2. Review of Literature

2.1 Prostate

Prostate is a firm grey to reddish, partly glandular, partly fibro-muscular body surrounding the beginning of the male urethra. It lies at a low level in the inferior border of the symphysis pubis and pubic arch and rectal ampulla through which it may be palpated. The gland is somewhat conical, the base measuring 4 cm transversely. It has 2 cm antero-posterior and 3 cm vertical diameters and weighs about 8 gm. It is enveloped in a thin, but strong fibrous capsule. The capsule is firmly adherent to the gland and continuous with a median septum in the urethral crest. It is also continuous with numerous fibromuscular septa enmeshing the glandular tissue.

The prostate is traversed by the urethra and ejaculatory ducts and contains the prostatic utricle. The urethra usually passes between its anterior and middle thirds. The ejaculatory ducts pass antero-inferiorly through its posterior region to open into the prostatic urethra (Fig.2.1).

Histological sections show two concentric zones of glandular tissue (Franks, 1954; Fergusson and Gibson, 1956). The larger peripheral zone has long, branched glands whose ducts curve posterior to open mainly into the prostatic sinuses. The internal zone consists of sub mucosal glands. Peripheral and internal zones are said to be separated by an ill-defined capsule. Carcinoma affects almost exclusively, the peripheral zone, the internal being prone to benign hypertrophy.

![Fig. 2.1: Longitudinal Section of Pelvic region showing Prostate gland](www.prostatehealthtips.com)
2.1.1 Age Changes in the Prostate

At birth the prostate has a system of ducts embedded in a stroma which forms a large part of the gland. At puberty, the prostate gland enters a maturation phase and in approximately 12 months during this time, it more than doubles in size, due almost entirely to follicular development. During the third decade, the glandular epithelium grows by irregular multiplication of the endothelial infoldings into the lumen of the follicles. The size remains unaltered until 45-50 years, when the epithelial foldings tend to disappear, follicular outlines become more regular and amyloidal bodies increase in number, all signs being of prostatic involution. After 45-50 years, the prostate may undergo benign hypertrophy, increasing in size until death, or alternatively it may undergo progressive atrophy.

2.1.2 Clinical Aspects of the Prostate

After middle age, the prostate often enlarges projecting into the bladder to impede urination by distorting the prostatic urethra. The median lobe may enlarge the most, with even a small enlargement obstructing the internal urethral orifice, the more the patient strains the more the prostatic mass, acting like a valve, blocks the opening. The hypertrophied part may be removed surgically (prostatectomy).

There are valve-less venous communications between the prostatic and extradural venous plexuses, which are probably an important factor in the metastasis of prostatic neoplasms to the vertebral bodies (Batson, 1940; Franks, 1953).

Prostate carcinoma is a major health problem in western industrialized countries, where around 10% of men are diagnosed with this cancer usually in their old age out of which 10% die from the disease. The introduction of good serum-marker, prostate specific antigen (PSA) has allowed detection of many cancers, while they are still locally confined and can be cured by surgery or radiotherapy (Stages I and II; Fig. 2.2A and 2.2B). Those cancers that have spread too extensively locally or have developed distant metastases cannot be cured by current procedures (Stage III; Fig.2.2C). Third and apparently large graph of prostate cancer takes a very slow course and will not lead to clinical symptoms during the life term of an elderly man and these need to be actively monitored.

Molecular research on prostate cancer, therefore, faces three major tasks:

1. Detecting the cancer
2. Distinguishing both locally confined and indolent, locally confined, but likely to progress, or has progressed beyond reach of current treatment
3. Developing effective treatment for metastasis and locally progressive cases

**Stage I**
- Tumor not detectable by imaging or clinical exam
- Low-grade tumor
- Less than 5% of tissue specimen

**Stage II**
- Tumor not detectable by imaging or clinical exam
- May be found in one or more lobes by needle biopsy
- Moderate/high grade tumor
- More than 5% of tissue specimen

**Stage III**
- Tumor extends beyond prostate capsule
- May invade seminal vesicles
- Any grade tumor

Fig.2.2: Different stages of development of prostate cancer: A, B: locally confined which can be cured by surgery or radio therapy; C: extensive local spread or distant metastasis
2.2 Benign Prostatic Hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) and prostate cancer are common diseases of aged men. Both diseases appear to be androgen dependant for growth, but BPH commonly arises in the central and transitional zone of the prostate while cancer is most often found in the peripheral zone of the gland.

It has been reported that more than half of the population aged 75 years has histologic evidence of benign prostatic hyperplasia (BPH), while prostate cancer is amongst the most common male cancers. Both share important anatomic, pathologic and genetic links. Prostate is now the most common site of cancer in men (excluding melanoma skin cancers), while BPH affects as many as 62% of men aged 75 yr (European National registry data). Studies have identified prolonged history of BPH as a risk factor for prostate cancer (Armenian et al., 1974).

