epithelial-mesenchymal transition</td>
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<td>EphA2</td>
<td>Ephrin type-A receptor 2</td>
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<td>ERBB2</td>
<td>Receptor tyrosine-protein kinase erbB-2</td>
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<td>GSTP1</td>
<td>Glutathione S-transferase</td>
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<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
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<td>HPC1</td>
<td>Hereditary prostate cancer</td>
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<td>Insulin like growth factor-1</td>
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<td>Acronym</td>
<td>Description</td>
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<td>IRAC</td>
<td>International Agency for Research on Cancer</td>
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<td>MAP Kinase</td>
<td>Mitogen-activated protein kinase</td>
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<td>Mouse double minute 2</td>
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<td>miRNAs</td>
<td>MicroRNAs</td>
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<td>MutL homolog 1</td>
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<td>MMPs</td>
<td>Matrix metalloproteinases</td>
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<td>MRI</td>
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<td>MSH2</td>
<td>Mismatch repair gene 2</td>
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<td>NF-κB</td>
<td>Nuclear factor-κB</td>
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<td>NMIIA</td>
<td>Nonmuscle Myosin IIA</td>
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<td>Tumor protein p53</td>
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<td>Prostatic acid phosphatase</td>
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<td>Prostate cancer antigen 3</td>
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<td>PCR</td>
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<td>PI-3K</td>
<td>Phosphatidylinositol 3-kinase</td>
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<td>PIN</td>
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<td>PSA</td>
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<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
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<td>RAGE</td>
<td>Receptor for advanced glycation end products</td>
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<td>RAS</td>
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<td>RB</td>
<td>Retinoblastoma gene</td>
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<td>2,5- oligoisoadenylate synthetase dependent ribonuclease L gene</td>
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<td>Statistical Package for the Social Sciences</td>
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<td>TMPRSS2</td>
<td>Transmembrane protease, serine 2</td>
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<td>TUR</td>
<td>Trans-urethral resection</td>
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Introduction

Worldwide, prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008 (Bock et al., 2009).

In Egypt, according to National Cancer Institute, Cairo University, it accounts for 8.5% of total malignancies being the fifth most common male cancer after urinary cancer, liver cancer, Non-Hodgkin lymphoma and lung cancer (Mokhtar et al., 2007). However, according to El Gharbia population based cancer registry, prostate cancer is uncommon. It represents 2% of all incident cancers, ranking seventh in males and the majority of cases (79%) present with advanced disease. (Seif Eldein et al., 2007).

Complete understanding of the causes of prostate cancer remains elusive (Hsing et al., 2006). The primary risk factors are obesity, age and family history. It is very uncommon in men younger than 45, but becomes more common with advancing age. The average age at the time of diagnosis is 70 (Hankey et al., 1999). Men who have first-degree family members with prostate cancer appear to have doubled the risk of getting the disease compared to men without prostate cancer in the family (Zeegers et al., 2003).

Being the most frequently diagnosed cancer in males, prostate cancer is a major health problem. Despite the fact that its mortality rate has been decreasing by about 4% per year since 1992, this cancer still kills 30,000 men annually in the US alone (Barry et al., 2009).
Prostate cancer presents in two distinct forms. One is a latent form and the second is an aggressive form. The disadvantages of the frequently used prostate-specific antigen (PSA) serum test are that this test detects indiscriminately both types of prostate cancer and that PSA can also be increased in nonmalignant prostatic diseases, such as benign prostatic hyperplasia and prostatitis leading to overdiagnosis (of latent forms) followed by overtreatment, with the associated side effects. Therefore, markers that can differentiate aggressive from nonaggressive disease are urgently needed (Andriole et al., 2009).

S100 proteins are involved in a variety of intracellular and extracellular functions including cell growth, cell-to-cell communication, energy metabolism and intracellular signal transduction (Heizmann et al., 2002 and Donato et al., 2001).

S100A4, a member of the S100 protein family, is overexpressed frequently in normal cells such as macrophages, neutrophils, and T-lymphocytes (Grigorian et al., 1993). It is also overexpressed in various cancer types such as those of the breast, ovary, and colon and is mainly associated with the invasion and metastasis of malignant tumors (Taylor et al., 2002; Garrett et al., 2006 and Sherbet et al., 2009).
AIM OF THE WORK

This study aims at:

1- Evaluation of the expression of S100A4 in benign prostatic hyperplasia and prostate adenocarcinoma cases.

2- Comparing the results with the clinicopathological data trying to assess the role of S100A4 in prostate carcinoma.
**Histoanatomy of the Prostate**

Adult prostate weighs approximately 20 gm. The prostate is a retroperitoneal organ encircling the neck of the bladder and urethra, and is devoid of a distinct capsule. In the adult, prostatic parenchyma can be divided into four biologically and anatomically distinct zones or regions: the peripheral, central, and transitional zones, and the region of the anterior fibromuscular stroma. The types of proliferative lesions are different in each region. For example, most hyperplasias arise in the transitional zone, whereas most carcinomas originate in the peripheral zone (Walz et al., 2011).

**FIGURE (1):** Adult prostate. The normal prostate contains several distinct regions, including a central zone (CZ), a peripheral zone (PZ), a transitional zone (TZ), and a periurethral zone. Most carcinomas arise from the peripheral glands of the organ and may be palpable during digital examination of the rectum. Nodular hyperplasia, in contrast, arises from more centrally situated glands and is more likely to produce urinary obstruction early than is carcinoma.
Histologically the prostate is composed of glands lined by two layers of cells: a basal layer of low cuboidal epithelium covered by a layer of columnar secretory cells. In many areas there are small papillary infoldings of the epithelium. These glands are separated by abundant fibromuscular stroma. Testicular androgens control the growth and survival of prostatic cells. Castration leads to atrophy of the prostate caused by widespread apoptosis (Fine et al., 2012).

FIGURE (2): Benign prostate gland with basal cell and secretory cell layer
Prostate cancer

Prostate cancer is the most common form of cancer in men, accounting for 29% of cancer in the United States in 2007 (Roehrborn et al., 2007). However, prostate cancer is tied with colorectal cancer in terms of cancer mortality, causing 9% of cancer deaths in the United States in 2007. There is a one in six-lifetime probability of being diagnosed with prostate cancer. Over the last 20 years, there has been a significant drop in prostate cancer mortality. It is one of the most remarkable tumors, exhibiting a wide range of clinical behaviors from very aggressive lethal cancers to incidentally discovered clinically insignificant cancers (Rosai et al., 2011).

Epidemiology

World wide, Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008 (Baade et al., 2009). Incidence rates vary by more than 25-fold worldwide, with the highest rates recorded primarily in the developed countries of Oceania, Europe, and North America, largely because of the wide utilization of prostate-specific antigen (PSA) testing in clinically detectable tumors as well as other slow-growing cancers that might otherwise escape diagnosis. In contrast, males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world, which is thought to reflect partly difference in genetic susceptibility (Bock et al., 2009).
Prostate cancer accounts for approximately 30% of cancers diagnosed each year in Australian men (Australian Institute of Health and Welfare, 2012). It is the second most common cause of cancer death, after lung cancer (Australian Institute of Health and Welfare, 2012). It has been estimated that around 120,000 Australian men are living with prostate cancer, and it has been predicted that the number will increase to 267,000 by 2017 (Smith et al., 2012).
In many of the countries of the Middle East, prostate cancer is already a problem and in Mauritanian males, it is the most frequent neoplasm (Kamel et al., 2006).

Arab Kuwaiti and Omani men were reported to have lower serum PSA levels and prostate volumes than those reported for Caucasians, but similar to those reported for Asians (Japanese and Chinese) (Kehinde et al., 2005).

In Egypt, according to National Cancer Institute, Cairo University, it accounts for 8.5% of total malignancies being the fifth most common male cancer after urinary cancer, liver cancer, Non-Hodgkin lymphoma and lung cancer (Mokhtar et al., 2007). However, according to El Gharbia population based cancer registry, prostate cancer is uncommon. It represents 2% of all incident cancers, ranking seventh in males and the majority of cases (79%) present with advanced disease (Seif Eldein et al., 2007).
Risk factors

Risk factors can be classified as endogenous or exogenous; however, some factors may reflect both endogenous and exogenous influences.

❖ **Endogenous Risk Factors**

Endogenous risk factors for prostate cancer include the following:

- **Age**

  Prostate cancer is a disease associated with aging. In the United States, 70% of all cases of prostate cancer have been diagnosed in men under the age of 65 years (American Cancer Society. 2003). It is relatively rare for prostate cancer to be diagnosed in men under the age of 50 years, but after this age, the incidence and mortality rates increase exponentially (Haas et al., 2003). The probability of developing prostate cancer increases from 0.005% among individuals aged below 39 years to 2.2% (1 in 45) for those aged 40 to 59 years and 13.7% (1 in 7) for those aged 60 to 79 years (American Cancer Society., 2003).

  **Carter et al., (2001)** showed that 20% of men aged 50 to 60 years and 50% of those aged 70 to 80 years had histologic evidence of malignancy. It has been estimated that a 50-year-old man has a lifetime risk of 42% for developing histologic evidence of prostate cancer, a 9.5% risk of developing clinical disease, and a 2.9% risk of dying of prostate cancer (Scher et al., 2000, Horner et al., 2009).

- **Ethnicity**

  Black men have a higher risk of prostate cancer than white men, while men of Asian and Chinese ethnicity have a lower risk than white
men (National Cancer Intelligence Network and Cancer Research UK, 2009). There are similar differences by major ethnic group in the United States (Howlader et al., 2012).

A cohort study shows that the risk increase associated with black ethnicity is higher at younger ages and that black men may be diagnosed on average three–five years younger than white men, although firm conclusions about differences in the average age at diagnosis between ethnic groups are hampered by differences in the underlying population age distribution within these groups (Ben-Shlomo et al., 2008, Metcalfe et al., 2008).

- **Family history and genetic susceptibility**

  The risk of developing prostate cancer doubles for men who have a father or brother affected by prostate cancer, and risk increases further when multiple first-degree relatives are affected (Kalish et al., 2000) and (Bratt, 2002). Epidemiologic studies indicate that men with a positive family history are diagnosed at an earlier age—on average 6 to 7 years earlier—than those without affected first-degree relatives. These studies estimate that 5% to 10% of all prostate cancer cases and up to 40% of those occurring at the age below 55 years may have a hereditary basis (Gronberg, 2003, Chen et al., 2008). Other than being diagnosed at an earlier age, hereditary prostate cancer does not differ clinically from disease that arises sporadically. The familial clustering of prostate cancer may be caused by inheritance of a susceptibility gene, but it may also be caused by exposure to common environmental factors or simply from chance alone because of the high incidence of this malignancy (Kicinski et al., 2011).

  There have been seven susceptibility loci for prostate cancer identified, but demonstrating linkage of currently known candidate genes
has proved to be problematic (Simard et al., 2002). Using linkage analysis based on a genome-wide search, Smith et al., (1996) first mapped prostate cancer susceptibility to the hereditary prostate cancer \textit{HPC1} locus on the long arm of chromosome 1 in high-risk families from Sweden and the United States. In these families, prostate cancer developed at an early age, affected less than five family members, and spanned 2 generations (Xu et al., 2000). A subsequent pooled meta-analysis of 772 families with hereditary prostate cancer showed weak evidence of a genetic linkage to \textit{HPC1} in only 6% of the families (Cotter et al., 2002). However, strong evidence of linkage was found in a subset of 8 families; in 2 of these families, a gene within the \textit{HPC1} locus (the 2,5-oligoisoadenylate synthetase dependent ribonuclease L (\textit{RNASEL}) gene) showed deleterious germline mutations (Carpten et al., 2002, Eeles et al., 2008). The \textit{RNASEL} gene is believed to be a tumor suppressor gene that regulates cellular proliferation and apoptosis. Although \textit{RNASEL} may represent a prostate cancer susceptibility gene, mutations of this gene will likely account for only a limited number of hereditary prostate cancer cases (Thomas et al., 2008).

- **Benign prostatic hyperplasia (BPH)**

Because of numerous similarities in pathophysiology, benign prostatic hyperplasia (BPH) has been investigated as a possible premalignant condition or precursor for prostate cancer. It has been suggested that BPH may predispose to cancer or that a common factor influences the development of both diseases. Studies to date, however, have been inconclusive (Kirby., 2000, Checkoway et al., 2006).

Benign prostatic hyperplasia and prostate cancer are often found concurrently, and the diagnosis of prostate cancer is frequently made
during the evaluation of obstructive symptoms associated with BPH. Both lesions are considered to be under similar androgenic hormone regulation (De Nunzio et al., 2011). Although BPH arises from the transition zone of the prostate and most prostate cancers originate in the peripheral zone, approximately 20% of cancers do develop from the transitional area. The frequency of incidental prostate cancer (stage A or TI) found at transurethral prostatectomy for BPH was as high as 20% to 25% in the pre-PSA era but has been declining as patients are evaluated for possible cancer before surgery (Buckley et al., 2011).

These findings led investigators to search for a link between BPH and prostate cancer. Men diagnosed with BPH have been reported to be more likely (by as much as fivefold) to develop prostate cancer than are age-matched controls (Checkoway et al., 2006). In contrast, another large scale study failed to identify any difference in prostate cancer incidence between the two groups (Kristal et al., 2007). Current efforts are directed toward establishing morphologic linkages between early lesions and clinical cancer and searching for common molecular markers of neoplastic transformation. Because both conditions are generally found in the same age groups, any increase of cancer detected in men who are symptomatic from BPH may be simply a reflection of the increased intensity of the evaluation (Buckley et al 2011).
Hormones

The prostate gland consists of stromal and epithelial elements under androgenic influence. Testosterone enters the prostate and is converted by the enzyme 5-alpha-reductase into dihydrotestosterone (DHT), the active metabolite influencing prostatic development. Other hormones, such as estrogen and prolactin, also may influence the growth of the prostate gland, either directly or indirectly by negative feedback inhibition (Dehm et al., 2007).

Eunuchs, prepubertal castrates, and men with congenital abnormalities of androgen metabolism do not develop either BPH or prostate malignancy. Surgical or chemical androgen blockade can cause involution of benign prostatic hyperplasia and carcinoma; such blockade has been a successful treatment modality for metastatic disease. All of this is compelling evidence for the role of hormones in the pathogenesis of prostate cancer (Tsai et al., 2006). Yet no consistent linkage has been established between steroid metabolism and development of prostate cancer (Muller et al., 2013.)

Several studies have measured plasma androgen levels among men with prostate cancer and age-matched controls. Some studies found elevated testosterone and DHT levels among those with cancer, whereas others either did not detect any differences or actually found lower testosterone levels (Harman et al., 2000, Williams et al., 2009). The incidence of prostate cancer is lower in men with cirrhosis of the liver, a condition associated with elevated levels of circulating estrogens and decreased testosterone (Roddam et al., 2008, Tobias et al., 2013).
• **Race**

Differences in prostate cancer risk by race may reflect three factors: differences in exposure, such as dietary differences (exogenous factors); differences in detection (reflecting exogenous factors); and genetic differences (endogenous factors). The highest incidence rates for prostate cancer in the world are among African-American men, who have a higher risk of prostate cancer than white American men (Ben-Shlomo et al., 2008).

However, racial differences may reflect differences in access to care (exogenous factors), differences in the decision-making process of whether to seek medical attention and follow-up, and differences in allelic frequencies of microsatellites at the androgen receptor (AR) locus or polymorphic variation (National Cancer Intelligence Network and Cancer Research UK, 2009).

❖ **Exogenous Risk Factors**

Exogenous risk factors for prostate cancer include the following.

• **Diet**

A wide variety of dietary factors have been implicated in the development of prostate cancer (Shirai et al., 2002).

Fat consumption, especially polyunsaturated fat, shows a strong, positive correlation with prostate cancer incidence and mortality, perhaps resulting from fat-induced alterations in hormonal profiles, the effect of fat metabolites as protein or DNA-reactive intermediates, or fat-induced elevation of oxidative stress (Harman et al., 2000, Crowe et al., 2008). High fat and caloric diets stimulate growth hormone and insulin
production and in turn IGF-1 production. This factor is known to regulate the proliferation and differentiation of cancer cells and to prevent them from undergoing apoptosis (Rowlands et al., 2009). In three prospective cohort studies, men in the highest quartile of IGF-1 concentrations had a 1.7- to 4.3-fold higher risk of prostate cancer than those in the lowest quartile (Roddam et al., 2008, Price et al., 2012).

Retinoids, including vitamin A, help to regulate epithelial cell differentiation and proliferation reducing prostate cancer risk (Mongan et al., 2007).