2.3 Tumour Specific Antigens and Antibodies

2.3.1 Prostate Specific Antigens

PSA is a serum protease that is secreted from prostate epithelial cells (Cohen et al., 1992). PSA levels have prognostic value for men with prostate cancer. Approximately 30 years ago, it was first proposed as serum marker for early detection of prostate cancer. The medial levels are approximately 0.7ng/ml in men aged 60 yr and modest elevation of blood level of PSA to 4.0ng/ml is strongly associated with an increased risk of cancer (Meitz et al., 2002). PSA is an important marker for diagnosis and follow up of prostate cancer patients (Ware, 1994). Specific germ line genetic polymorphisms in the promoter region of the PSA gene have been reported to be associated with higher serum PSA levels. The same may be used for screening of prostate cancer. However, the specificity of total serum PSA is limited particularly in rising incidence of clinically relevant prostate cancer in patients with low PSA serum levels (less than 4.0ng/ml). The specificity of PSA determination is essentially required in cancer patients for which there is a dire need of new tools.

2.3.2 Tumour Specific Antibodies

Proteomic serum profiling as a diagnostic tool and platform for biomarker discovery in prostate cancer is an emerging research area (Banez et al., 2005). Changes in serum protein composition reflecting the pathological state of organs/tissues will facilitate the discovery and quantification of protein biomarkers for differentiation between malignant and normal cells (Petricoin et al., 2002). Tumours are known to induce release of many proteins into the blood and due to various
modifications, they appear as foreign molecules and thus lead to the activation of immune system. The presence of auto-antibodies in the sera of high risk individuals foretells the onset of cancer development and marks their significance as molecular signatures for useful clinical diagnostic and prognostic information. Multiple prostate cancer specific antigens were identified via the detection of auto-antibodies in the serum of patients with prostate cancer via high throughput phage peptide microarray analysis. The measurement of serum auto-antibodies against a panel of 22 tumour associated peptides detected prostate cancer with 88.2% specificity and 81.6% sensitivity in a case-control study (Leushner, 2001). Compared to PSA, this auto-antibody signature had significantly better performance suggesting its use against peptides derived from prostate cancer tissue as better tool for screening of prostate cancer.

2.4 Methylated DNA as Biomarker

The regulation of gene expression by aberrant methylation has been well established in tumour biology. The epigenetic phenomenon of hypermethylation in tumour-related genes has been implicated in cancer development and progression (Nelson et al., 1997; Lou et al., 1999; Sasaki et al., 2002) and is, therefore, one of the most promising means of identifying marker candidates for the early detection of cancer. The diagnostic potential of cancer specific methylated markers for prostate and bladder cancers have been evaluated in urine and serum samples (Nelson et al., 1997).

2.5 Role of Estrogen

Prostate growth depends on synergistic interactions between oestrogen and androgens. The ratio of estrogen to androgens in the prostate increases by 40% in ageing men, and this may influence the natural history of both Benign Prostatic Hyperplasia (BPH) and prostate cancer (Preziso et al., 2007). Asian men who follow a traditional diet (which provides rich supply of phytoestrogens) have a lower prevalence of BPH and prostate cancer than men following a modern western diet (Sim and Cheng, 2005) and that is why prostate cancer incidence among Asian immigrants to the United States is higher than in their respective native population (Cook et al., 1999).

2.6 Role of Inflammation

Inflammation contributes to the development of BPH. In one study, it was suggested that inflammation plays a role in the pathogenesis of prostate cancer.
Needle biopsy specimens from men with clinical signs to suggest malignancy revealed a significant link between inflammation and serum PSA (MacLennan et al., 2006).

2.7 Genetic Changes

Mapping the whole genome made it possible to identify genes related to specific disease states and using biomarker technology based on molecular signatures of gene expression feasibility for discovering and predicting cancer classes has been demonstrated (Golub et al., 1999). Genetic alterations damage the structure of DNA and, thereby, induce mutations which manifest in abnormally functioning proteins that, thereby, precipitate diseased conditions. Malignancy is characterized by genomic alterations that allow proliferation. A number of such alterations have been identified in prostate cancer. Genes that encode enzymes involved in steroid hormone synthesis or function such as cytochrome P-45017 alpha (CYP17), steroid 5-X reductase type II (SRD5A2) and prostate specific antigen (PSA) have attracted attention as potential markers of prostate cancer and BPH (Habuchi et al., 2000; Salam et al., 2005). Gene rearrangements have also been implicated in a number of cancers and have recently been uncovered in patients with prostate cancer, however, similar incidences have been rarely reported in BPH (Laxman et al., 2006; Perner et al., 2007).