Vitamin D deficiency may be a risk factor for prostate cancer; the hormonal form, 1-25-dihydroxyvitamin D, inhibits invasiveness and has antiproliferative and antidifferentiative effects on prostate cancer. Vitamin E (α-tocopherol) is an antioxidant that inhibits prostate cancer cell growth through apoptosis, and daily intake decreased the risk of prostate cancer by 32% in a large, controlled, clinical trial from Finland (Lippman et al., 2009).

High calcium intake was also related to an increased prostate cancer risk in the Physicians’ Health Study (Chan et al., 2001).

Zinc (Zn) concentration is higher in the prostate than in any other organ in the body; although it is reduced > 90% in prostates with cancer; the relation of dietary zinc and prostate cancer risk is uncertain (Costello et al., 2005).

Selenium is an essential trace element that inhibits viral and chemical carcinogen-induced tumors in animals. A chemopreventative role for selenium is plausible, but the evidence in humans is limited (Ferrís-Tortajada et al., 2012).
Lycopene, an abundant constituent of tomato-based products and the most efficient carotenoid antioxidant, has a significant protective effect (Mariani et al., 2014).

- **Environmental agents**

One class of environmental agents that has received a lot of attention is the endocrine disrupting chemicals (EDCs). EDC is an environmental agent that positively or negatively alters hormone activity and ultimately leads to effects on reproduction, development, and/or carcinogenesis, particularly of reproductive organs (Euling et al., 2001). Exposure to EDCs can occur through ingestion of food or water or through inhalation (Calafat et al., 2007).

Studies have shown that certain pesticide residues on foods, chemicals used in plastics production, and phytoestrogens in dietary plant products (e.g., soy) behave as EDCs (Jenkins et al., 2001).

Individuals or groups with relatively high endogenous estrogen or androgen concentrations (serum or prostate tissue levels) may have a greater susceptibility to EDC exposure, because exposure to an EDC could add effectively to the endogenous activity (Maffini et al., 2006, Tang et al., 2011).

- **Occupation and heavy metal exposure**

Many industrial and occupational exposures have been studied in relation to prostate cancer risk, but the findings are inconclusive; of greatest concern is farming and, to a lesser extent, working in the rubber industry (Rushton et al., 2012).
The prostate gland contains the highest level of zinc of all the organs in the body. Zinc is an essential ingredient of proper enzyme function and is required for DNA and RNA replication and repair. Cancerous prostate glands contain less zinc than do normal glands (Julin et al., 2012). It has been postulated that zinc is somehow involved with retinoic acid metabolism and that diminished intraprostatic zinc levels may decrease the availability of retinol-binding protein. The cause and effect relationship of zinc levels and prostate cancer is yet to be confirmed (Costello et al., 2005, Julin et al., 2012).

Another trace element, cadmium, is an inhibitor of zinc metabolism. Prostate cancer mortality in workers with long-term exposure to high levels of cadmium has been disproportionately high, and cadmium workers often have clinically more aggressive tumors. Cadmium exposure may contribute to prostate cancer risk directly, or the risk may result from the effect of cadmium on zinc availability (Cogliano et al., 2011).

❖ Other factors:

- Socioeconomic status

Several studies failed to show an association between socioeconomic class and prostate cancer epidemiology or between educational level and cancer incidence (Giovannucci et al., 2007). However, existing data suggest that the incidence of different types of cancer, including that of the prostate, increases with decreasing socioeconomic status (Berglund et al., 2012).

Economic factors may influence access to health care, the type of care available, and even the attitudes and concern over health matters exhibited by the various populations (National Cancer Intelligence
Network and Cancer Research UK. 2009). Interestingly, American Indians, who generally have a lower standard of living, have significantly lower rates of prostate cancer compared with other ethnic groups living in the same areas with a shared economic base (Howlader et al., 2012).

- Cigarette smoking

Cigarette smoking has been well established as a cause of human malignancies such as lung cancer and has been associated with the development of many others, such as kidney and bladder cancer (Lee et al., 2002). Some of the studies link prostate cancer to known carcinogens in tobacco smoke; others consider the effect of tobacco on serum hormone fluctuations. The data are not convincing that cigarette consumption results in an increased risk of prostate cancer (Huncharek et al., 2010, Cogliano et al., 2011).

- Sexual activity and sexually transmitted infections

In different studies, the frequency of sexual activity has been found to have both a direct and an indirect relationship to the consequent development of prostate cancer (Dennis et al., 2002).

Some studies suggest that prostate cancer are related to early intercourse or sexual precocity, number of sexual partners, venereal disease and fertility as established by the number of children reported, and married versus unmarried life. By contrast, several studies found no change in the incidence of prostate cancer among Catholic priests and no association with the frequency of sexual activity, venereal disease, marital status, or number of children; some even found a decreased incidence of venereal disease among prostate cancer patients (Huang et al., 2008).
Bacterial prostatitis, particularly a history of gonorrhea, has been suggested as an increased risk factor for prostate cancer (Shiels et al., 2009). Several types of viruses have been isolated from cancer cells, such as human papillomavirus, cytomegalovirus, HIV virus and herpesvirus, but so far only association, rather than causation, has been shown (Cheng et al., 2010).

- **Vasectomy**

Recently, a history of vasectomy has been associated with an increased risk for prostate cancer. The risk correlated with the length of time elapsed since vasectomy. Many ongoing studies are addressing this issue, but deficiency of experimental design and methodologic discrepancies limit their utility (Tang et al., 2011).

Vasectomy may increase serum androgen levels or may induce antisperm antibodies, and an immunologic reaction may be responsible for elevation of prostate cancer rates. Vasectomy may cause an imbalance in the growth hormones or their inhibitors reaching the prostate (Dennis et al., 2002). In addition, patterns of sexual activity, frequency of sexually transmitted diseases, or infections also may be altered (Huang et al., 2008).

- **Body mass index:**

Recent results from epidemiologic studies suggest that a high body mass index (BMI) and bone mass may be associated with prostate cancer (Calle et al., 2003, Discacciati et al., 2012).

Risk of prostate cancer mortality increased significantly in association with higher baseline BMI. Men with a BMI of 35.0 to 39.9
had a 34% greater risk of dying of prostate cancer than those with a normal BMI (Cao et al., 2011). In contrast, BMI and other measures of body size at age 21 were unrelated to prostate cancer risk in an Australian case-control study (Golabek et al., 2014).

- **Height**

  Risk of advanced, aggressive, or fatal prostate cancer increases by 12% for every 10cm increase in height, according to a meta-analysis (Zuccolo et al., 2008, Wiren et al., 2013).

- **Previous cancers**

  Men with a previous lung adenocarcinoma have a 56% increased risk of prostate cancer, according to an international registry study (Charles-Edwards et al., 2014).

  A previous thyroid cancer has also been associated with an increased risk (Subramanian et al., 2007).

  Men with a previous renal cell (kidney) cancer have an increased risk of prostate cancer and the risk is higher for men with a family history of prostate cancer (Zhang et al., 2009, Liu et al., 2011).

  A 14–15% increased risk has been shown in various studies for men with a previous bladder cancer (Hayat et al., 2007). This is at least partly due to detection bias, although genetic factors may also play a role (Lehnert et al., 2012).

  Men with a previous melanoma have a 15–50% increased risk of prostate cancer (Bradford et al., 2010).
• **Radiation**

  The risk increase for prostate cancer in atomic bomb survivors is consistent with that for all solid cancers *(Preston et al., 2007)*.

  The International Agency for Research on Cancer (IARC) states that there is limited evidence that exposure to thorium-232 and its decay products, gamma and x-rays increases prostate cancer risk *(Cogliano et al., 2011)*.
Pathogenesis

- Androgens in prostatic oncogenesis:

  Like their normal counterparts, the growth and survival of prostate cancer cells depends on androgens, which bind to the androgen receptor (AR) and induce the expression of pro-growth and pro-survival genes (Albertelli et al., 2006). Of interest with respect to differences in prostate cancer risk among races, the X-linked AR gene contains a polymorphic sequence composed of repeats of the codon CAG (which codes for glutamine) (Dehm et al., 2007). ARs with the shortest stretches of polyglutamine have the highest sensitivity to androgens. The shortest polyglutamine repeats on average are found in African Americans, while Caucasians have an intermediate length and Asians have the longest, paralleling the incidence and mortality of prostate cancer in these groups. More directly, the length of the repeats is inversely related to rate at which prostate cancer develops in mouse models (Albertelli et al., 2006, Ben-Shlomo et al., 2008).

  The importance of androgens in maintaining the growth and survival of prostate cancer cells can be seen in the therapeutic effect of castration or treatment with anti-androgens, which usually induce disease regression (Nieto et al., 2007). Unfortunately, most tumors eventually become resistant to androgen blockade. Tumors escape through a variety of mechanisms, including acquisition of hypersensitivity to low levels of androgen (e.g., through AR gene amplification); mutations in AR that allow it to be activated by non-androgen ligands; and other mutations or epigenetic changes that activate alternative signaling pathways, which may bypass the need for AR altogether. Among the latter are changes that lead to increased activation of the P1-3 kinase/AKT signaling pathway,
which is observed most often in tumors that have become resistant to anti-androgen therapy (Nieto et al., 2007, Muller et al., 2013).

- **Germline mutations involved in the pathogenesis of cancer prostate**

  There is much interest in the role of other inherited polymorphisms in the development of prostate cancer (Tomlins et al., 2006). Compared with men with no family history, men with one first-degree relative with prostate cancer have twice the risk and those with two first-degree relatives have five times the risk of developing prostate cancer. Men with a strong family history of prostate cancer also tend to develop the disease at an earlier age (Prowatke et al., 2007). Men with germline mutations of the tumor suppressor BRCA2 have a 20-fold increased risk of prostate cancer, but the vast majority of familial prostate cancers are due to variation in other loci that confer a small increase in cancer risk (Kicinski et al., 2011).

  Family and genome-wide association studies have identified a number of risk-associated loci, including one at 8q24 that appears to selectively increase the risk among African American men (Wiklund et al., 2003). A number of the candidate genes in these regions are involved in innate immunity, leading to speculation that inflammation may set the stage for the development of prostate carcinoma, as has been shown with respect to other human cancers (Cheng et al., 2010).

- **Acquired somatic mutations and epigenic changes in prostate cancer**

  Somatic mutations are very common in prostate cancer giving rise to chromosomal rearrangements that juxtapose the coding sequence of an
ETS family transcription factor gene (most commonly ERG or ETV1) next to the androgen-regulated TMPRSS2 promoter (Iljin et al., 2006). These rearrangements place the involved ETS gene under the control of the TMPRSS2 promoter and lead to their over-expression in an androgen-dependent fashion. Over-expression of ETS transcription factors makes normal prostate epithelial cells more invasive, possibly through the upregulation of matrix metalloproteases. In addition, tumors with rearranged ETS genes have certain distinctive morphologic features and a different gene expression signature than those lacking ETS gene rearrangements (Mosquera et al., 2007). ETS rearrangements may also have implications for prostate cancer screening and early diagnosis, as it is possible to detect ETS fusion genes in the urine using sensitive PCR assays (Kumar-Sinha et al., 2008).

The most common epigenetic alteration in prostate cancer is hypermethylation of glutathione S-transferase (GSTP1) gene which down-regulates GSTP1 expression. The GSTP1 gene is located on chromosome 11q13 and is an important part of the pathway that prevents damage from a wide range of carcinogens. Other genes silenced by epigenetic modifications in a subset of prostate cancers include a number of tumor suppressor genes, including PTEN, RB, p16/INK4a, MLH1, MSH2, and APC (Carmen et al., 2001).

- **Biomarkers in prostate cancer**

In addition to prostate specific antigen (PSA), other genes and proteins that may serve as biomarkers in prostate cancer have emerged, and some of these appear to play a direct role in the biology of the disease. Three worthy of brief mention are EZH-2 (enhancer of zeste-2), alpha-methylacyl-CoA racemase (AMACR), and PCA3 (Varambally et al., 2001).
Prostate cancers show a relatively frequent loss of E-cadherin. Loss of E-cadherin from prostate cancer cells is associated with expression of high levels of EZH-2, a transcriptional repressor that may contribute to prostate cancer progression (Schalken et al., 2005). Alpha-methylacyl-CoA racemase (AMACR), an enzyme involved in the beta-oxidation of branched chain amino acids, is selectively upregulated in prostate cancer and its possible precursor lesions as compared to normal prostate. (Luo et al., 2002, Jiang et al., 2004 and Groskopf et al., 2006).

- **MicroRNAs (miRNAs) in pathogenesis of prostatic cancer**

  In recent years, the discovery of microRNAs has laid a new layer of complexity over the mechanisms regulating gene expression and function (He and Hannon 2004). MicroRNAs (miRNAs or miRs) are endogenous non-coding RNAs that can interfere with protein expression either by inducing the cleavage of specific target mRNAs or, in most cases, by inhibiting their translation. Thus, miRs offer a fast fine-tuning and energy-saving mechanism for post-transcriptional control of protein expression (Bartel, 2004, Chen and Rajewsky, 2007).

  Some studies state a widespread down-regulation of miRs in tumours, consistently with a documented role of miRs in terminal differentiation and a general tendency of tumour cells to a more anaplastic state (Lu et al. 2005). Other studies report on a cancer miR signature composed mostly by overexpressed miRs, as the one conducted by Volinia et al., (2006) that verified a general up-regulation of miRs in cancer: specifically, 39 miRs were up regulated and six miRs were down regulated in prostate cancer. These results were in partial agreement with a more focused study conducted with a similar experimental setting by Ambs et al., (2008) in which total RNA extracted from 60
macrodissected prostate cancers and 16 surrounding non-tumour tissues was analyzed. Both studies found an up-regulation of miR-32, miR-26a, miR-196a, miR-181a, miR-25, miR-93, miR-92 and let-7i and a down-regulation of miR-218 and miR-128. In addition, both studies identified some miRs associated with extraprostatic extension of the tumours, including miR-101, miR-30c and miR-195, which were also part of the prostate cancer signature (Volinia et al., 2006, Ambs et al., 2008). The general overexpression of miRs in prostate cancer was also supported by a computational study that verified a reduced abundance of putative miR targets in human prostate tumours by comparing three different gene expression datasets (Sun et al., 2009).

As can be summerized from the multiplicity of abnormalities, prostate carcinoma (like other cancers) is the product of some critical combination of acquired somatic mutations and epigenetic changes. A putative precursor lesion, prostatic intraepithelial neoplasia (PIN), has been described. Prostates containing cancer have a higher frequency and a greater extent of PIN, which is also often seen in proximity to cancer (Cerveira et al., 2006). Studies have revealed that many of the molecular changes seen in invasive cancers are present in PIN, for example, rearrangements involving ETS genes are found in a subset that strongly supporting the argument that PIN is a precursor of invasive cancer. What remains unclear is whether PIN inevitably progresses to cancer, or instead sometimes remains latent or even regresses (Epstein et al., 2006, Perner et al., 2007).
**Pathologic features**

Approximately 80% of cases with prostatic tissue removed for carcinoma harbors presumptive precursor lesions, referred to as high-grade prostatic intraepithelial neoplasia (PIN) (Gonzalez-Berjon et al., 2012).

- **Prostatic intraepithelial neoplasia (PIN)**

  Prostatic intraepithelial neoplasia (PIN) is the currently preferred term for a process involving prostatic ducts and acini, which has also been described as intraductal or ductal–acinar dysplasia (Bostwick et al., 2004). It is often multicentric and may even extend to the prostatic utricle (Sakr et al., 2012).

**Histopathological and immunohistochemical features of PIN:**

PIN consists of architecturally benign prostatic acini lined by cytologically atypical cells with prominent nucleoli. Originally, it was divided into three grades, depending on the severity of the following alterations: cell crowding and stratification; nuclear enlargement, pleomorphism, and chromatin pattern; and nucleolar appearance. These three grades (I, II, and III) are currently grouped into two categories: low-grade PIN (corresponding to grades I and II) and high-grade PIN (corresponding to grade III). The key feature in distinguishing high-grade from low-grade PIN is the nuclear (and particularly the nucleolar) appearance, regardless of architecture (Bostwick et al., 2012).

Morphologic variations of PIN include tufting, micropapillary, cribriform, and flat/atrophic patterns at the architectural level, and inverted (hobnail) and foamy types at the cytologic level (Ayala et al.,
Of these, the cribriform pattern is the most difficult to distinguish from invasive tumors, particularly in biopsy specimens (Kronz et al., 2001).

**Mimicking of high grade PIN to prostate adenocarcinoma:**

The degree of cytologic (particularly nuclear and nucleolar) alterations in high-grade PIN (PIN III), pattern of intraluminal mucin staining, keratin immunoreactivity profile, expression of racemase, frequency of neuroendocrine cells, frequency of ERBB2 and ERBB3 expression, expression of EphA2 receptor tyrosine kinase and expression (or lack thereof) of other molecular markers are analogous to those seen in invasive carcinoma, hence its alternate designation as carcinoma in situ (Zeng et al., 2003., Wu et al., 2004 and Cohen et al., 2007). Parenthetically, the existence of a prostatic adenocarcinoma in-situ distinct from high-grade PIN has been proposed, but it remains a contentious issue (Cohen et al., 2007).

AgNOR counts have not proved useful in the distinction between the grades of PIN, and between PIN and adenocarcinoma. The majority of low-grade PIN has a diploid DNA, whereas 50% of high-grade PIN is aneuploid (Iwata et al., 2010).

- **PIN as a risk factor to prostate adenocarcinoma:**

Several studies have shown a statistical association between high-grade PIN and prostatic carcinoma, in the sense that PIN has been found in 59–100% of step-sectioned radical prostatectomy specimens (De Nunzio et al., 2009). It has also been shown that in prostates containing both PIN and adenocarcinoma, there is a good degree of concordance in
the DNA ploidy pattern of both lesions (Iwata et al., 2010). These findings have led to the suggestion that PIN may have a high predictive value as a marker for carcinoma and to the recommendation to follow closely patients in whom PIN is identified in a prostatic biopsy (Algaba et al., 2012).

In this regard, it has been claimed that PIN in and of itself does not result in elevated PSA levels (Wu et al., 2004). It has also been shown that low-grade PIN is a relatively common finding in young male patients (Montironi et al., 2011).

In a study by Kronz et al., (2001), a repeated prostatic biopsy in patients with previous biopsy of high-grade PIN revealed invasive carcinoma in 32.2% of the cases. The incidence was 30.2% if only 1 or 2 cores had showed PIN, 40% with 3 cores, and 75% with >3 cores. If invasive cancer was not diagnosed on the first two follow-up biopsies, it was unlikely to develop later. If PIN was accompanied by the presence of adjacent small atypical glands, the risk of cancer on repeated biopsy was 46% (Kronz, Shaikh et al., 2001).

However, Netto et al., (2006) reestablished the prognostic significance of PIN by showing a 39% risk of finding prostatic carcinoma on repeat biopsies if the original biopsy had shown widespread (present in 4 or more biopsy cores) high-grade PIN. It would seem then as if the risk of cancer were directly related to the number of cores involved by PIN and not so much to the morphologic subtype (Bishara et al., 2004).

Whereas the relationship between high-grade PIN and adenocarcinoma seems well established, this is not the case for low-grade PIN (Gonzalez-Berjon et al., 2012), and it is therefore questionable whether the latter should be even mentioned in the pathology report.
- **Prostatic carcinomas**

Prostatic carcinomas can be divided based on the different sites of origin into two major categories: (1) adenocarcinoma of peripheral (‘secondary’) ducts and acini, and (2) carcinoma of large (‘primary’) ducts. The two patterns are sometimes seen together in the same tumor, and the two types may coexist in the same prostate as anatomically separate lesions (Srigley., 2009). Therefore, the alternative proposal has been advanced that it is the site of the growth rather than the origin that governs the tumor architecture. The large majority of the tumors belong to the first category, and most studies dealing with grading, staging, prognosis, and therapy of prostatic carcinoma refer exclusively to them (Rosai et al., 2011).

**Adenocarcinoma of peripheral ducts and acini**

Most prostatic carcinomas arise in the peripheral zone, whether posteriorly, laterally, or anteriorly, with sparing of the periurethral region except for the late stages of the disease (Montironi et al., 2003, Al-Ahmadie et al., 2008). However, a small percentage of tumors do actually arise in the prostatic transition zone (Garcia et al., 2008).

**Grossly,** the tumor may be difficult to see but usually can be identified as a gray or yellowish, poorly delineated, firm area. Residual carcinoma may be unidentifiable grossly or even microscopically in a radical prostatectomy specimen performed because of a positive biopsy (so-called ‘vanishing cancer phenomenon’ or ‘minimal residual cancer’) (Egevad et al., 2006). The frequency of this finding has increased in recent years, probably due to earlier diagnosis and therefore smaller size of the original tumors (Duffield et al., 2009, Egevad et al., 2011).

**Microscopically,** prostatic adenocarcinomas exhibit a wide spectrum of appearances, ranging from anaplastic tumors to highly
differentiated neoplasms that are distinguished from the non-neoplastic gland only with great difficulty (Lee et al., 2002, van der Kwast et al., 2011). Four major cytoarchitectural patterns occur. These patterns are: medium-sized glands, small glands, diffuse individual cell infiltration, and cribriform. Carcinomas composed of medium-sized glands are detected on low-power examination by virtue of the closely spaced arrangement of those glands, irregular outline, smooth inner surface, and scanty intervening stroma. Tumors made up of small glands appear as expansive nodules on low power, the individual glands having a regular round configuration and small size. Both of these architectural patterns (but particularly the latter) are accompanied by cytologic abnormalities in the form of nuclear enlargement, irregularity of contour, hyperchromasia, and – most important – prominent nucleoli (‗macronucleoli‘, defined as measuring >1 µ in diameter) (Lee et al., 2002, Kench et al., 2010) Mitoses are also of significance, but they are rarely found in well-differentiated tumors composed of either medium-sized or small glands. The pattern of diffuse cell infiltration resembles somewhat that of invasive lobular carcinoma of the breast, whereas the cribriform pattern is highly reminiscent of that seen in the homonymous type of breast carcinoma. It has been stated that the cribriform pattern represents intraductal carcinoma, as evidenced by the preservation of the epithelial basal layer (Truskinovsky et al., 2004). Although this observation is probably correct, it is as well to remember that this pattern is accompanied by clearcut invasive carcinoma in the overwhelming majority of cases, and therefore use of the term ‘intraductal carcinoma’ under these circumstances may be misleading (Nunez-Mora et al., 2009, van der Kwast et al., 2011).

A single cell layer usually lines the gland-forming types of prostatic adenocarcinoma but occasionally they exhibit a stratified
epithelium that may simulate PIN (Parwani et al., 2004, Hameed et al., 2006). An additional pattern of growth that has recently been described is that referred to as glomeruloid. It is characterized by the presence of intraluminal ball-like clusters of tumor cells and is regarded by many as a pathognomonic sign of malignancy (Egevad et al., 2006). The claim has been made that the rare prostatic adenocarcinomas arising in the transition zone have a distinct morphology, characterized by glands of variable size with tall columnar cells displaying basally located nuclei and clear to pale pink cytoplasm, but these features are neither specific nor consistent (Garcia et al., 2008). Squamous metaplasia is an uncommon but well-documented finding in prostatic carcinoma (especially of the high-grade type). It is often, but not always, seen in the setting of prior hormonal or radiation therapy, and is associated with a poor prognosis (Parwani et al., 2004, Garcia et al., 2008).

The presence of prostatic glands within perineurial spaces is common in these tumors. This finding is a strong indicator of malignancy but is not pathognomonic (Humphrey et al., 2007). It does not represent permeation of perineurial lymphatic vessels, as formerly believed, but rather spread of glandular tissue along planes of lesser resistance. Its presence in a needle biopsy specimen is a good predictor of capsular invasion by the tumor (Rosai et al., 2011).

The stroma surrounding the neoplastic glands may show a combination of hypercellularity and deposition of a basophilic ground substance (mucinous fibroplasia or collagenous micronodules) (Christian et al., 2005). Both intraluminal and stromal calcification may be seen in association with prostatic cancer, but the incidence of the latter is much lower than in benign prostates (Berney et al., 2007). Protein crystalloid structures morphologically and immunocytochemically similar to
Bence Jones crystals are seen in the glandular lumina of 10–23% of prostatic carcinomas and are particularly common in tumors composed of medium-sized glands. Their presence usually indicates malignancy, but their occasional occurrence in benign glands has been documented. When the latter is the case, their presence should not be viewed as indicating a significant risk factor for the subsequent development of cancer (Delahunt et al., 2012). Electron probe x-ray microanalytic studies have shown that they are predominantly composed of inorganic sulfur. Exceptionally, these crystalloids are also found in metastatic foci (Berney et al., 2007, Cheng et al., 2012). The intraluminal secretion of malignant glands often has a bluish hue (‘wispy blue mucin’), indicative of a mucinous composition. The presence of corpora amylacea in the glandular lumen is not necessarily a sign of benignancy, as formerly believed; these formations can also be exceptionally found in association with malignant glands, particularly in extensive, well to moderately differentiated tumors (Gleason patterns 3, 4, or 5) (Christian et al., 2005, Bostwick et al., 2012).

Many variations of this complex ‘basic’ pattern have been described. Some are distinctive enough to be regarded as separate microscopic types. Others are variations of a lesser degree, although still important because of the diagnostic errors they may engender. These treacherous variations of prostatic adenocarcinoma include:

1 Foamy gland carcinoma. Grossly, the tumor acquires a bright yellow color and a soft consistency, making it difficult to detect on rectal palpation even when it is extensive. Microscopically, the cytoplasm of the carcinoma cells usually has a finely granular appearance, but on
occasion it is clear or foamy (xanthomatous) because of the massive accumulation of lipids. When this feature is prominent, the tumor is known as foamy gland carcinoma (Arista-Nasr et al., 2008). Its microscopic recognition can also be difficult, particularly at metastatic sites. The voluminous tumor cells are cuboidal to columnar, and the nuclei are small and hyperchromatic. The nucleoli are not particularly conspicuous (Zhuo et al., 2009). Most foamy gland carcinomas are assigned a low Gleason score (3 + 3 = 6), but a high-grade form of this variant exists. The behavior of foamy gland carcinomas is often aggressive (Amanda et al., 2014).

2 Prostatic adenocarcinoma with atrophic features. This variant is composed of tumor cells with an attenuated cytoplasm, such that the nuclei occupy almost the entire cell height. These cells are identifiable as malignant because of their infiltrative pattern of growth, nuclear enlargement, macronucleoli, and sometimes the presence of adjacent carcinoma of the ordinary type (Yaskiv et al., 2010).

3 Pseudohyperplastic prostatic adenocarcinoma. This variant resembles many of the features of hyperplastic glands at the architectural level, including papillary infoldings, branching, and corpora amylacea. On low power, the tumor has a deceptively bland microcystic appearance, a feature that it shares with the atrophic type (Levi et al., 2000). Clues, which identify the lesion as malignant, are nuclear enlargement, macronucleoli, mitoses, intraluminal crystalloids, and sometimes the presence of adjacent PIN (So et al., 2014).

Tumor multicentricity

Multiple tumor foci have been demonstrated in 75–85% of radical prostatectomy specimens studied by step-section or whole-mount techniques (Takimoto et al., 2001). These remarkably high percentages
hold even for the small volume cancers (<0.5 ml) (Cheng et al., 2005). They are probably an expression of true multicentricity rather than intraglandular tumor spread, as supported by their frequent genetic heterogeneity. It is noted that, multicentricity is less common in centrally located tumors (Andreoiu et al., 2010).

**‘Minimal adenocarcinoma’ and atypical small acinar proliferation (ASAP)**

A particularly frequent problem in the handling of prostatic needle biopsies is represented by the foci of small atypical glands that are suspicious but not diagnostic of carcinoma. Minimal criteria for the unequivocal diagnosis of malignancy have been presented (Iczkowski et al., 2000). For the cases in which the recommended threshold is not reached, terms such as ‘atypical gland suspicious of malignancy’ and ‘atypical small acinar proliferation (ASAP) suspicious of malignancy’ have been proposed (Girasole et al., 2006).

The fact remains that a certain number of prostatic biopsies (about 4–6%) cannot be confidently placed into a benign or malignant category, either with plain morphology or after immunostaining with 34ßE12 keratin and/or racemase (Herawi et al., 2005). One thing is certain: a patient with such a diagnosis warrants a second biopsy (Bostwick et al., 2006).

**Carcinoma of large (‘primary’) ducts**

The other major (but numerically less significant) type of prostatic carcinoma originates from (or is located within) the large (primary) ducts that are normally found in a periurethral location (Orihuela et al., 2008). Cystoscopic examination often shows a polypoid villous or an infiltrative
urethral component. Microscopically, the following types have been recognized:

1. **Large (prostatic) duct adenocarcinoma.** This tumor is characterized by malignant changes in large dilated ducts, with a cribriform and/or papillary architecture lined by columnar pseudostratified malignant epithelium, occasionally with a clear cell (mesonephroid) look (**Aydin et al., 2009**). Sometimes the tumor is accompanied by pagetoid spread in the prostatic urethra. Some cases of this entity have been reported in the past as Paget disease and Bowen disease of this region. Positivity for PSA and PAP is the rule (**Hammer et al., 2008**). The tumors tend to have a more advanced stage at presentation and a higher short-term survival rate than peripheral duct–acinar carcinomas. This comment also applies to the cases diagnosed on prostatic needle biopsy (**Tu et al., 2009**). On occasion, large duct carcinoma simulates high-grade PIN, from which it is distinguished by the presence of cystically dilated glands, a greater predominance of flat architecture, a lesser frequency of macronucleoli, absence of basal cells on high molecular weight keratin immunostain, and higher Ki-67 index (**Tavora et al., 2008**).

   Endometrial-type (endometrioid) adenocarcinoma was originally described as arising from the prostatic utricle (a müllerian remnant thought to represent the male homolog of the female uterus and vagina) but is currently regarded as a variant of large duct prostatic adenocarcinoma (**Bostwick et al., 2008**). Microscopically, glands and papillae are seen, lined by tall, pseudostratified columnar epithelium. Microscopic studies, immunocytochemical determinations (positivity for PAP and PSA), and the response that has been observed after orchiectomy indicate that this tumor is truly of prostatic origin.
2 Primary urothelial (transitional cell) carcinoma of the prostate.

The existence of this tumor type is explained by the fact that the outer portion of the prostatic (periurethral) ducts emptying into the urethra is lined by urothelium (Oliai et al., 2001). This variant comprises less than 2% of all prostatic carcinomas. The microscopic appearance of this neoplasm is identical to that of the homonymous bladder tumor. The diagnosis can be made in prostatic needle biopsies and in TUR specimens (Grignon., 2004). Before a diagnosis of primary urothelial carcinoma of prostate is made, the possibility of prostatic extension from a bladder or urethral carcinoma should be excluded (Huguet et al., 2012).

3 Mixed adenocarcinoma–urothelial (transitional cell) carcinoma exhibits a combination of types 1 and 2 (Huguet et al., 2012).

Sometimes, tumors having any of the appearances listed previously are seen associated with an ordinary prostatic adenocarcinoma or with an independent urothelial tumor of the bladder. The mode of presentation, initial stage, and response to hormone therapy for the carcinomas arising from large ducts (with the possible exception of pure urothelial carcinoma) are similar to those of the conventional prostatic adenocarcinoma. However, the presence of a ductal component in a prostatic adenocarcinoma is associated with an increased incidence of extraprostatic extension (Epstein et al., 2009). Atypical hyperplasia and carcinoma in situ of periurethral glands, presumably representing the precursors of large duct carcinomas, have been observed (Samaratunga et al., 2010).
Spread and metastases

- **Local spread**

Prostatic carcinoma spreads initially within the various compartments of the prostate itself, including ducts and acini, fibromuscular stroma, perineurial spaces, and blood vessels. Immunostaining for EMA can be helpful in distinguishing true perineurial invasion from mere perineurial ‘indentation’ (Tsuzuki et al., 2005).

Invasion of the capsule (i.e., the outer fibromuscular layer of the prostate) is very common. The probability of tumor having extended outside the prostate into the neurovascular bundles was found to be zero if the ‘capsular margin’ was negative, 12% if it was equivocal, and 60% if it was positive (Chou et al., 2008).

Advanced tumor may extend into the seminal vesicles, apex (distal aspect) of the gland, prostatic urethra (very rarely), and bladder. Tumors should be listed as exhibiting seminal vesicle invasion only if the muscular wall of this organ is infiltrated by tumor (Owens et al., 2007).

Rectal invasion is much less common, because of the resistance offered by the tough fibromuscular structure covering the posterior aspect of the prostate known as Denonvilliers’ fascia. This invasion can manifest in the form of an anterior rectal mass (with an intact or ulcerated mucosa), an annular rectal stricture resulting from circumferential infiltration, or subserosal implants. The diagnosis can be made from a colorectal biopsy, but only if the pathologist thinks of the possibility (Lane et al., 2008).
Lymph node spread

The most common pathway of nodal involvement is to the pelvic chains, from which the tumor spreads to the retroperitoneal nodes. However, in some instances, retroperitoneal node metastases may occur in the absence of pelvic node metastases; these patients are less likely to have metastases to bladder and rectum but more likely to have them in lungs and liver. Rarely, metastases are also found in periprostatic/periseminal vesicle lymph nodes or in the perirectal lymph node basin (Murray et al., 2004).