2.8 Epigenetic Changes

Epigenetic processes such as DNA methylation represent other mechanism of gene regulation. A number of genes are commonly hypermethylated and, therefore, inactivated during prostate cancer progression (Doboxy et al., 2007). Some of these are tumour suppressor genes (Adenomatous polyposis coli; APC and Ras-association domain family 1A gene; RASSF1A), while others have a role in cell cycle regulation (14-3-3σ) or heavy metal binding (MTG) or encode for proteins such as ATP-binding cassette (ABC) transporters (MDR 1) glutathione-S-transferase (GSTP) and glutathione peroxidases (GPX3). Some genes like endothelin receptor type B (EDNRB), cell adhesion molecules (cadherin-4; CDH4), estrogen receptor (ER) are selectively methylated in many prostate cancers in their 5’ promoter regions. Epigenetic technologies in cancer studies are helping to increase the number of cancer candidate genes and allow to examine changes in 5-methyl cytosine DNA and histone modifications at a genome wide level.
2.9 Epidemiology of Prostate Cancer

Aging population as well as introduction of more sensitive diagnostic procedures and cancer registration have resulted in an increase in incidence of prostate cancer at a higher pace (Vercelli et al., 2000). Worldwide, a remarkable increase in the identification of prostate cancer cases has been reported. The use of new diagnostic techniques including serum PSA, transrectal ultrasound guided needle biopsy, computer tomography and greater frequency of operation for benign diseases may be the major reasons of this increase. This has led to the substantial increase in number of cancer cases observed as compared to earlier time (Hankey et al., 1990; Jacobson et al., 1995). Also the mortality rate has declined mainly due to incidental discovery and timely detection (Miller et al., 1993). Besides, rising incidence rate and increased public awareness of prostate cancer has created an explosion of prostate cancer screening in western countries. It has certainly affected several epidemiologic features of the disease like its incidence, tumour staging, patient characteristics as well as care and outcome (Mettlin, 2000).

2.9.1 International Statistics

Prostate cancer is the second most common cancer in the Europe and the US. Its incidence and mortality rates vary worldwide. It accounts for 33% of all the recently diagnosed malignancies among men in United States. It is considered as most common malignancy in men followed by lung and colorectal cancer in European Union (Ferlay et al., 2007). More than 30,000 men are diagnosed with prostate cancer every year in UK (http://info.cancerresearchuk.org.pdf). Fifteen percent higher incidence rate and 38% higher death rate is reported in African-American men than in white men. As compared to whites, the death rate from prostate, stomach and cervical cancers is more than double in black Americans. Factors known to contribute to racial disparities in mortality, vary by cancer site (Jemal et al., 2007). According to World Health Organization, death from cancer is expected to increase manifold worldwide by the year 2020. People living in developing countries (Latin America, the Caribbean, Asia, the Middle East and Africa) are predicted to be at higher risk than those in developed countries. Although actual cancer incidence rates are still lower in developing countries than in North America and Europe, the rise in cancer-related deaths will represent a significant burden to the already overwhelmed health systems in developing countries (Rastogi et al., 2004). Thus, prostate cancer is going to be an actual health burden worldwide.
2.9.2 Asian Statistics

Although Asian people have the lowest incidence and mortality rates of prostate cancer in the world, these rates have rapidly risen in the past two decades in most Asian countries. Prostate cancer has become one of the leading male cancers in some Asian countries. In 2000, the age-adjusted incidence was over 10 per 100,000 men in Japan, Taiwan, Singapore, Malaysia, the Philippines and Israel (Pu et al., 2004). According to a recent study, the incidence of prostate cancer in Asian countries is still increasing rapidly due to a more lifestyle. Prostate cancer mortality is expected to continue to increase in Asian countries as the percentage of advanced-stage prostate cancers remains high (Namiki et al., 2010). There has been a significant increase in the incidence of prostate cancer in Korea (Jung et al., 2010). High incidence rate of prostate cancer has also been reported in Turkey (Ceber et al., 2008). The increase in the incidence and mortality rates in Asian countries from 1973-1997 was estimated to be 4.8 - 9.8 (Singapore), 4.9 - 9.0 (Miyagi, Japan), 5.1 - 7.9 (Hong Kong), 1.6 - 2.3 (Shanghai, China), and 6.8 - 7.9 (Bombay, India) per 100,000 men. In Japan, Taiwan, Singapore, Malaysia, Philippines and Israel, the age-adjusted incidence of prostatic cancer was over 10 per 1,00,000 men in 2002. In Taiwan and Singapore, prostate cancer is the sixth leading male cancer, population of both the places being 23 and 4.6 millions respectively (as per the 2003 data) and Chinese people being the largest proportion of this population (Pu et al., 2004).

2.9.3 Indian Statistics

In the year 2000, nearly 5,50,000 deaths due to cancer occurred in Indian population. While in 2001, 6,605 cases of prostate cancer and a total of 5,65,682 cases of all types of cancer were predicted in India. In developing countries like India, 80% of victims when detected are already at incurable stage (Pal and Mittal, 2004). The epidemiology of prostate cancer is poorly understood in India and very few reports are available on the survival data in spite of being the largest country in south-central Asia (population 1.05 billion in 2003). This may be because of poor patient follow up and inadequate reporting system of cancer incidence rate among all male cancers in India. The survival of prostate cancer patients is relatively poor in India, being 40% for localized disease, 24% for direct extension and regional node involvement, and 13% for metastasis diseases (Sunny et al., 2004).