Latent prostatic carcinomas detected at autopsy are almost never accompanied by nodal metastases. A system has been proposed (the ‘Hamburg algorithm’) for predicting the likelihood of lymph node metastases based on the needle biopsy findings (Kothari et al., 2001, Haese et al., 2002).

Nodal metastases in patients with clinically localized palpable prostatic carcinoma are most likely to be found on the same side as the tumor and are very unlikely to be present on the contralateral side alone. Many patients with nodal metastases lack evidence of concurrent bony or visceral dissemination, thus contradicting the traditional concept that nodal metastases always represent a secondary phenomenon from skeletal metastases. Detection of nodal involvement by lymphangiography has proved notoriously inaccurate. Instead, high-resolution MRI with magnetic nanoparticles seems extremely promising (Harisinghani et al., 2003).

Metastases can also occur in supradiaphragmatic lymph node groups. Sometimes, involvement of a left supraclavicular or a mediastinal node is the first manifestation of the disease. Most of these tumors are poorly
differentiated and not particularly suggestive of a prostatic origin on microscopic examination; immunohistochemical staining for PSA or PAP is extremely valuable in establishing the prostatic origin of a metastatic adenocarcinoma, but it has not proved useful as a screening method for occult nodal metastases (Geisinger et al., 2008).

**Distant spread**

The most common sites of metastatic spread of prostatic carcinoma are the skeletal system and lymph nodes (Carlin et al., 2000). Bone metastases are usually multiple but can be solitary. They are characteristically osteoblastic and can radiographically simulate Paget disease or even osteosarcoma, but they can also be mixed or entirely osteolytic. Sometimes, the appearance of a bone metastasis precedes by several years the urologic manifestations (Roodman et al., 2004). Lumbar spine, sacrum, and pelvis are the most common locations, supposedly because of tumor spread via Batson's vertebral venous system. However, any other bone can be involved through the systemic circulation. Metastases to the spine may result in epidural masses with spinal cord compression, and metastases to the base of the skull may result in severe cranial nerve defects (Weinfurt et al., 2005).

Microscopically, clusters of malignant glands are seen surrounded by exuberant new bone formation (Ye et al., 2007).

Immunohistochemical reactions for PAP and PSA are usually positive, even after decalcification (Roodman et al., 2004).

Lung metastases are not as rare as formerly believed; most of them exhibit a lymphangitic pattern of spread. Massive pleural effusion may be the initial symptom. On occasion, the process simulates primary lung
carcinoma. The pattern of growth may be microacinar, tubulopapillary, or carcinoid-like. Adenocarcinoma of large ducts may simulate metastatic colonic adenocarcinoma (Copeland et al., 2002).

Rarely, metastatic prostatic carcinoma is found unexpectedly in orchiectomy specimens. Prostatic carcinoma also may metastasize to the breast, sometimes bilaterally, particularly in patients taking estrogens. PSA and PAP immunostains are important to confirm the metastatic nature (Tu et al., 2002).

Other metastatic sites include liver, adrenal gland, central nervous system (including dura), eye, skin, and unusual locations such as umbilicus (‘Sister Mary Joseph nodule’), penis and salivary gland (Bubendorf et al., 2000, Tu et al., 2002). In general, the degree of microscopic differentiation of the metastases and of PSA expression follows quite closely that of the primary tumor. However, in almost half of the cases the Gleason score is higher than in the primary tumor (Tremont-Lukats et al., 2003).
Staging and grading

**Staging of prostatic carcinoma:**

Carcinomas of the prostate have been traditionally divided into clinical, latent, and occult types. The clinical tumor produces local symptoms and signs; the *latent* (incidental) carcinoma is unsuspected clinically and found incidentally at autopsy or in prostatectomy specimens performed for nodular hyperplasia or some other condition; *occult* carcinomas result in distant metastases, whereas the primary tumor remains clinically undetected. Some of the criteria of this old-fashioned but eminently sound scheme have been incorporated into the currently used staging systems. In the initial staging, stage A tumors correspond to the latent neoplasms of the previous classification; stage B carcinomas are clinically detectable but confined within the prostatic capsule; in stage C the disease has spread outside the capsule; and in stage D there are distant metastases (*Cancer fact and figures 2012*).

Subdivisions within each category have been proposed, based on the amount or extent of tumor as determined clinically, serologically (PSA levels), and radiographically, sometimes incorporating technology such as MRI and ultrasonography (*Eble et al., 2004*). This must be distinguished from the pathologic staging done microscopically in biopsies, TUR, and radical prostatectomy specimens. A particularly important subdivision which incorporates an element of grading exists in pathologic stage A tumors. Stage A1 tumors, which receive no additional therapy and have a good prognosis, are defined as small tumors that are well or moderately differentiated. Stage A2 tumors (large and/or poorly differentiated) require added therapy (often prostatectomy) and have a worse prognosis than stage B1 disease (*Edge et al., 2010*). Recently, the
American system has been modified to accommodate PSA-detected cancers (new stage BO). The current stage divisions are similar to those of the TNM system (Figure ) but do not include tumor grade except in the separation of stages A1 and A2 (Cheng et al., 2012).

TNM classification for prostate cancer according to the AJCC 6th edition (2002) and UICC 6th edition

_Evaluation of the (primary) tumor (‘T’)_
TX: cannot evaluate the primary tumor
T0: no evidence of tumor
T1: tumor present, but not detectable clinically or with imaging
   T1a: tumor was incidentally found in less than 5% of prostate tissue resected (for other reasons)
   T1b: tumor was incidentally found in greater than 5% of prostate tissue resected
T1c: tumor was found in a needle biopsy performed due to an elevated serum PSA
T2: the tumor can be felt (palpated) on examination, but has not spread outside of the prostate
   T2a: the tumor is in half or less than half of one of the prostate gland’s two Lobes
   T2b: the tumor is in more than half of one lobe, but not both
   T2c: the tumor is in both lobes
T3: the tumor has spread through the prostatic capsule (if it is only part-way through, it is still T2)
   T3a: the tumor has spread through the capsule on one or both sides
   T3b: the tumor has invaded one or both seminal vesicles
T4: the tumor has invaded other nearby structures

_Evaluation of the regional lymph nodes (‘N’)_
NX: cannot evaluate the regional lymph nodes
N0: there has been no spread to the regional lymph nodes
N1: there has been spread to the regional lymph nodes

_Evaluation of distant metastasis (‘M’)_
MX: cannot evaluate distant metastasis
M0: there is no distant metastasis
M1: there is distant metastasis
   M1a: the cancer has spread to lymph nodes beyond the regional ones
   M1b: the cancer has spread to bone
   M1c: the cancer has spread to other sites (regardless of bone involvement)

**Figure (5):** TNM staging of cancer prostate according to American Joint Committee on cancer, Manual of staging cancer, 6th edition (Greene et al, 2002).
**Figure (6): AJCC Prostate cancer stage grouping, (Edge et al., 2010)**

**Grading of prostatic carcinoma:**

The microscopic grading system developed by Gleason in conjunction with the Veterans Administration Cooperative Urological Research Group (Gleason et al., 1974, Gleason, 1992) is currently preferred with the modifications to the other grading systems that have been proposed over the years (Bain et al., 1982, Lilleby et al., 2001 and Humphrey, 2004). It is based on the degree of glandular architectural differentiation and the growth pattern of the tumor in relation to the stroma as evaluated on low-power examination. The predominant tumor pattern (referred to as ‘primary’) is graded from 1 to 5, and the ‘secondary’ pattern (if present) is graded similarly, with the two numbers being added to obtain the Gleason score or sum. If the tumor has the same pattern throughout (i.e., it has only a ‘primary’ pattern), the number is multiplied by 2 in order to
obtain the final score. Some tumors have a tertiary pattern. This is to be reported only if it is a grade 5 (Mosse et al., 2004, Trpkov et al., 2009). The AJCC considered Gleason < or = 6 grade 1, Gleason 7 as grade 2, and Gleason 8-10 as grade 3 (El-Bolkainy et al, 2013).

In general, Gleason high score tumors (8–10) are usually abundantly represented in the biopsy cores, but on occasion they present in the form of easy-to-miss minute foci (Allsbrook et al., 2001). In cases of multicentric involvement by tumor, there is often a great heterogeneity of the Gleason score (Arora et al., 2004). The interobserver reproducibility in Gleason grading has been found to be in an acceptable range both among urologic pathologists and among general pathologists (Fine et al., 2005).
<table>
<thead>
<tr>
<th>GRADE</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>1</td>
<td>Single, separate, uniform glands in closely packed masses with a definite, usually rounded, edge limiting the area of tumor</td>
</tr>
<tr>
<td>2</td>
<td>Single, separate, slightly less uniform glands, loosely packed (separated by small amounts of stroma), with less sharp edge</td>
</tr>
<tr>
<td>3a</td>
<td>Single, separate, much more variable glands; may be closely packed but usually irregularly separated; ragged, poorly defined edge</td>
</tr>
<tr>
<td>3b</td>
<td>Like 3a, but very small glands or tiny cell clusters</td>
</tr>
<tr>
<td>3c</td>
<td>Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumor (‘papillary intraductal tumor’)</td>
</tr>
<tr>
<td>4a</td>
<td>Raggedly outlined, raggedly infiltrating, fused glandular tumor</td>
</tr>
<tr>
<td>4b</td>
<td>Like 4a, with large pale cells (‘hypernephroid’)</td>
</tr>
<tr>
<td>5a</td>
<td>Sharply circumscribed, rounded masses of almost solid cribriform tumor, usually with central necrosis (‘comedocarcinoma’)</td>
</tr>
<tr>
<td>5b</td>
<td>Ragged masses of anaplastic carcinoma with only enough gland formation or vacuoles to identify it as adenocarcinoma</td>
</tr>
</tbody>
</table>

Figure (7): Gleason's microscopic grading system of prostatic carcinoma (Gleason., 1977).
Figure (8): Gleason grade Lower grades are associated with small, closely packed glands. Cells spread out and lose glandular architecture as grade increases (Edge et al., 2010).
Prognostic factors of prostatic carcinoma:

Many parameters have been evaluated for their ability to predict outcome in patients with prostatic carcinoma, and they are listed below. Because of the usually long natural history of the disease, the PSA-free survival and PSA recurrence have been used in some of the studies as indicators for actual survival and recurrence, respectively.

1 Clinical stage. This is a very important prognostic determinant, and it has become even more so with the incorporation of newer technology.

2 Pathologic stage. This represents the ultimate indicator of tumor extent and, as such, the most accurate predictor of prognosis currently available (Ahlering et al., 2002).

There is a strong association between the level of tumor invasion into or through the prostatic capsule and the grade, volume, and rate of recurrence of the tumor. There is also an association between the radial distance of extraprostatic extension and PSA recurrence (Sung et al., 2007). Conversely, microscopic bladder neck involvement is not a significant prognostic factor (Zhou et al., 2009).

In cases with nodal metastases, the prognosis is worse when they are multiple rather than solitary, when they are detectable grossly rather than only microscopically, when their overall volume is large, and when they are accompanied by extracapsular extension (Cheng et al., 2000). Their prognostic significance seems to be the same regardless of whether they are found in the usual pelvic location or around the prostate/seminal vesicles (Kothari et al., 2001).

3 Microscopic grading. There is convincing evidence that microscopic grading using the Gleason score system is superior to the others as an independent prognostic variable (Lilleby et al., 2001, Shah., 2009). Generally, direct correlation exists between clinical or pathologic
staging and microscopic grading regardless of the grading system used (El-Bolkainy et al., 2013).

4 Surgical margins. Positive margins are strongly correlated with tumor progression (Kausik et al., 2002). However, the extent of surgical margin positivity does not seem to influence the rate of PSA recurrence (Marks et al., 2007). Positive margins indicative of extraprostatic extension should be distinguished from those resulting from capsular incision, in which the surgeon transects either benign or malignant prostatic tissue and leaves the edge of the prostate within the patient, a not particularly easy determination (Chuang et al., 2008).

5 Tumor volume. It has been shown that tumor volume, as measured in whole sections of prostatectomy specimens with morphometric techniques, correlates with Gleason score, capsular penetration, capsular margins of resection, seminal vesicle invasion, and lymph node metastases (Nobumichi et al., 2012). As a matter of fact, Vollmer, (2009) has shown that a plain visual estimate (‘eyeballing’) of tumor volume is more closely associated with overall survival than PSA level or Gleason score (Kunz et al., 2003). General speaking, a reasonably good prediction of tumor volume can be made from needle biopsy specimens by assessing a combination of morphologic and laboratory-based data (Sebo et al., 2001). It has even been proposed that a good estimate of tumor volume can be obtained by the following formula: cancer-specific serum PSA/amount of PSA leaking into the serum per cm$^3$ of cancer (Lewis et al., 2002).

6 Age. Overall, the patient's age is not an important prognostic determinant. It is true that the few reported cases of prostatic carcinoma in men less than 35 years of age have been usually
characterized by poor differentiation and a very aggressive behavior. However, statistical analysis of prostatic carcinomas occurring after the age of 40 years (the overwhelming majority) has not shown a definite relationship between age and survival (Saadettin et al., 2011).

7 Race. Black males have mortality from prostatic carcinoma that is almost twice that of white males. This is due, at least in part, to the fact that they are more likely to have a more advanced stage at presentation. When the disease is stratified for grade and stage, survival is similar in the two races (Howlader et al., 2012).

8 Method of initial diagnosis. Patients in whom the prostatic carcinoma was diagnosed by TUR have a higher incidence of tumor dissemination than those diagnosed by needle biopsy; it is not yet clear whether this is the result of the TUR procedure itself (unlikely) or a reflection of the fact that TUR-diagnosable tumors are usually more advanced. Conversely, the large majority (over 80%) of the prostatic carcinomas found incidentally in radical cystoprostatectomy specimens done for bladder carcinomas are clinically insignificant (Mazzucchelli et al., 2009).

9 PSA serum levels. The serum level of PSA is related to prognosis in prostatic carcinoma, as an indirect indicator of tumor volume, tumor extension, and response to therapy (Albertsen et al., 2005).

10 PSA and PAP immunoreactivity. Prostatic carcinomas with areas of weak or negative reactivity for PSA or PAP behave as a group more aggressively than the others (Humphrey., 2004).

11 Perineurial invasion. Perineurial invasion is a time-honored clue for the diagnosis of carcinoma, but its prognostic value remains controversial (Ohori et al., 2004, Merrilees et al., 2008).
12 *Lymphovascular invasion*. Permeation of vascular channels as detected in whole-mount specimens of radical prostatectomy has been found to correlate with Gleason score, extraprostatic extension, seminal vesicle involvement, and likelihood of tumor progression (Herman et al., 2000). Furthermore, peritumoral lymph vessel invasion is associated with an increased likelihood of regional lymph node metastases (Roma et al., 2006).

13 *Neovascularity*. It has been claimed that microvessel density is an independent predictor of pathologic stage and of progression in prostatic carcinoma. Examination of CD34-immunostained sections has been found to be the most reliable in this regard (Taille et al., 2000).

14 *Neuroendocrine features*. Neuroendocrine features correlate with poor differentiation and poor prognosis in all types of prostatic carcinoma. Furthermore, a relationship has been claimed to exist between the degree of neuroendocrine differentiation and tumor progression. It is not clear, however, that they carry independent prognostic significance. Curiously, the few prostatic carcinomas composed entirely of Paneth cell-like neuroendocrine cells seem to be associated with a generally favorable prognosis (Tamas et al., 2006).

15 *Prominent reactive stroma*. Tumors with abundant (grade 3) reactive stroma (stromogenic) are more likely to recur, regardless of whether this feature is evaluated in radical prostatectomy specimens or in needle biopsies (Yanagisawa et al., 2007).

16 *Androgen-receptor status*. High levels of androgen receptor as measured immunohistochemically are associated with aggressive clinicopathologic features and decreased PSA-free survival. Mutations of the androgen-receptor gene have been detected in
metastatic prostatic carcinoma and postulated to be the reason for the androgen independence of such tumors (Li et al., 2004).

**17 DNA ploidy.** Tumor aneuploidy, as determined by image or flow cytometry, correlates both with a higher Gleason score and with local and distant spread. It also predicts an increased probability of grade shifting from the biopsy to the prostatectomy specimen (Lapointe et al., 2007).