A Delhi urban resident population based cancer registry reveals an age standardized rate (ASR) of 9.0 per 1,00,000 men in prostate cancer and the incidence
of prostate cancer in males was the highest among the Indian registries (Manoharan et al., 2009).

2.10 Risk Factors Involved in Prostate Cancer

In the past several decades, the epidemiology and screening studies have shown concern about the pathogenesis of prostate cancer, but no definitive cause has been established. The clinical effect of prostate cancer is increasing. Growing public awareness has raised questions concerning the cause of prostate cancer as well as ways to screen for and prevent this disease. The ultimate goal of epidemiologic studies is to identify risk factors to guide disease prevention strategies. The prevailing risk factors that may be important in the development of prostate cancer supported by scientific literature are being cited as under:

2.10.1 Age and Ethnicity

Diagnosis and mortality rate of prostate cancer is very rare in men below 50 years of age, which after this age shows an exponential increase (Haas & Sakr., 1997). The probability of developing prostate cancer increases with age. Studies have revealed that the probability increases from 0.005% among individuals who are less than 39 years of age to 2.2% for those aged 40-59 years and 13.7% for those aged 60-79 years. Twenty percent of men in the age of 50-60 years and 50% of those in the age of 70-80 years showed histological evidence of malignancy (Carter et al., 1990). In United States, more than 70% of all cases of prostate cancer are diagnosed in men above 65 years of age.

As far as ethnicity is concerned, the highest rates of prostate cancer in the world have been found in African-Americans (275.3 per 1,00,000 men). This incidence is 60% higher than among whites which in turn is higher than the rates in Hispanics and Asian/Pacific Islanders (Table 2.1).

Table 2.1: Rate of incidence of prostate cancer amongst different ethnic groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Incidence (per 1,00,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>172.9</td>
</tr>
<tr>
<td>African-American</td>
<td>275.3</td>
</tr>
<tr>
<td>Asians/Pacific Islander</td>
<td>107.2</td>
</tr>
<tr>
<td>American Indian/ Alaskan Native</td>
<td>60.7</td>
</tr>
<tr>
<td>Hispanics</td>
<td>127.6</td>
</tr>
</tbody>
</table>
Scandinavian countries show highest rate of prostate cancer, while Asian countries show the lowest (American Cancer Society, 2003; Crawford, 2003). Prostate cancer is ranked as second leading cause of deaths in Spain (11%) and fourth in Italy (8%). It is the most common cancer among males in France (19%) (Reddy et al., 2003). Age-adjusted incidence rates as well as death rates from clinical prostate cancer vary dramatically from country to country, considering the difference in and availability of screening programmes (Haas and Sakr, 1997). Waterhouse (1982) and Muir et al. (1991) found a 25 fold difference between incidence rates in American black men living in San Francisco and in Japanese men. In 1988, the age-adjusted death rate per 1,00,000 population was 15.7 for men in the United State and 3.5 for those in Japan. This interpretation is further supported by the observation that immigrants moving from low-risk areas to the United States gradually assume higher risk of the U.S population (Haenszel and Kirihoro, 1968; Dunn, 1975; Flanders, 1984; Miekle and Smith, 1990; Muir et al., 1991; Yu et al., 1991; Shimizu et al., 1991). Thus, despite the presence of histologic cancer appearing to be related to age, other risk factors that increase the development of prostate cancer probably affect the ‘Promotion’ steps of the transformation pathway.

2.10.2 Family History

The incidence of prostate cancer in male relatives of patients with prostate cancer is increased. Higher incidence of prostate cancer was found among male relatives of patients with breast cancer (Woolf, 1960; Krain, 1974; Theisseu, 1974; Schuman et al., 1977; Cannon et al., 1982; Miekle and Stanish, 1982; Miekle et al., 1985; Steinberg et al., 1990; Carter et al., 1990, 1992; Spitz et al., 1991).

Familial clustering of prostate cancer was also reported by Canon et al. (1982) in Utah Mormons. Men with a father or brother with prostate cancer have double the risk of developing prostate cancer as men without affected relatives and the risk increases with increasing number of affected relatives (Carter et al., 1990; Steinberg et al., 1990; Carter et al., 1992). Studies in Japan reported that the age at diagnosis of prostate cancer was significantly lower in patients with a positive family history than those without it (69.4±7.5 vs 74.2±8.2 years; p 0.001) (Smith et al., 1996; Xu et al., 1998). Segregation and linkage analysis have shown that early-onset of prostate cancer may be inherited in an autosomal-dominant fashion of a rare high-risk allele suggesting that the autosomal-dominant form of prostate cancer accounts for a significant number of early-onset cases (Steinberg et al., 1990), though these cases
represent only a small proportion of prostate cancer (Carter et al., 1993). To date, two familial susceptibility loci have been mapped to the X chromosome and to a region of chromosome 1q. About 0.6% of white men inherit a mutated allele of one or more predisposing genes (Gronberg et al., 1996; Ghadirian et al., 1997).