**18 Proliferation index.** The Ki-67 labeling index of prostatic carcinoma has been said to predict tumor-specific mortality both in cases of limited disease and in cases associated with lymph node metastases (Whitfield et al., 2006). The combined determination of Gleason score and proliferation index constitutes a particularly powerful prognostic tool (Zellweger et al., 2009).

**19 Chromosomal abnormalities.** Patients with clonal karyotypic abnormalities are said to have shorter survival rates than those with normal karyotypes (Tomlins et al., 2007).

**20 P53 expression.** The TP53 tumor suppressor gene has been found to be mutated in a subset of advanced-stage prostatic carcinomas. It remains to be seen whether this finding is of value independent of stage and grade (Laurora et al., 2005).

**21 RAS oncogene.** Expression of the RAS oncogene p21 has been found to correlate with the degree of nuclear anaplasia and therefore with microscopic grading, a feature closely related to prognosis. However, there is no indication that the expression of this oncogene has independent prognostic value (Whitfield et al., 2002).

**22 BCL2.** Positivity for this oncoprotein is statistically related to the probability of recurrence of prostatic carcinoma (Keshgegian et al.,
23 *Circulating tumor cells*. The detection of circulating tumor cells through the measurement of PSA transcripts with the RT-PCR technique may indicate a greater likelihood of tumor recurrence (Detchokul et al., 2011).

24 *Other molecular genetic markers*. In addition to the above prognostic factors, genetic molecular markers claimed to have prognostic relevance in prostatic carcinoma are gain of distal 8q, p21-waf1, p27-kip1, p34-cdc-2, p120, the various cyclins, and cathepsin-D (Dekken et al., 2003).

In 1999, the College of American Pathologists (CAP) convened a group of clinicians, pathologists and statisticians to produce a consensus statement on the relative significance of these parameters. The result was the establishment of the categories listed below, most of which have remained valid at present (Bostwick et al., 2000, Rosai et al., 2011).

I Proven to be of prognostic importance and useful in clinical patient management:

- Preoperative serum PSA level
- TNM stage grouping
- Histologic grade as Gleason score
- Surgical margin status

II Extensively studied but whose importance remains to be validated:

- Tumor volume
- Histologic type
- DNA ploidy

III Not sufficiently studied to demonstrate their prognostic value:
Perineurial invasion
Neuroendocrine differentiation
Microvessel density
Nuclear roundness
Chromatin texture
Other karyometric factors
Proliferation markers
PSA derivates
Other factors (oncogenes, tumor suppressor genes, apoptosis genes, etc.).
Molecular basis of cancer

The literature on the molecular basis of cancer continues to proliferate at such a rapid pace that it is easy to get lost in the growing forest of information. We list here some fundamental principles.

- **Nonlethal genetic damage lies at the heart of carcinogenesis.** Such genetic damage (or mutation) may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line (Howe et al., 2002). The term environmental, used in this context, involves any acquired defect caused by exogenous agents or endogenous products of cell metabolism. Not all mutations, however, are “environmentally” induced. Some may be spontaneous and stochastic, falling into the category of bad luck (Mitchell et al., 2012).

- A tumor is formed by the clonal expansion of a single precursor cell that has incurred genetic damage (i.e., tumors are monoclonal). The most commonly used method to determine tumor clonality involves the analysis of methylation patterns adjacent to the highly polymorphic locus of the human androgen receptor gene, AR (Ostrovnaya et al., 2011). The frequency of such polymorphisms in the general population is more than 90%, so it is easy to establish clonality by showing that all the cells in a tumor express the same allele. For tumors with acquired cytogenetic aberrations of any type (e.g., a translocation) their presence can be taken as evidence that the proliferation is clonal (Aparicio et al., 2013).
• Four classes of normal regulatory genes: the growth-promoting proto-oncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repair, are the principal targets of genetic damage (Paugh et al., 2011). Mutant alleles of proto-oncogenes are considered dominant, because they transform cells despite the presence of a normal counterpart. In contrast, typically, both normal alleles of the tumor suppressor genes must be damaged before transformation can occur. However, there are exceptions to this rule; sometimes, loss of a single allele of a tumor suppressor gene reduces levels or activity of the protein enough that the brakes on cell proliferation and survival are released. Loss of gene function caused by damage to a single allele is called haploinsufficiency. Such a finding indicates that dosage of the gene is important, and that two copies are required for normal function (Santarosa et al., 2004). Genes that regulate apoptosis may behave as proto-oncogenes or tumor suppressor genes. Mutations of DNA repair genes do not directly transform cells by affecting proliferation or apoptosis. Instead, DNA-repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and genes that regulate apoptosis. A disability in the DNA-repair genes can predispose cells to widespread mutations in the genome and thus to neoplastic transformation. Cells with mutations in DNA repair genes are said to have developed a mutator phenotype (Zhang et al., 2007). Interestingly, a new class of regulatory molecules, called microRNAs (miRNAs), has recently been discovered. Even though they do not encode proteins, different
families of miRNAs have been shown to act as either oncogenes or tumor suppressors. They do so by affecting the translation of other genes (Zhang et al., 2007, Rana et al., 2007).

- Carcinogenesis is a multistep process at both the phenotypic and the genetic levels, resulting from the accumulation of multiple mutations (Loeb et al., 2003). Malignant neoplasms have several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases. Furthermore, it is well established that over a period many tumors become more aggressive and acquire greater malignant potential. This phenomenon is referred to as tumor progression and is not simply a function of an increase in tumor size (Wu et al., 2012).

At the molecular level, tumor progression and associated heterogeneity most likely result from multiple mutations that accumulate independently in different cells, generating subclones with varying abilities to grow, invade, metastasize, and resist (or respond to) therapy. Some of the mutations may be lethal; others may spur cell growth by affecting additional proto-oncogenes or tumor suppressor genes. Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident their constituent cells are extremely heterogeneous. During progression, tumor cells are subjected to immune and nonimmune selection pressures. A growing tumor therefore tends to be enriched for subclones that “beat the odds” and are adept at survival, growth, invasion, and metastasis (Yongvanit et al., 2012).
**S100A4 antibody**

S100-A4 is a protein that in humans is encoded by the S100A4 gene. The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21. This protein may function in motility, invasion, and tubulin polymerization. Chromosomal rearrangements and altered expression of this gene have been implicated in tumor metastasis (Bresnick et al., 2015).

**Mode of action of S100A4**

- **S100 proteins role in cancer cell proliferation**

- **A- S100A4 proteins role in RAGE (receptor for advanced glycation end products) signaling**

  One of the general receptors of S100 proteins is RAGE. As S100A4 binds to RAGE, it trigger RAGE-mediated cellular signaling which involves in MAP Kinase, NF-κB, and phosphatidylinositol 3-kinase (PI-3K)/AKT signaling pathway. Therefore, S100 proteins are involved in the regulation of diverse cellular processes including inflammation, cell proliferation and cancer development (Leclerc et al., 2009, Siddique et al., 2013).
• **S100A4 protein role in Epidermal Growth Factor (EGF) signaling**

Extracellular S100A4 was found to interact with a variety of EGFR ligands and have the highest affinity for amphiregulin and stimulate EGFR/ErbB2 receptor signaling and enhance the amphiregulin-mediated cell proliferation (Chen et al., 2014).

• **S100 proteins role in p53 signaling**

Overexpression of S100A4 drives cells into G2/M phase by sequestering p53 and upregulating the expression of p53 target gene as p21/WAF1, thrombospondin-1, MDM2. Furthermore, S100A4 can interact with p53 and interfere with p53 transcriptional activity. However, S100A4 exhibits a differential modulation of the p53 target gene (Chen et al., 2014).

❖ **S100A4 proteins role in cell apoptosis**

Apoptosis is a tightly regulated cell suicide program, defects and evasion of cell apoptosis promote malignant transformation and have been recognized as a hallmark of cancer (Hanahan et al., 2011).

Accumulating evidence shows that S100A4 proteins play central roles in the regulation of cell apoptosis. Knockdown of S100A4 induces cell apoptosis and enhances chemosensitivity through the induction of BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) expression. In contrast, S100A4 sensitizes tumor cells to IFN-gamma-mediated induction of apoptosis in parallel with activating NF-κB (Chen et al., 2014).
S100A4 protein role in tumor metastasis

S100A4 regulates cell motility and adhesion by interacting with Nonmuscle Myosin IIA (NMIIA) and Ephrin-beta 1, thus contributing to the metastatic behavior of tumor cells. In addition, extracellular S100A4 activates the transcription factor NF-κB and induces a series of gene products including Ephrin-A1 and optineurin in a subset of human cancer cells, which represent possible candidates responsible for S100A4-mediated metastatic progression (Grotterød et al., 2010).

In addition to stimulating the motility of tumor cells, S100A4 may affect cell invasive properties through influencing the expression of matrix metalloproteinases (MMPs) and their endogenous inhibitors (Matsuura, 2010).

Therefore, S100A4 protein is involved in many steps of metastasis and it has been well recognized as a metastasis marker, enhancing the migratory phenotype of tumor cell. Thus, it is considered an epithelial-mesenchymal transition (EMT) mediator (Lo et al., 2011).
Figure (10): Role of S100A4 in invasion and metastasis (Sherbet et al., 2009).

In brief, S100A4 is involved in tumorigenesis and metastatic potential of multiple cancers as melanoma, pancreatic adenocarcinoma, osteosarcoma, and prostate cancer. Thus, it could be used as a therapeutic target for cancer (Chen et al., 2014).
Materials and methods

This is a retrospective controlled study performed on formalin fixed paraffin embedded biopsy specimens from 42 Egyptian patients with different types of prostatic lesions. The specimens of 36 cases were obtained by radical prostatectomy, 4 cases were prostatic chips via TURB and 2 cases were prostatic cores.

They included 8 cases of benign prostatic hyperplasia were taken as a normal control and 34 cases of different Gleason grades of prostate adenocarcinoma.

The material included archival formalin fixed paraffin embedded blocks processed during the years 1997-2010. The blocks were collected from Department of Pathology, Benha faculty of medicine. From each block, two sections of 4um thickness were cut. One for H&E staining and the other section was maintained on positive charged slide for S100A4 immunohistochemical staining.

Histopathological studies:

Sections stained by conventional hematoxylin and eosin (H&E) stain were evaluated for reviewing of cases. Malignant prostatic lesions were evaluated and graded according to Gleason, (1992) into well, moderately and poorly differentiated tumors. Thirteen cases were of low grade, 11 cases were intermediate grade with Gleason score 7 and 10 cases were high-grade adenocarcinoma.

Immunohistochemical studies:

Immunohistochemical studies S100A4 antibody staining was performed for all biopsies using Avidin Biotin complex technique. For
such immunohistochemical marker the steps were as follow (Yong-Wook et al., 2010):

- Formalin-fixed, paraffin-embedded, 4 μm-thick sections were dewaxed in xylene,
- The sections then were rehydrated in graded solutions of alcohol.
- Sections then were placed in an endogenous peroxide-blocking solution for 15 min.
- Sections were placed in a citrate buffer (10% citrate buffer stock in distilled water, pH 6.0).
- Nonreactive staining was blocked by incubation with 1% horse serum in Tris-buffered saline (pH 6.0) for 3 min.
- The primary antibodies for S100A4 (Thermo Scientific, Fremont, CA 94538, USA) were incubated with the sections overnight.
- Antibody-binding was detected by use of a standard labeled streptavidin-biotin system (DakoCytomation, Denmark, A/S).
- Tonsil tissue was used as an external positive control.
- For negative controls, the primary antibodies were omitted.

**Immunohistochemical assessment:**

Sections were screened on low power. S100A4 expression was assessed according to Huang et al., (2011) using a scoring system that incorporates two variants, the intensity and extent of staining. The intensity of staining was scored as 0 (no staining), 1 weak (light yellow staining), 2 moderate (yellow to brown staining) and 3 strong (brown staining). The extent of staining was based on the percentage of positive tumor cells in the tow most predominant Gleason grades: 0 (less than 5%), 1 (6%-25%), 2 (26%-50%) and 3 (51%-100%). The final score was assessed by multiplying the results of intensity and extent of staining. The
case was considered negative if the final score was 0 or 1 (-) and positive if the score was 2 or 3 (+), 4 or 5 (++) or 6 or 7 (+++).  
The results were tabulated and analyzed using SPSS (version 16) statistical package for Microsoft windows (SPSS Inc., Chicago, IL, USA). The person correlation coefficient was used for statistical analysis. P value < 0.05 was considered statistically significant.
Results

This is a retrospective study including 42 cases of prostatic lesions. Eight cases (19%) were benign prostatic hyperplasia (BPH) were taken as control and the other 34 cases (81%) were prostatic adenocarcinoma. Thirteen cases (38.2%) of them were grade I, 11 cases (32.4%) were grade II and 10 cases (29.4%) were grade III.

Graph (1): Histopathological classification of malignant studied cases.
Clinico-pathological data:

- Age distribution in the examined cases:

  The age of all examined cases ranged between 45-81 years with the mean age 67 years. The age of the benign prostatic hyperplasia cases ranged from 45-69 years with the mean age 57 years, while the age of prostatic carcinoma cases ranged between 49-81 years with the mean age 70 years.

Table (1): Age groups of different examined cases.

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Age in years</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45-55</td>
<td>56-65</td>
</tr>
<tr>
<td>Grade I canceroma</td>
<td>13</td>
<td>1(7.7%)</td>
<td>6(46.2%)</td>
</tr>
<tr>
<td>Grade II canceroma</td>
<td>11</td>
<td>1(9%)</td>
<td>4(36.4%)</td>
</tr>
<tr>
<td>Grade III canceroma</td>
<td>10</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34</strong></td>
<td><strong>2(6%)</strong></td>
<td><strong>10(29%)</strong></td>
</tr>
</tbody>
</table>
**Histopathological results**

Among the examined 34 cases of prostatic adenocarcinoma, 13 of them (31%) were Grade I (low grade), 11 of them (26%) were Grade II (intermediate grade) and 10 of them (24%) were Grade III (high grade). Being 2 cases (6%) of Gleason score 4, 5 cases (15%) of score 5, 6 cases (18%) of score 6, 11 cases (32%) score 7, 4 cases (12%) of score 8 and 6 cases (18%) of Gleason score 9.

Sixteen (47%) out of the 34 malignant cases showed capsular invasion, 3 (18.8%) of them were low grade, another 3 (18.8%) were intermediate grade and the other 10 (62.5%) were high grade.

Eleven cases (32.4%) showed lymph nodal metastasis, one (9%) was intermediate grade carcinoma and 10 (91%) were high-grade carcinoma.

Out of the 34 malignant cases, 28 (82%) showed perineural invasion and 21 (62%) were associated with high grade PIN.

The staging of the 34 examined malignant cases revealed 8 cases (24%) stage I, 19 cases (56%) stage II and 7 cases (21%) stage III.

**Other clinico-pathological results:**

The preoperative serum PSA level in the 34 patients of prostatic adenocarcinoma revealed 12 cases (35%) with the level ≤ 20ng/dl, 10 cases (29%) with level >20-40ng/dl, 8 cases (24%) with level >40-60ng/dl and 4 cases (12%) with level > 60ng/dl.

The available 5 years follow up data of the 34 patients with prostatic adenocarcinoma revealed 25 survived cases (74%) and 9 cases (26%) couldn't survive.
Correlation between tumor Gleason grade and capsular invasion:

Out of 13 cases of Grade I (Gleason score 4-6) adenocarcinoma; three (23.1%) showed capsular invasion. All of them were scored as 5 on Gleason grading system. Out of 11 cases of Grade II adenocarcinoma (Gleason score 7), three (27%) showed extra capsular extension. However, all the 10 cases (100%) of Grade III adenocarcinoma (Gleason score 8-10) showed capsular invasion. Four of them were scored 8 and six were scored as 9.

There was a statistically significant positive correlation between Gleason grade of the tumor and capsular invasion (p value < 0.05).

Table (2): Correlation between Gleason grade of the tumor and presence of capsular invasion:

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Capsular invasion</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>absent</td>
<td>Present</td>
</tr>
<tr>
<td>Low grade (13 cases)</td>
<td>10(77%)</td>
<td>3(23%)</td>
</tr>
<tr>
<td>Intermediate grade (11 cases)</td>
<td>8(73%)</td>
<td>3(27%)</td>
</tr>
<tr>
<td>High grade (10 cases)</td>
<td>0</td>
<td>10(100%)</td>
</tr>
<tr>
<td>Total (34 cases)</td>
<td>18(53%)</td>
<td>16(47%)</td>
</tr>
</tbody>
</table>
Results

❖ **Correlation between Gleason grade of the tumor and presence of lymph node metastasis.**

Out of 11 cases of intermediate grade adenocarcinoma, only 2 (18%) showed lymph node metastasis while all the 10 cases of high grade adenocarcinoma (100%) had lymph node spread, however no case of low grade adenocarcinoma showed lymph node metastasis.