2.10.3 Socio-Economic Conditions

Baquet et al. (1991) at the National Cancer Institute while investigating the incidence of prostate cancer and correlating it with population density, education and income level in African-Americans and Whites, found it to be higher in former than in latter group. No statistically significant association was found between socio-economic status and prostate cancer incidence (Baquet et al., 1991). Ernster et al. (1978) using data from Third National Cancer Survey (1969-1971 in Alameda County) also found no association of prostate cancer incidence and socio-economic status in African-American white men. Similar studies by McWhorter et al. (1989), Mishina et al. (1985) and many others with positive and negative results (Clemmesen & Nielsen., 1951; Buell et al., 1960; Richardson., 1965; Seidman., 1970; Ernster et al., 1977) in general, support the view that socio-economic status is not an important risk factor for the development of prostate cancer. No doubt, factors like poverty, lack of education and health insurance are important causative factors of cancer because they activate the risk and affect the prognosis and diagnosis as also palliative care. Mortality rate has also been found to be more in developing countries than developed ones (Ward et al., 2004).

2.10.4 Occupation

One consistent data from a large body of literature show that farmers and other agricultural workers have a 7-12% increased risk of cancer (Steinberg et al., 1990; Carter et al., 1990). This may be due to life-style factors such as increased intake of meat and fats. It can be attributed to exposure to chemicals also. Though the epidemiologic evidence linking specific pesticide or herbicide exposure to prostate cancer is weak, organochlorines present in these can affect circulating hormone levels. Workers in heavy industry, rubber manufacturing and newspaper printing may also have higher risk of prostate cancer (Steinberg et al., 1990).

2.10.5 Physical Activity

Overweight and obesity due to lack of physical activity increases the risk of certain cancers. Physical activity and sports play an important role in preventing genitourinary tumours (Sommer et al., 2004). This may be attributed to the fact that
physical activity decreases levels of total and free testosterone, reduces obesity and enhances immune protection (Canon et al., 1982). Physical activity reduces physical role limitation, decreases falls, elevates mood, reduces fatigue and attenuates losses in bone density promoting weight loss for cancer risk.

2.10.6 Diet

Research in epidemiology and studies on migrants have revealed the influence of diet on prostate cancer risk. Japanese men, who in their native country manifested low incidence of prostate cancer, and had migrated to the United States in younger age started reflecting the prevailing local incidence and mortality rates (Shimizu et al., 1991; Whittemore et al., 1995; Cook et al., 1999). This may have been attributed to higher intake of fat, meat and dairy products. Studies on Whites, African Americans and Asian Americans have shown association of prostate cancer risk with total fat intake (Giovannucci et al., 1993). Red meat rich diet has been linked with prostate cancer risk (Veierod et al., 1997). Non vegetarian diet has been associated with an increased risk of prostate cancer (Sobti et al., 2009).

Dairy products and beef are major sources of dietary branched fatty acids. An enzyme, α-methyl-coenzyme-M-reductase, that plays a key role in the peroxisomal oxidation of these fatty acids, has been found to be up regulated in prostate cancer, but not in normal prostate. Hydrogen peroxide, generated by oxidation process may be a source of oxidative damage to the prostate genome (Gronberg, 2003). A low fat, high fibre diet has been shown to affect male sex hormone metabolism by decreasing circulating testosterone. Altered hormone metabolism plays a role in the progression of prostate cancer from histologic to clinically significant forms and it has been observed that the incidence of prostate cancer is very low in eunuchs and castrated men as the growth and differentiation of prostate is under androgenic control (Hill et al., 1979; Wynder et al., 1984).

Soybean products are found to be rich in isoflavones such as genistin and daidzin. It has been suggested that isoflavones inhibit protein tyrosine kinase which are important for cell proliferation and transformation as also for angiogenesis, thus limiting the development and metastasis of prostate tumours (Shirai et al., 2002). This factor may be one of the major reasons for lower incidence of prostate cancer in Japan than in the United States as Japanese consume soybean rich diet. In general, a high fibre and low-fat diet may protect men against the development of prostate cancer.
High calcium intake has also been related to an increased risk of prostate cancer (Rodriguez et al., 2003).

Armstrong and Doll (1975) found that prostate cancer deaths from 32 countries were highly correlated with total fat consumption. Rose et al. (1986) determined this correlation to animal fat and not vegetable fat.

Consumption of beans, lentils, pear, tomatoes, raisins, dates and dried fruits have been reported to decrease risk for prostate cancer significantly (Mills et al., 1989). Increased vitamin A intake has been reported with an increased risk for prostate cancer (Armenian et al., 1974; Graham et al., 1983; Heshmet et al., 1985; Kolonel et al., 1988; Ohno et al., 1988; Mills et al., 1989). Some studies have contradicted these results (Hirayama, 1979; Ohno et al., 1988). Vitamin A found in plants (beta carotene) has been observed to be protective, whereas its intake from animal sources increases the risk (Shekelle et al., 1981; Mettlin et al., 1989).

Slightly reduced risk with regular supplements of vitamin E has also been reported (Heinonen et al., 1998). Polyphenols in green tea act as powerful antioxidants, reducing the risk of prostate cancer. The increased frequency, duration and quantity of green tea decline prostate cancer risk (Gupta et al., 1999). It has been suggested that a high body mass index (BMI) and bone mass may be associated with prostate cancer. Men with BMI of 35.0 to 39.9 had a 34% greater risk of dying of prostate cancer than those with a normal BMI (Calle et al., 2003).

Selenium, an essential trace element found largely in grains, fish and meat is protective against prostate cancer (Leitzmann et al., 2003; Klein, 2004).

Interestingly, men with diabetes mellitus have been observed to have a lower risk of developing prostate cancer. In a hospital based case-control study on Whites and Hispanics, diabetes was associated with a 40% lower risk of prostate cancer and a 53% lower risk of regional or advanced prostate cancer (Rosenberg et al., 2002a).

2.10.7 Smoking

A number of studies have reported that cigarette smoking may be a risk factor for the development of prostate cancer (Shaarawy and Mahmoud, 1982; Dai et al., 1988; Honda et al., 1988; Fincham et al., 1990; Hsing et al., 1990). Relative risks of 1.8 and 2.1 for cigarette smoking and tobacco chewing respectively have been reported by Hsing et al. (1990). Significantly higher risk in prostate cancer cases and in BPH cases has been reported in recent studies (Sobti et al., 2009, 2010; Thakur et al., 2010).
A study has also proposed a higher risk in smokers with an increased exposure to cadmium (Honda et al., 1988). Cadmium is a trace element found in cigarette smoke and alkaline batteries. People working in the welding and electroplating occupations are exposed to high levels of cadmium. However, a case-control study by Fincham et al. (1990) found no link between smoking and prostate cancer. It has also been proposed that cigarette smoking may alter circulating levels of steroid hormones. It is associated with enhanced levels of bio-available testosterone and also lowers estradiol in men. Significant positive association between cigarettes smoked per day and total serum androstenedione and total and free testosterone in men has been reported (Dai et al., 1988). The overall data for cigarette smoking risk are complicated by conflicting reports on the effect of cigarette smoking on serum sex hormone (Shaarawy et al., 1982; Dai et al., 1988).

2.10.8 Vasectomy

Vasectomized men have higher levels of circulating testosterone and this may have an increased risk for prostate cancer. A large cohort study on 5332 men, each matched with three non vasectomized comparison controls, showed no increased risk either for prostate cancer or benign prostatic hypertrophy (Giovannucci et al., 1992).

Metllin et al. (1990) reported a relative risk of 1.7 for reporting a vasectomy at any age and a relative risk of 2.2 for men reporting vasectomy 13 to 18 years before being diagnosed with cancer. Honda et al. (1988) have also reported the same association. Two other larger studies have confirmed this positive trend between the number of years since vasectomy and prostate cancer risk (Giovannucci et al., 1992). Thus, vasectomy appears to confer an increased risk for the development of prostate cancer.

2.10.9 Benign Prostatic Hyperplasia

It is difficult to determine the role of BPH in the risk of prostate cancer. History of benign prostatic hyperplasia has been reported to carry a relative risk of 13.5% by Mischina et al. (1985) and a relative risk of 5.1 for prostate cancer by Armenian et al. (1974). Not knowing the reasons, authors of retrospective and prospective studies have found a higher death rate from prostate cancer in men with a history of benign prostatic hyperplasia (Armenian et al., 1974). However, Greenwald et al. (1974) found no association between BPH and prostate cancer.
2.10.10 Alcohol Consumption

Alcohol affects hormone metabolism disturbing the balance between androgen and estrogen. Repeated high dose of alcohol in non-alcoholic men suppresses testicular production and increases clearance of testosterone. It also increases circulating estrogen levels leading to development of feminine characters (Purohit, 2000).

Alcohol may influence risk of prostate cancer by enhancing the solubility and absorption of mutagens and inhibiting cytochrome P-450 detoxification enzymes. Acetaldehyde, the product of ethanol metabolism, also inhibits enzymes in the DNA methylation pathway.

Dennis (2000) found no association between prostate cancer risk and alcohol consumption. Certain cohort studies have reported a moderately increased risk for prostate and bladder cancers from specific types of alcohol (Sommer et al., 2004).