There was a statistically significant positive correlation between the Gleason grade of the tumor & the lymph node metastasis (p value < 0.01).

**Table (3): Correlation between Gleason grade of the tumor and presence of lymph node metastasis.**

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Lymph node metastasis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Low grade (13 cases)</td>
<td>13(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate grade (11 cases)</td>
<td>9(82%)</td>
<td>2(18%)</td>
</tr>
<tr>
<td>High grade (10 cases)</td>
<td>0(0%)</td>
<td>10(100%)</td>
</tr>
<tr>
<td>Total (34 cases)</td>
<td>22(65%)</td>
<td>12(35%)</td>
</tr>
</tbody>
</table>
Results

*Correlation between Gleason grade of the tumor and perineural invasion:*

All the 10 high-grade cases (100%) showed perineural invasion, 10 cases (91%) out of the 11 of the intermediate grade adenocarcinoma showed perineural invasion and 8 (62%) out of the 13 cases of low grade adenocarcinoma showed perineural invasion.

There was a statistically significant positive correlation between the Gleason grade of the tumor and perineural invasion (p value < 0.05).

**Table (4): Correlation between Gleason grade of the tumor and the perineural invasion:**

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Neural invasion</th>
<th></th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>5(38%)</td>
<td>8(62%)</td>
<td>13(38.2%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>1(9%)</td>
<td>10(91%)</td>
<td>11(32.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High grade</td>
<td>0</td>
<td>10(100%)</td>
<td>10(29.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6(18%)</td>
<td>28(82%)</td>
<td>34(100%)</td>
<td></td>
</tr>
</tbody>
</table>
Correlation between Gleason grade of the tumor and preoperative serum PSA level:

In the 13 low-grade adenocarcinoma cases, seven patients (54%) had serum PSA level <20 ng/dl, 5 patients (38%) had level 21-40 ng/dl and only one (8%) had the level of 41-60 ng/dl.

Among the 11 cases of intermediate carcinoma, 5 (45%) had the PSA level below 20 ng/dl, another 5 (45%) had it between 21ng/dl & 40 ng/dl and only one (9%) had the level 41-60 ng/dl.

Out of the 10 high-grade cases, 6 (60%) had 41-60 ng/dl PSA serum level and the other four (40%) had it >60 ng/dl.

There was a statistically significant positive correlation between Gleason grade of the tumor and preoperative serum level of PSA (p value < 0.05).

Table (5): Correlation between Gleason grade of the tumor and preoperative serum PSA level.

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>PSA before</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-20ng/dl</td>
<td>21-40ng/dl</td>
<td>41-60ng/dl</td>
<td>&gt;60ng/dl</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>7(54%)</td>
<td>5(38%)</td>
<td>1(8%)</td>
<td>0</td>
<td>13(38.2%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>5(45%)</td>
<td>5(45%)</td>
<td>1(9%)</td>
<td>0</td>
<td>11(32.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High grade</td>
<td>0</td>
<td>0</td>
<td>6(60%)</td>
<td>4(40%)</td>
<td>10(29.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12(35%)</td>
<td>10(29%)</td>
<td>8(24%)</td>
<td>4(12%)</td>
<td>34(100%)</td>
<td></td>
</tr>
</tbody>
</table>
Correlation between Gleason grade of the tumor and presence of High grade PIN:

Six (46%) Out of 13 cases of low grade adenocarcinoma were associated with high grade PIN, 7 (64%) out of 11 cases of intermediate grade adenocarcinoma were associated with high grade PIN and 8 cases (80%) out of 10 high grade adenocarcinoma were associated with high grade PIN.

There was a statistically significant positive correlation between the Gleason grade of the tumor and the presence of high grade PIN (p value < 0.01).

Table (6): Correlation between Gleason grade of the tumor and presence of High grade PIN.

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Total</th>
<th>High PIN</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absent</td>
<td>present</td>
</tr>
<tr>
<td>Low grade.</td>
<td>13</td>
<td>7(54%)</td>
<td>6(46%)</td>
</tr>
<tr>
<td>Intermediate grade.</td>
<td>11</td>
<td>4(36%)</td>
<td>7(64%)</td>
</tr>
<tr>
<td>High grade.</td>
<td>10</td>
<td>2(20%)</td>
<td>8(80%)</td>
</tr>
<tr>
<td>Total.</td>
<td>34</td>
<td>13(38%)</td>
<td>21(62%)</td>
</tr>
</tbody>
</table>
Results

- **Correlation between Gleason grade and stage of adenocarcinoma cases:**

  Out of the 13 low-grade cases, 8 (62%) were stage II, 4 (31%) were stage I and only one (8%) was stage III.

  Five (45%) out of intermediate grade cases were stage II, 4 (36%) were stage I and only 2 (18%) were stage III.

  Out of the high-grade cases, 6 (60%) were stage II and four (40%) were stage III.

  There was no significant statistical correlation between the Gleason grade and the stage of the tumor in all studied malignant cases (p value > 0.05).

**Table (7): Correlation between Gleason grade and stage of adenocarcinoma cases.**

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Total</th>
<th>Stage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Low grade</td>
<td>13(38.2%)</td>
<td>4(31%)</td>
<td>8(62%)</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>11(32.4%)</td>
<td>4(36%)</td>
<td>5(45%)</td>
</tr>
<tr>
<td>High grade</td>
<td>10(29.2%)</td>
<td>0</td>
<td>6(60%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>8(23.5%)</td>
<td>19(56%)</td>
</tr>
</tbody>
</table>
Results

◆ Correlation between Gleason grade of the tumor and 5 years survival:

Nine (69%) out of the 13 low-grade cases survived for 5 years, 8(73%) among the 11 cases of intermediate grade adenocarcinoma could survive for 5 years and eight (80%) out of the 10 high-grade cases survived for 5 years.

There was no significant statistical correlation between the Gleason grade and the 5 years survival (p value > 0.05).

Table (8): Correlation between Gleason grade of the tumor and 5 years survival.

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>5 years survival</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survived patients</td>
<td>Not survived patients</td>
</tr>
<tr>
<td>Low grade</td>
<td>9(69%)</td>
<td>4(31%)</td>
</tr>
<tr>
<td>High grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>8(73%)</td>
<td>3(27%)</td>
</tr>
<tr>
<td>High grade</td>
<td>8(80%)</td>
<td>2(20%)</td>
</tr>
<tr>
<td>Total</td>
<td>25(74%)</td>
<td>9(26%)</td>
</tr>
</tbody>
</table>
Table (9): Correlation between Gleason grade of the tumor and other Clinico-pathological parameters:

<table>
<thead>
<tr>
<th>Clinico-pathological parameters</th>
<th>Gleason grade</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low grade (13 cases)</td>
<td>Intermediate Grade (11 cases)</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>10 (77%)</td>
<td>8 (73%)</td>
</tr>
<tr>
<td>Present</td>
<td>3 (23%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>13 (100%)</td>
<td>9 (82%)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>High grade PIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>7 (54%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>Present</td>
<td>6 (46%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5 (38%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (62%)</td>
<td>10 (91%)</td>
</tr>
<tr>
<td>PSA before resection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20ng/dl</td>
<td>7 (54%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>21-40ng/dl</td>
<td>5 (38%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>41-60ng/dl</td>
<td>1 (8%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>&gt;60ng/dl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stage of the tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 (30.8%)</td>
<td>4 (36.3%)</td>
</tr>
<tr>
<td>II</td>
<td>8 (61.5%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>III</td>
<td>1 (7.7%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>5 years survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>9 (69%)</td>
<td>8 (73%)</td>
</tr>
<tr>
<td>Didn't survive</td>
<td>4 (31%)</td>
<td>3 (27%)</td>
</tr>
</tbody>
</table>
Results

Figure (11): Benign prostatic hyperplasia (BPH) (H&E X100).

Figure (12): Grade I prostatic adenocarcinoma Gleason score 6 showing infiltrating irregular glands (H&E x 200).
Results

Figure (13): Grade I prostatic adenocarcinoma Gleason score 6 showing infiltrating irregular glands (H&E x 600).

Figure (14): Prostatic glands showing high grade PIN lined by atypical pleomorphic cells with prominent nuclei and preserved myoepithelial cell layer (H&E x 200).
Figure (15): Grade II prostatic adenocarcinoma Gleason score 7 showing infiltrating irregular glands and small solid masses of malignant cells (H&E x 200).

Figure (16): Grade II prostatic adenocarcinoma Gleason score 7 showing infiltrating irregular glands and small solid masses of malignant cells (H&E x 400).
Figure (17): Grade II prostatic adenocarcinomas Gleason score 7 showing perineural invasion (H&E x 400).

Figure (18): Grade III prostatic adenocarcinomas Gleason score 9 showing solid sheets of malignant cells (H&E x 200).
Results

Figure (19): Grade III prostatic adenocarcinomas Gleason score 9 showing solid sheets of malignant cells (H&E x 400).

Figure (20): Grade III prostatic adenocarcinomas Gleason score 8 showing solid masses of malignant cells and irregular malignant glands. (H&E x 200).
Results

- **Immunohistochemical staining results:**

  S100A4 was detected as cytoplasmic and occasionally nuclear brown granules with different expression patterns in different prostatic studied cases.

  - **S100A4 expression in control cases:**

    In benign prostatic hyperplasia (BPH), scattered rare epithelial cells were S100A4 immunoreactive which were considered negative except for only 2 cases which showed low S100A4 positive expression score (+).

  - **S100A4 expression in adenocarcinoma cases:**

    Out of 34 adenocarcinoma cases, 8 (24%) showed score (+), another eight (24%) showed score (++), 11 (32%) showed score (+++) and 6 (18%) showed score (++++) of S100A4 positivity, however only one case was negative for S100A4.

  
  Table (10): Immunohistochemical staining results.

<table>
<thead>
<tr>
<th>Case type</th>
<th>No. of cases</th>
<th>S100A4 score</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign hyperplasia</td>
<td>8</td>
<td>6(75%)</td>
<td>2(25%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>34</td>
<td>1(3%)</td>
<td>8(24%)</td>
<td>8(24%)</td>
<td>11(32%)</td>
<td>6(18%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>7(17%)</td>
<td>10(24%)</td>
<td>8(19%)</td>
<td>11(26%)</td>
<td>6(14%)</td>
<td></td>
</tr>
</tbody>
</table>
Results

*Correlation between Gleason grade and score of S100A4 expression in prostatic adenocarcinoma cases:*

Out of 13 cases of low-grade adenocarcinoma, only one (8%) was negative score (-) and 12 cases (92%) showed positive expression. Six cases (46%) showed score (+), five cases (38%) showed score (+++) and only one (8%) showed score (+++).

All the 11 cases (100%) of intermediate grade adenocarcinoma showed positive expression. Two (18%) showed score (+), 3 (27%) showed score (++) and 6 cases (55%) showed score (+++).

All the 10 cases (100%) of high-grade adenocarcinoma showed positive expression. Four cases (40%) showed score (+++) and the other 6 (60%) showed score (++++).

There was a significant statistical positive correlation between the Gleason grade and the score of S100A4 expression (P value < 0.05).

**Table (11): Correlation between Gleason grade and the score of S100A4 expression in prostatic adenocarcinoma cases.**

<table>
<thead>
<tr>
<th>Gleason grade</th>
<th>Immunohistochemical score of S100A4 expression</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>-</td>
</tr>
<tr>
<td>Low grade</td>
<td>13</td>
<td>1(8%)</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>High grade</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
</tr>
</tbody>
</table>
Results

Graph (2): Correlation between Gleason grade and score of S100A4 expression in prostatic adenocarcinoma cases.
Results

❖ Correlation between Gleason score and score of S100A4 expression in prostatic cancer cases:

The two cases (100%) with Gleason score 4 expressed S100A4 by score (+), two (40%) out of the 5 cases with Gleason score 5 expressed S100A4 with score (+), another 2 cases (40%) showed score (++) and only one case (20%) was negative (-).

Out of the 6 cases with Gleason score 6, 3 (50%) showed S100A4 expression score (++), 2 (34%) showed score (+) and only one case (17%) showed score (+++).

Among the 11 cases of Gleason score 7, six (66%) showed S100A4 score (+++), 3 (27%) expressed S100A4 with score (++) and only 2 cases (18%) showed score (+).

The 4 cases with Gleason score 8 showed S100A4 expression scores (+++) and (++++) by (50%) for each. Out of the 6 cases with Gleason score 9, four (67%) showed S100A4 score (++++) and 2 (33%) showed score (+++).

There was a significant statistical correlation between the Gleason score of the prostatic adenocarcinoma and the score of S100A4 expression (P value ≤ 0.01).
Table (12): Correlation between Gleason score and score of S100A4 expression in prostate cancer cases.

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>Total</th>
<th>(-)</th>
<th>(+)</th>
<th>(++)</th>
<th>(+++)</th>
<th>(++++)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 4</td>
<td>2</td>
<td>0(0%)</td>
<td>2(100%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>≤ 0.05</td>
</tr>
<tr>
<td>Score 5</td>
<td>5</td>
<td>1(20%)</td>
<td>2(40%)</td>
<td>2(40%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Score 6</td>
<td>6</td>
<td>0(0%)</td>
<td>2(33.3%)</td>
<td>3(50%)</td>
<td>1(16.7%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Score 7</td>
<td>11</td>
<td>0(0%)</td>
<td>2(18%)</td>
<td>3(27%)</td>
<td>6(55%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Score 8</td>
<td>4</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>2(50%)</td>
<td>2(50%)</td>
<td></td>
</tr>
<tr>
<td>Score 9</td>
<td>6</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>2(33%)</td>
<td>4(67%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
<td>8(23.5%)</td>
<td>11(32.4%)</td>
<td>6(17.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Graph (3): Correlation between Gleason score and score of S100A4 expression in prostate cancer cases.
Results

*Correlation between capsular invasion and score of S100A4 expression in prostate cancer cases:*

Out of the 16 malignant cases with capsular invasion, 6 showed score (+++), 6 showed score (++), 3 were score (+) and only one case was score (-) S100A4. However none of the eight cases with score (++) showed capsular invasion.

There was a significant positive statistical correlation between capsular invasion and the score of S100A4 expression (p value < 0.05).

Table (13): Correlation between capsular invasion and score of S100A4 expression in prostate cancer cases.

<table>
<thead>
<tr>
<th>Capsular invasion</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>(-)</td>
</tr>
<tr>
<td>Absent</td>
<td>18(53%)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>16(47%)</td>
<td>1(6%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
</tr>
</tbody>
</table>
Graph (4): Correlation between capsular invasion and score of S100A4 expression in prostate cancer cases.
Results

- **Correlation between presence of lymph node metastasis and score of S100A4 expression in prostate cancer cases:**

  Six (55%) out of the 11 cases of score (+++) and all the 6 cases (100%) with score (++++) showed lymph node metastasis, however there were no cases with (-), (+) and (++) S100A4 score showed lymph node metastasis.

  There was a significant statistical positive correlation between the presence of lymph node metastasis and S100A4 expression score (p value < 0.05).

**Table (14): Correlation between presence of lymph node metastasis and score of S100A4 expression in prostate cancer cases.**

<table>
<thead>
<tr>
<th>Lymph node metastasis</th>
<th>Total</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>Absent</td>
<td>22(65%)</td>
<td>1(100%)</td>
<td>8(100%)</td>
</tr>
<tr>
<td>Present</td>
<td>12(35%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
</tr>
</tbody>
</table>
Graph (5): Correlation between presence of lymph node metastasis and score of S100A4 expression in prostate cancer cases.
Results

*Correlation between preoperative serum PSA level and score of S100A4 expression in all prostate cancer cases:*

Out of the 34 prostate cancer cases:

There were 12 cases (35%) with preoperative serum PSA level of 0-20ng/dl. Five (42%) out of them were S100A4 score (+), 3 (25%) were score (++), 3 (25%) showed score (+++) and only one case showed score (-). While no cases showed score (+++).

Ten cases (29%) had serum PSA level >20-40 ng/dl, 5 (50%) out of them showed (+) score, 4 (40%) showed score (+++) and only one case (10%) showed (+) score. So, no cases showed (-) or (+++) score.

Eight cases (24%) had >40-60 ng/dl PSA level. Four of them (50%) expressed S100A4 with score (+++), two (25%) with score (+++) and another two cases (25%) were score (+). Thus, no cases showed score (-) nor (+).

Four cases (12%) showed serum PSA level > 60 ng/dl, 2 (50%) out of them were score (+++) and the other two cases (50%) were score (+++). While none of them showed score (-), (+) or (++).

There was a significant statistical positive correlation between preoperative serum PSA level and score of S100A4 expression (p value < 0.05).
Results

Table (15): Correlation between preoperative serum PSA level and score of S100A4 expression in prostate cancer cases.

<table>
<thead>
<tr>
<th>Preoperative serum PSA level</th>
<th>Total</th>
<th>(-)</th>
<th>(+)</th>
<th>(++)</th>
<th>(+++)</th>
<th>(++++)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20 ng/dl</td>
<td>12(35%)</td>
<td>1(8%)</td>
<td>5(42%)</td>
<td>3(25%)</td>
<td>3(25%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;2-40 ng/dl</td>
<td>10(29%)</td>
<td>0</td>
<td>1(10%)</td>
<td>5(50%)</td>
<td>4(40%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;40-60 ng/dl</td>
<td>8(24%)</td>
<td>0</td>
<td>2(25%)</td>
<td>0</td>
<td>2(25%)</td>
<td>4(50%)</td>
<td></td>
</tr>
<tr>
<td>&gt;60 ng/dl</td>
<td>4(12%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(50%)</td>
<td>2(50%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
<td>8(23.5%)</td>
<td>11(32%)</td>
<td>6(18%)</td>
<td></td>
</tr>
</tbody>
</table>

Graph (6): Correlation between preoperative serum PSA level and score of S100A4 expression in all prostate cancer cases.
Correlation between stage and score of S100A4 expression in prostate cancer cases:

In the 34 studied malignant cases there were 8 (24%) stage I, 19 (56%) stage II and 7 cases (21%) stage III.

Among the stage I cases, three (38%) showed score (+) of S100A4 expression, another 3 (38%) showed score (++) and two cases (25%) showed score (+++). However no cases showed score (-) nor (++++).

Out of the 19 cases of stage II, 5 (26%) showed score (++) , another 5 (26%) showed score (++++), 4 (21%) showed score (+) 4 other showed score (++ ++), however only one case (5%) was negative score (-).

In stage III cases; 4 (57%) showed score (+++), 2 (29%) showed score (++++) and only one case (14%) showed score (+). However no cases showed score (-) or score (++).

There was no significant statistical correlation between stage and score of S100A4 expression in prostate cancer cases (p value >0.05).
Table (16): Correlation between stage and score of S100A4 expression in prostate cancer cases.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>I</td>
<td>8(24%)</td>
<td>0</td>
<td>3(38%)</td>
</tr>
<tr>
<td>II</td>
<td>19(56%)</td>
<td>1(5%)</td>
<td>4(21%)</td>
</tr>
<tr>
<td>III</td>
<td>7(21%)</td>
<td>0</td>
<td>1(14%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
</tr>
</tbody>
</table>

Graph (7): Correlation between stage and score of S100A4 expression in prostate cancer cases.
Results

*Correlation between neural invasion and score of S100A4 expression in prostate cancer cases:*

In the whole 34 studied malignant cases, 28 (82%) showed perineural invasion. Six cases were score (++++), 11 were score (+++), five were score (++), 5 were score (+) and only one case was negative score (-).

There was no significant statistical correlation between the presence of perineural invasion and S100A4 score in malignant cases (p value >0.05).

**Table (17): Correlation between neural invasion and score of S100A4 expression in prostate cancer cases.**

<table>
<thead>
<tr>
<th>Neural invasion</th>
<th>Total</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>Negative</td>
<td>6(18%)</td>
<td>0</td>
<td>3(38%)</td>
</tr>
<tr>
<td>Positive</td>
<td>28(82%)</td>
<td>1(100%)</td>
<td>5(63%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
</tr>
</tbody>
</table>
Graph (8): Correlation between perineural invasion and score of S100A4 expression in prostate cancer cases
Correlation between presence of high grade PIN and score of S100A4 expression in prostate cancer cases:

Three cases (38%) out of the eight of score (+), 5 (63%) out of 8 cases of score (++) , 9 (82%) out of 11 cases of score (+++) and 4 (67%) out of 6 cases with score (++++) were associated with high grade PIN. The only S100 A4 negative case was not associated with high grade PIN.

There was no significant statistical correlation between associated high grade PIN and score of S100A4 expression in all examined malignant cases (p value >0.05).

Table (18): Correlation between presence of high grade PIN and score of S100A4 expression in prostate cancer cases:

<table>
<thead>
<tr>
<th>High grade PIN</th>
<th>Total</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>Absent</td>
<td>13</td>
<td>1(8%)</td>
<td>5(38%)</td>
</tr>
<tr>
<td>Present</td>
<td>21</td>
<td>0</td>
<td>3(14%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
</tr>
</tbody>
</table>
Graph (9): Correlation between presence of high grade PIN and score of S100A4 expression in prostate cancer cases.
Results

Correlation between 5 years survival and score of S100A4 expression in prostate cancer cases:

Out of the 25 survived cases for 5 years, 7 (28%) expressed S100A4 (+) score, 10 showed S100A4 score (+++), four (16%) showed score (++) and 4 other cases (16%) showed score (++__). Thus, no cases showed (-) score.

Four cases (44%) out of the nine who didn’t survive showed S100A4 score (++), 2 (22%) showed score (++++) and only 3 case (33%) distributed evenly between the scores of (-), (+) and (+++).

There was no significant statistical correlation between 5 years survival and score of S100A4 expression in prostate cancer cases (p value >0.05).
Table (19): Correlation between 5 years survival and score of S100A4 expression in prostate cancer cases.

<table>
<thead>
<tr>
<th>5 years survival</th>
<th>Total</th>
<th>S100A4 score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Survived</td>
<td>25(74%)</td>
<td>0</td>
<td>7(28%)</td>
</tr>
<tr>
<td>Didn't survive</td>
<td>9(26%)</td>
<td>1(11%)</td>
<td>1(11%)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>1(3%)</td>
<td>8(23.5%)</td>
</tr>
</tbody>
</table>

Graph (10): Correlation between 5 years survival and score of S100A4 expression in prostate cancer cases.
Table (20): Correlations between score of S100A4 expression and other Clinico-pathological parameters:

<table>
<thead>
<tr>
<th>Clinico-Pathological parameters</th>
<th>Score of S100A4 expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>Gleason grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>1(8%)</td>
<td>6(46%)</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>0</td>
<td>2(18%)</td>
</tr>
<tr>
<td>High grade</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Capsular invasion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
<td>5(55%)</td>
</tr>
<tr>
<td>Present</td>
<td>1(100%)</td>
<td>4(45%)</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1(100%)</td>
<td>8(100%)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Serum PSA before resection of the tumor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-20 ng/dl</td>
<td>1(8%)</td>
<td>5(42%)</td>
</tr>
<tr>
<td>21-40 ng/dl</td>
<td>0</td>
<td>1(10%)</td>
</tr>
<tr>
<td>41-60 ng/dl</td>
<td>0</td>
<td>2(25%)</td>
</tr>
<tr>
<td>&gt;60 ng/dl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Stage of adenocarcinoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>0</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>1(5.2%)</td>
<td>4(21.1%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>0</td>
<td>1(14%)</td>
</tr>
<tr>
<td><strong>Perineural invasion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td>Positive</td>
<td>1(100%)</td>
<td>5(62.5%)</td>
</tr>
<tr>
<td><strong>High grade PIN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1(100%)</td>
<td>5(62.5%)</td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td><strong>5 years survival</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>0</td>
<td>7(28%)</td>
</tr>
<tr>
<td>Didn't survive</td>
<td>1(11.1%)</td>
<td>1(11.1%)</td>
</tr>
</tbody>
</table>
Figure (21): Benign prostatic hyperplasia showing (-) score of S100A4 expression (streptavidin-biotin x 100).

Figure (22): Grade I prostatic adenocarcinoma (Gleason score 6) showing (+++) score of S100A4 expression (streptavidin-biotin x 400).
Figure (23): Grade I prostatic adenocarcinoma (Gleason score 6) showing (++) score of S100A4 expression (streptavidin-biotin x 400).

Figure (24): Grade II prostatic adenocarcinoma (Gleason score 7) showing (+++) score of S100A4 expression (streptavidin-biotin x 400).
Results

Figure (25): Grade II prostatic adenocarcinoma (Gleason score 7) with (+++) score of S100A4 expression showing perineural invasion (streptavidin-biotin x 400).

Figure (26): Grade II prostatic adenocarcinoma (Gleason score 7) showing (++) score of S100A4 expression (streptavidin-biotin x 400).
Results

Figure (27): Grade II prostatic adenocarcinoma (Gleason score 7) showing (++) score of S100A4 expression (streptavidin-biotin x 400).

Figure (28): Grade III prostatic adenocarcinoma (Gleason score 8) showing (++++) score of S100A4 expression (streptavidin-biotin x 200).
Figure (29): Grade III prostatic adenocarcinoma (Gleason score 8) showing (++++) score of S100A4 expression (streptavidin-biotin x 400).

Figure (30): Grade III prostatic adenocarcinoma (Gleason score 8) showing (++++) score of S100A4 expression (streptavidin-biotin x 600).
Results

Figure (31): Grade III prostatic adenocarcinoma (Gleason score 8) showing (++++) score of S100A4 expression (streptavidin-biotin x 400).

Figure (32): Grade III prostatic adenocarcinoma (Gleason score 9) showing (++++) score of S100A4 expression (streptavidin-biotin x 400)
Discussion

Worldwide, Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males. (Baade et al., 2009). More than 1.1 million cases of prostate cancer were recorded in 2012, accounting for around 8% of all new cancer cases and 15% in men (Ferlay et al., 2013).

In Egypt, according to National Cancer Institute, Cairo University, it accounts for 8.5% of total malignancies being the fifth most common male cancer after urinary bladder cancer, liver cancer, Non-Hodgkin lymphoma and lung cancer (Mokhtar et al., 2007).

However according to El-Bolkainy et al, (2013), prostate cancer formed the majority of male genital cancer (60.7%) in Egypt in the last 10 years with median age 72.8 years.

The present study included 42 cases of prostatic lesions. Eight cases (19%) of benign prostatic hyperplasia (BPH) were taken as control and 34 cases (81%) of prostatic adenocarcinoma.

In the present study, there was a statistically significant positive correlation between the Gleason grade of the tumor & the capsular invasion (p value < 0.05). This result was in agreement with a study carried by Helpap et al., (2008), who found a statistically significant positive correlation between the Gleason grade of the prostate adenocarcinoma & the capsular invasion.

Eleven cases (32.4%) out of the 34 malignant cases showed lymph nodal metastasis, one case (9%) was intermediate grade carcinoma and 10 cases (91%) were high-grade carcinoma. This provides a statistically significant positive correlation between the Gleason grade of the tumor & the lymph node metastasis (p value < 0.01). The same results was stated by Ross et al., (2012) who found that prostate cancers with Gleason grade ≤ 6 rarely metastasize to the lymph nodes.
There was also a statistically significant positive correlation between Gleason grade of the tumor and perineural invasion (p value < 0.05) and this was in agreement with study of Liebig et al., (2009) who found similar results suggesting that perineural invasion might predict occult, high-grade disease in otherwise low-risk patients.

The preoperative serum levels of PSA were higher in patients having tumors with higher Gleason grades in a statistically significant positive correlation (p value < 0.05). Attard et al., (2008) agreed with the current study regarding such correlation.

In contrast to the current study were Hessels et al., (2010) found no significant correlation between the Gleason grade and the preoperative serum level of PSA in prostate adenocarcinoma cases.

The contrasting results may be due to the retrospective manner of the current study and differences in lab. kits used in measuring PSA level.

In the current study, a statistically significant positive correlation was found between adenocarcinoma grade and frequency of high-grade PIN association (p value < 0.01). In contrast were Moussa et al., (2009) found statistically significant negative correlation between the Gleason grade of the tumor and the presence of high grade PIN. These contrasting results may be due the limited number of cases in the current study.

There were no statistical significant correlations between Gleason grade and prostatic carcinoma stage. This was in accordance with Hessels et al., (2010).

The present work found no significant correlation between Gleason grade of prostate adenocarcinoma and 5 years survival of the patients. In contrast to studies carried out by Attard et al., (2008), Epstein et al., (2010) and Penney et al., (2011) in which they found a significant
positive correlation between the Gleason grade and 5 years survival of prostate cancer.

The contrasting findings between the current study and other studies may be referred to the different number of cases and follow up methods.

S100A4, a member of the S100 family of proteins, is involved in tumorigenesis and metastatic potential of multiple cancers as melanoma, pancreatic adenocarcinoma and osteosarcoma (Chen et al., 2014).

According to Immunohistochemical results, S100A4 was detected as cytoplasmic and occasionally nuclear brown granules with differences in the intensity and prevalence of expression in benign prostatic hyperplasia and different Gleason grades of prostatic adenocarcinoma.

In benign prostatic hyperplasia (BPH), scattered rare glandular epithelial cells were S100A4 immunoreactive. These results were in agreement with Kwon et al., (2010) and Shrawan et al., (2011), who found that S100A4 immunoreactivity in benign prostatic hyperplasia was scattered and rare.

In addition, Yutaka et al., (2000) observed that S100A4 expression was faint in the cytoplasm of the normal mucosa, but strong in the cytoplasm of lymphocyte and smooth muscle of gastric tissue specimens. Wang et al., (2010) reported that S100A4 protein was detected in 12 of 92 (13.04%) human non-tumorous gastric mucosa samples, and all samples expressed the protein at a low level.

In the present study there was a significant statistical positive correlation between Gleason score and that of S100A4 expression, as the score of expression increases with the progression from lower Gleason scores to higher scores (P value < 0.05).

These results were in agreement with results reported by Yong-Wook et al., (2010) who noticed a progression in S100A4
immunoreactivity according to the pathologic grade (Gleason score) (p<0.05). These were the same results of Hifzur et al., (2013) who found that S100A4 is not merely a metastatic protein but also an oncoprotein that plays a critical role in the development of prostate cancer, as S100A4 showed progressively increasing expression in prostatic tissues with the advancement of the grade of prostate cancer.

Cho et al., (2005) found that S100A4 expression has been proved to be a highly significant and independent prognostic marker showing a significant correlation between over-expression of S100A4 and grade progression of colorectal cancer. This may indicate tumor cell aggressiveness.

Ran et al., (2007) on his study on endometrium found that expression of S100A4 was expressed in the tumor cell cytoplasm of poorly differentiated tumors, but was not detected in low-grade tumors.

In addition, Hua et al., (2013) showed a significant positive correlation between S100A4 over expression and gastric carcinoma grade increase, indicating the significant role of S100A4 in tumor cell prognosis.

In contrast, Rud et al., (2012) found in his work on non-small cell lung cancer (NSCLC) that S100A4-positive tumors were smaller and more differentiated than S100A4 negative tumors. What might explain these unexpected results that S100A4 is associated with a non-aggressive phenotype in NSCLC, could be that in contrast to one of the most important biological functions contributing to S100A4-induced metastasis which is increased invasive capacity. However, induction of S100A4 has also been shown to decrease motility and invasiveness in some tumors such as in squamous cell carcinoma (Uozumi et al., 2000). Down-regulation of S100A4 in astrocytes was found to increase their migratory capacity in vitro (Fang et al., 2006).
Furthermore, certain lines of evidence suggest that S100A4 may have tumor suppressor functions in the lung. S100A4 knockout mice, that were otherwise phenotypically normal, were prone to spontaneous tumor development, and the most frequent tumor observed was carcinoma of the lung (El-Naaman et al., 2004).

Taken together, these results indicate that the biological function of S100A4 is cell type-dependent, and possibly, S100A4 may not play a pro-metastatic role in all tumor types. One might also speculate that S100A4 could inhibit tumor progression in the early stages of NSCLC development, while promoting metastasis at later disease stages (Massague., 2008).

In the present study, there was a significant statistical correlation between S100A4 score and the presence of capsular invasion and/or lymph node metastasis (p value < 0.05). However there was no significant statistical correlation between the score of S100A4 and the presence of perineural invasion (p value >0.05).

In an agreement with the current study, a study carried out by Yong-Wook et al., (2010) who observed high expression of S100A4 in invasive prostate cancer. These results indicate that S100A4 protein may be associated with invasion, and metastasis of cancer prostate.

Pedersen et al., (2002) also found an association between S100A4 expression and an aggressive tumor phenotype in his study on breast cancers.