Antioxidant and anticarcinogenic properties of red wine due to high concentration of polyphenols have been observed to decrease tumour formation. Prostate cancer risk is reduced by 6% by consuming a glass of red wine each week. Schuurman et al. (1999) observed an increased risk with white and fortified, but no association with red wine. Statistically significant rise in relative risk was observed in men consuming 22 or more drinks per week (Hayes, 2001). Direct association was suggested in some studies (Knowles et al., 2000), while inverse association by others (Albertsen and Gronbeck, 2002). Men taking 5-6 drinks of alcohol per week showed a moderately high risk of prostate cancer than those who were non-alcoholics or who drank less than 1 day per week (Sesso et al., 2001).

2.11 Polymorphisms of DNA Repair Genes

Repair mechanisms protect the genome from DNA damage caused by endogenous and environment agents. Polymorphisms and defects in many genes affect the efficiency and accuracy of DNA repair. Genetic polymorphisms of DNA repair genes have been reported to lead to amino acid substitution in various cancers. The discovery of an increasing number of single nucleotide polymorphisms (SNPs) in perhaps all the genes of an organism highlights the bewildering diversity between individuals and the potential differences in molecular responses of humans to DNA lesions.

Estimates of daily number of DNA lesions in a human cell ranges from 100-500 spontaneous deaminations to 20,000-40,000 single strand breaks (Mullaart et al.,
The repair of damage to DNA is essential for the survival of the cell and the health of the organism and over evolutionary periods, the cell has developed a diverse set of defense mechanisms to deal with a wide range of DNA lesions and adducts. Individuals with defects in a mutation repair pathway are often susceptible to spontaneous or induced cancers. The molecular machinery of the repair pathways has slowly been unraveled over the past few decades.

A number of DNA repair pathways are involved in the maintenance of genetic stability. Nucleotide excision repair pathway is the most versatile and important (Sarasin, 2003) especially for DNA damage induced by cigarette smoking. Many diseases are associated with dysfunction of this pathway suggesting it to be important in general population also. The base excision pathway involves removal of modified bases such as single strand breaks, non-bulky adducts, oxidative damage, alkylation or methylation. The oxoguanine glycosidase 1 (hOGG1) gene, encodes a DNA glycosylase/ AP lyase. It suppresses the mutagenic effects of 8-hydroxyguanine by catalyzing its removal from reactive oxygen species. In this regard Xu et al. (2002) found two sequence variants of this gene and showed an association between these polymorphisms and risk of prostate cancer. Mismatch repair pathway removes unrepaird bases and partially recognizes bulky adducts. Finally recombination pathways are able to remove or bypass bulky lesions allowing the cells to tolerate them (Hoeijmakers, 2001). Abnormal recombination may produce mutations or genetic instability leading eventually to cancer. Susceptibility to cancer is determined by two types of genes, low penetrance and high penetrance genes. Alterations in high penetrance DNA repair genes, generally result in inherited disorders such as xeroderma pigmentosum and Hereditary Non-Polyposis colorectal cancer. Genetic polymorphisms are generally found in low penetrance genes in which the sensitivity is more subtly affected (Shields and Harris, 2000).

2.11.1 DNA Repair Mechanisms and Role of Xeroderma Pigmentosum Group D (XPD) Gene

The nucleotide excision repair (NER) pathway repairs bulky DNA adducts and includes xeroderma pigmentosum group D (XPD) and xeroderma pigmentosum group C (XPC) repair genes. NER is especially important as it plays a critical role in repairing DNA damage induced by several suspected human prostate carcinogens, including tobacco related polycyclic aromatic hydrocarbons (PAHs) and heterocyclic
aromatic amines (HAAs) from well done meats and pesticides. Prostate cells can activate PAHs and HAAs.

Khan et al. (2000) and Van Hoffen et al. (2003) explained the basic mechanism of NER in which recognition of DNA lesion is the first step followed by single strand incision at both sides of the lesion. The lesion containing the single stranded DNA fragment undergoes excision and the excised nucleotides are replaced by the DNA repair synthesis and ligation of the remaining single stranded nick. The two NER sub pathways, Global genome repair (GGR) pathway (which repairs DNA lesions across the genome) and Transcription coupled repair (TCR) pathway (which repairs DNA lesions that are specific to the transcribed strand of active genes) differ in damage recognition step. The XPD protein is absolutely necessary in nucleotide excision repair. Once the DNA lesion has been recognized by specific proteins, the helicase activity of XPD, in concerted action with the xeroderma pigmentosum group B helicase, allows the opening of double helix so that the damaged strand can be cut and removed. XPD activity is essential for life, total absence of the XPD gene results in embryonic lethality (Friedberg, 2003).