The same results were reached by Zou et al., (2005) in his study on thyroid tissue as he found that S100A4 expression was much higher at the tumour-invading front and in the metastatic tumors as compared to the primary tumors and suggested that overexpression of S100A4 is associated with thyroid tumor invasion and metastasis and may be a potential target for therapeutic intervention.
Yanqiong et al., (2013) also found that there is a significant association between high S100A4 expression and the presence of lymph node metastasis in his study on colorectal carcinoma.

The increase of the expression of S100A4 with cancer progression and metastasis could be explained by the ability of S100A4 to stimulate motility of endothelial cells, promoting tumor-induced angiogenesis and plasmin formation, and stimulating remodeling of the extracellular matrix via production of extracellular-matrix-degrading enzymes from endothelial cells, through transcriptional activation of collagenase 3 (MMP-13) mRNA (Semov et al., 2005, Saleem et al., 2006 and Wang et al., 2010).

In contrast to the current work kim et al., (2009), Kwak et al., (2010) and Wang et al., (2010) in their studies on colorectal carcinoma (CRC) and Ai et al., (2008) on his work on pancreatic carcinoma didn't find any correlation between S100A4 expression and either aggressiveness or lymph node metastasis.

These discrepancies could arise from subtle variations in the number of patients, observation period, stage distribution, tissue fixation methods, use of antibodies, and cutoff of positivity.

In the present study, there was a significant statistical correlation between the score of S100A4 expression and the preoperative serum level of PSA as the score of S100A4 increase with higher levels of serum PSA (p value < 0.05). However There was no significant statistical correlation between the score of S100A4 expression and the tumor stage of examined malignant cases (p value >0.05).

On the contrary, Ai et al., (2008) in his work on pancreatic cancers found that pancreatic cancer with a large size and high TNM stage had a higher S100A4 expression.
Kwon et al., (2010) observed that the PSA level of patients was significantly different among groups according to clinical stage, but not significantly different among groups according to pathologic grade. A significant progressive increase in S100A4 expression was observed in cancer specimens according to clinical stage and the pathologic grade (Gleason score) \( (p < 0.05) \).

Liu et al., (2013) found that there was a significant association between high S100A4 expression and advanced TNM stage in his work on colorectal carcinoma. Also Ran et al., (2007) in his work on endometrial carcinoma found an increase of S100A4 expression with the stage being significantly higher in stage III and IV tumors compared with stage I.

There was no significant statistical correlation between the score of S100A4 expression and the 5 years survival of the patients in the current work \( (p \text{ value} > 0.05) \).

However Kwon et al., (2010) found that during about 5 years of follow-up, 10 of 67 (14.9%) patients had a biochemical relapse based on PSA levels. He found that the length of biochemical relapse-free survival was shorter for those with higher expression of S100A4.

Also Kang et al., (2012) found that S100A4 protein expression is an independent prognostic marker correlated with low overall survival in patients with Dukes stage A and B colorectal cancer.

There was no significant statistical correlation between the score of S100A4 expression and the presence of associated high grade PIN in the current study.
Summary

Cancer prostate is the fifth most common male cancer in Egypt after urinary cancers, liver cancer, Non-Hodgkin lymphoma and lung cancer.

S100A4 is a marker that has been proposed to be implicated in tumorigenesis, invasion and metastasis of many malignant tumors.

The aim of this study was to evaluate the expression of S100A4 in benign prostatic hyperplasia and prostate adenocarcinoma cases and to compare these results with the clinicopathological data trying to assess the role of S100A4 in prostate adenocarcinoma. Statistical analysis was made for all grades.

This retrospective study was carried on 42 cases of prostatic lesions. Eight cases of benign prostatic hyperplasia (BPH) were taken as control and 34 cases of prostatic adenocarcinoma, 13 cases of them were grade I, 11 cases were grade II and 10 cases were grade III.

Histopathological examination of the cases revealed significant positive statistical correlations between Gleason grade of prostate cancer and capsular invasion, lymph node metastasis, associated high grade PIN, perineural invasion and preoperative PSA level. However, no significant statistical correlations were found between Gleason grade and the stage or 5 years survival.

S100A4 immunostaining was performed for each case and was correlated with clinicopathological parameters. S100A4 was detected as cytoplasmic and occasionally nuclear brown granules and was scored as 0 or 1 (-) and 2 or 3 (+), 4 or 5 (++), 6 or 7 (+++) or 8 or 9 (++++).

There was a significant statistical correlation between the Gleason score of prostate carcinoma and the score of S100A4 expression (P value < 0.05). This means that S100A4 might be involved in prostate adenocarcinoma progression and carcinogenesis.

There was a significant statistical correlation between the score of S100A4 expression and the presence of capsular invasion and/or lymph node metastasis (p value <0.5). This suggests that S100A4 playing a role in tumor invasion and metastasis.
There was a significant statistical positive correlation between the preoperative serum level of PSA and the score of S100A4 expression (p value < 0.05).

There was insignificant statistical correlation between the stage of prostatic adenocarcinoma and the score of S100A4 expression (p value >0.05) which needs further work to find out if S100A4 protein has a role in prostatic adenocarcinoma aggressiveness.

There was insignificant statistical correlation between the presence of perineural invasion and the score of S100A4 expression (p value >0.05).

There was insignificant statistical correlation between the score of S100A4 expression and the presence of associated high grade PIN in malignant cases (p value >0.05).

There was insignificant statistical correlation between 5 years survival and the score of S100A4 expression (p value >0.05).

Further studies may be needed to detect prognostic value of S100A4 in prostate adenocarcinoma.
Conclusions and Recommendations

**Conclusions**

1. S100A4 might be involved in prostate adenocarcinoma progression and carcinogenesis.
2. S100A4 may play a role in tumor invasion and metastasis.
3. Higher Gleason grade of prostate cancer is associated with local, nodal and perineural invasion.
4. High grade PIN and higher levels of preoperative serum PSA are associated with higher Gleason grade prostatic cancers.

**Recommendations**

Further studies using different molecular methods on S100A4 on larger number of cases are recommended:

- To clarify its prognostic value as determined by free recurrence and overall survival.
- To help the hope of developing new strategies in prostate cancer therapy.
References


- Algaba and Ferran: "Evolution of isolated high-grade prostate intraepithelial neoplasia in a Mediterranean patient population." European urology 35, no. 5-6: 496-497; (2012).


• Attard, Gerhardt, Jeremy Clark, Laurence Ambroisine, Gabrielle Fisher, Gyula Kovacs, P. Flohr, D. Berney et al. "Duplication of the fusion of TMPRSS2 to ERG sequences


- Cerveira N, et al: TMPRSS2-ERG gene fusion causing ERG overexpression precedes chromosome copy number changes in


• Chuang AY, Epstein JI: Positive surgical margins in areas of capsular incision in otherwise organ-confined disease at radical


- **Cotter MP,** et al.: Role of family history and ethnicity on the mode and age of prostate cancer presentation., (2002).


• Epstein JI, Walsh PC, Carmichael M, Brendler CB: Pathologic and clinical findings to predict tumor extent of nonpalpable (stage T1c) prostate cancer. JAMA; 271:368-374., (1994).


References

- **Helpap, Burkhard, and Lars Egevad.** "Correlation of modified Gleason grading of prostate carcinoma with age, serum prostate


Based on November 2008 SEER data submission, posted to the SEER web site., (2009).


- **Hua Li, Ziquan Liu, Chuanxiang Xu, Yunyun Chen, Jianwei Zhang, Bo Cui, Xuewei Chen, Gaihong An, Xiaojun She, Hongtao Liu, Zifeng Jiang and Tianuui Wang:** Overexpression of S100A4 is closely associated with the progression and prognosis of gastric cancer in young patients, ONCOLOGY LETTERS 5: 1485-1490., (2013).


• **Iwata, Tsuyoshi, Denise Schultz, Jessica Hicks, Gretchen K. Hubbard, Laura N. Mutton, Tamara L. Lotan, Carlise Bethel** et al.: "MYC overexpression induces prostatic intraepithelial neoplasia and loss of Nkx3.1 in mouse luminal epithelial cells." PloS one 5, no. 2: e9427., (2010).


• Kunz GM, Epstein JI: Should each core with prostate cancer be assigned a separate Gleason score?. Hum Pathol; 34:911-914., (2003).


• Kwon, Yong-Wook, In Ho Chang, Kyung Do Kim, Young Sun Kim, Soon-Chul Myung, Mi-Kyung Kim, and Tae-Hyoung Kim. "Significance of S100A2 and S100A4 Expression in the
References


- **Lewis JS, Vollmer RT, Humphrey PA**: Carcinoma extent in prostate needle biopsy tissue in the prediction of whole gland


• **Mariani S, Lionetto L** et al: Low prostate content of lycopene is associated with development of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia., (2014).


References


• **Ran Xie, David S Loose, Gregory L Shipley, Susu Xie, Roland L Bassett Jr and Russell R Broaddus**: Hypomethylation-induced expression of *S100A4* in endometrial carcinoma, Modern Pathology 20, 1045–1054; doi:10.1038/modpathol.3800940; published online., 3 August (2007).


• **Ross, Hillary M., Oleksandr N. Kryvenko, Janet E. Cowan, Jeffry P. Simko, Thomas M. Wheeler, and Jonathan I. Epstein.** "Do adenocarcinomas of the prostate with Gleason score (GS)≤ 6 have the potential to metastasize to lymph nodes?." The American journal of surgical pathology 36, no. 9: 1346., (2012).


• **Rubin MA, Dunn R, Kamlblam N, Misik CP, O’Toole KM:** Should a Gleason score be assigned to a minute focus of carcinoma on prostate biopsy?. Am J Surg Pathol; 24:1634-1640., (2000).


• Shrawan Kumar Mishra & Hifzur Rahman Siddique & Mohammad Saleem: S100A4 calcium-binding protein is key player in tumor progression and metastasis: preclinical and clinical evidence, Cancer Metastasis Rev (2012) 31:163–172, Published


References


• **Yanqiong Liu, Weizhong Tang, Jian Wang, Li Xie, Taijie Li, Yu He, Xue Qin and Shan Li**: Clinicopathological and prognostic significance of S100A4 overexpression in colorectal cancer : a meta-analysis, Diagnostic Pathology, 8:181., (2013).

• **Yaskiv, Cao, Peter A.**: Microcystic Adenocarcinoma of the Prostate: A Variant of Pseudohyperplastic and Atrophic Patterns,
References


• **Yong-Wook Kwon, In Ho Chang, Kyung Do Kim, Young Sun Kim, Soon-Chul Myung, Mi-Kyung Kim1, Tae-Hyoung Kim**: Significance of S100A2 and S100A4 Expression in the Progression of Prostate Adenocarcinoma, Korean Jornal of Urology, DOI:10.4111/kju. 51.7.456., (2010).


الملخص العربي

مقدمة:

يعتبر سرطان البروستاتا من أكثر السرطانات تشخيصًا في العالم، و من أكثر أسباب الموت بين الذكور و يمثل 14% من كل حالات السرطان و 6% من حالات الوفاة بالسرطان بين الذكور في عام 2008.

اما في مصر، وفقًا لآخر إحصائيات المعهد القومي للأورام، جامعة القاهرة، فإنه يمثل 8.5% من كل حالات الأورام السرطانية و خامس أكثر السرطانات شيوعًا بين الرجال، و وفقًا لاحصائيات مركز الجريدة لأبحاث السرطانات الطبية للأورام السرطانية، فإن سرطان البروستاتا يعد غير شائعًا إذ يمثل 2% من كل الأورام السرطانية و يحتل المركز السابع بين أكثر الأورام السرطانية شيوعًا بين الذكور. ووجد أن معظم حالات سرطانات البروستاتا تكتشف في مرحلة متأخرة.

تهدف هذه الدراسة إلى تقييم دور تمرکز4 S100A4 سواء في حالات التضخم الحميد أو سرطان البروستاتا و مقارنة هذه النتائج ببيانات المريض الباطنية و الاكستروفيلكية في محاولة للتعرف على دور دلالة S100A4 في أورام البروستاتا السرطانية. وقد تم تحليل النتائج الإحصائية.

اشتملت هذه الدراسة - التي تمت بتأشر رجعي - على 42 حالة من أمراض البروستاتا حميدة و خبيثة، منهم 8 حالات بيعان من التضخم الحميد للبروستاتا و قد استخدموا لغضن المقارنة، و 13 حالة تم تشخيصهم كسرطان البروستاتا من الدرجة الأولى، و 11 حالة من الدرجة الثانية و 10 حالات من الدرجة الثالثة.

و بالفحص الميكيروسكوبى للحالات تبين وجود علاقات إيجابية إحصائية بين درجة سرطان البروستاتا و كلا من: اختراق الورم لل kapsولة، انتقال الورم للغدد الليمفاوية، وجود ورم عالى الدرجة داخل ظاهرة البروستاتا، اختراق الورم للغشاء المحيطي بالاعصاب و نسبة المستضد الخاص بالبروستاتا في الدم قبل استئصال الورم. في حين تبين عدم وجود اية علاقات ذات أهمية إحصائية بين درجة سرطان البروستاتا و مرحلته أو نسبة نجاة المرضى من السرطان في خلال 5 سنوات.
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و قد تم صباغة كل الحالات بالدلالات المناعية S100A4 و تم مقارنتها بالبيانات الالكليميكية والباثولوجية للمريض. و ظهرت تلك الصباغة المناعية على هيئة حبيبات بنية اللون بصفة اساسية في هيولى الخلايا و بنسبة اقل في النواة. و قد أعطيت تصنيفاً كما يلي: 0-1 (-)
2-3(+) ، 4-5(++) ،6-7(+++) و 8-9(++++)

و لقد وجد في هذه الدراسة علاقة احصائية إيجابية بين درجة سرطان البروستاتا على تصنيف جليسون و درجة التصبغ بالدلالات المناعية S100A4 مما يشير لتورط S100A4 في سرطان البروستاتا.

و لقد تم التوصل لعلاقة اخرى ايجابية على صعيد الإحساس بين درجة تصبغ الورم بالدلالات S100A4 و اختراق المرض للكبسولة و/أو انتشاره فالغدد الليمفاوية مما قد يدل على وجود دور ل S100A4 في انتشار الورم و اختراقه للانسجة.

و علاقة اخرى إيجابية توصلت إليها العمليات الإحصائية بين نسبة المستضد الخاص بالبروستاتا في دم المرضى قبل استئصال سرطان البروستاتا و درجة تصبغ النسيج السرطاني S100A4.

في حين لم تستطع العمليات الإحصائية التوصل لاي علاقات ذات اهمية بين درجة تصبغ الأنسجة السرطانية في البروستاتا بالدلالات S100A4 و مرحلة السرطان، اختراق الورم للغشاء المحيط بالاصاب، وجود ورم عالي الدرجة داخل ظهارة البروستاتا أو نسبة نجاة المرضى من السرطان في خلال 5 سنوات.
و من هذه الدراسة نستنتج:

1. أن الدرجات العالية من سرطان البروستاتا طبقًا لتصنيف جليسون لها علاقة باختراق المرض للكبسولة و الغشاء المحيط بالاعصاب و انتشاره في الغدد الليمفاوية.

2. إن هناك علاقة طردية بين وجود ورم عالي الدرجة داخل ظهارة البروستاتا و نسبة المستضد الخاص بالبروستاتا في دم المرضى قبل استئصال الورم السرطاني و بين درجة سرطان البروستاتا في تصنيف جليسون.

3. احتمالية تورط الدلالات المناعية S100A4 في مسار تطور سرطان البروستاتا.

4. احتمالية وجود دور للدلالة المناعية S100A4 في انتشار سرطان البروستاتا و اخترقه للأنسجة.

و أخيرا نوصي بإجراء مزيد من الدراسات على الدلالات المناعية S100A4 باستخدام طرق جزئية مختلفة لمزيد من الأيضاح لما يلي:

- دور الدلالات المناعية S100A4 في تطور البروستاتا.
- دور الدلالات المناعية S100A4 في تطور و انتشار أورام البروستاتا السرطانية.
- قيمة الدلالات المناعية S100A4 الانخراطية كما تحددها نسبة انتكاس أو نجاة المرضى من السرطان في خلال 5 سنوات.

و مما قد يساعد على تطوير استراتيجيات جديدة لعلاج سرطان البروستاتا.
دور التعبير بالدلاله المناعية S100A4 في أورام البروستاتا السرطانية

 رسالة مقدمة من الطبية/ رنا "محمد السعيد" أبو الفتوح
 بكالوريوس الطب والجراحة
 توطئة للحصول علي درجة الماجستير في الباثولوجي
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