Point mutations in the human XPD protein play a causative role in DNA repair-deficiency diseases (Xeroderma pigmentosum, trichothiodystrophy, and Cockayne syndrome), which are characterized by high ultraviolet-light hypersensitivity, a high mutation frequency, and cancer-proneness, as well as some mental and growth retardation and probably ageing (Stary et al., 2002). Most of these mutations are located in the C-terminal part of the protein, which is the domain of interaction, inside the transcription factor IIH complex, with the p44 protein being necessary for activating the helicase activity (Tirode et al., 1999). The very high cancer-proneness of Xeroderma pigmentosum patients shows clearly the relevant association between DNA repair efficiency and cancer risk. Because the XPD protein is absolutely necessary for efficient nucleotide excision repair, DNA repair-deficient cells arising from a mutation in the XPD gene exhibit low unscheduled DNA synthesis and low survival following ultraviolet irradiation.

Besides point mutations that cause diseases and are found in the homozygous state in patients or on only one XPD allele in asymptomatic parents, seven polymorphisms in exons 6,8,10,17,22, and 23 of the XPD gene have been identified by sequencing the DNA of individuals (Broughton et al., 1996; Shen et al., 1998; Mohrenweiser et al., 2002). Three of these polymorphisms are silent, and the
remaining four result in amino acid changes. Whereas the codons 199, 201, and 575 polymorphisms are rare (allele frequency ~1 percent), those in codons 156, 312, 711 and 751 are common (allele frequencies > 25 percent) (Shen et al., 1998; Mohrenweiser et al., 2002). Following the study by Shen et al. (1998), three XPD polymorphisms, arg156arg, asp312asn, and lys751gln, were mainly investigated in genetic epidemiologic studies because of their high frequencies and amino acid substitution variants.

With regard to the XPD lys751gln polymorphism, the gln allele is common in Europe and North America; approximately 50 percent of the subjects carrying the heterozygous lys/gln genotype and 10-15% carrying homozygous glngln genotype. The gln allele was less frequently reported in African Americans, with 5.6 percent glngln homozygosity (David-Beabes et al., 2001). This allele is uncommon in China (Liang et al., 2003), South Korea (Park et al., 2002) and Japan (Hamajima et al., 2002) with nearly 90 percent of the subjects carrying the lys/lys genotype, while the glngln genotype was rarely observed. However, this pattern of genotype frequencies was very different in another Chinese population (Chen et al., 2002), with approximately 18 percent of subjects carrying the homozygous gln/gln genotype. The discrepancy in results may be due to technical errors or ethnic differences in the pattern of mutations.

A large number of SNPs in different DNA repair genes have been identified and some of them have been studied for human cancer susceptibility (Mohrenweiser et al., 2002). However, only a few of them have been evaluated in prostate cancer risk. SNPs in a base excision repair (BER) gene, oxoguanine glycosidase 1 (hOGG1) were associated with prostate cancer in both sporadic and familial cases (Sanyal et al., 2004).

A genetic variant in another BER gene, X-ray cross complementation group 1 (XRCC1) R399G, was associated with elevated prostate cancer risk in individuals with lower vitamin E and lycopene intake (Hu et al., 2005).

To date more than 40 genes and 200 SNPs have been identified in the NER pathway (Rybczki et al., 2004). Rybczki et al. (2004) while evaluating a NER polymorphism and prostate cancer risk demonstrated that combined variant genotypes of ERCC2/XPD D312N in NER and XRCC1 R399G in BER greatly increased the risk of prostate cancer.
2.11.2 DNA Repair Mechanisms and Role of Xeroderma Pigmentosum Group C (XPC) Gene

The XPC protein, involved in the NER pathway binds to HR23B to form the XPC-HR23B complex and is thought to be an early damage detector and initiator of NER (Melton et al., 1998). The XPC codon 939 polymorphism (A-C transition, exon 15) results in a Lys to Gln alteration, which has been found to be associated with an increased risk of bladder and lung (Sanyal et al., 2004; Hu et al., 2005) cancer.

The XPC gene product contributes to the global genome repair pathway (GGR). It is a member of the NER pathway and is tightly associated with one of the two human homologues of Saccharomyces cerevisiae RAD23 protein (HR23B) (Melton et al., 1998). The XPC-HR23B complex has a structure specific affinity for certain defined lesions, including UV-induced photoproducts, the acetyl amino fluorescence adduct (AAF) and artificial cholesterol moieties. There are six core NER factors (XPC HR23B, TF11H, XPA, RPA, XPG and ERCC 1-XPF). Among these factors, only the XPC-HR23B complex can bind damaged DNA, changing the DNA conformation around the lesion (Melton et al., 1998; Sanyal et al., 2004). Studies have shown that in global genome repair (GGR), damage is initially recognized by the XPC-hHR23B complex in association with the XPA-RPA complex as well as XPE protein (Chavanne et al., 2000; Khan et al., 2000; Gozukara et al., 2001). XPA-RPA may aid in positioning other repair factors and guiding the nucleases to proper incision sites between single stranded and duplex DNA (Goode et al., 2002). Lesions on the transcribed strand of DNA result in the stalling of RNA polymerase II. Thus XPC-HR23B complex is the DNA damage detector and initiator of the GGR reaction (Melton et al., 1998; Sanyal et al., 2004).

Amongst all identified SNPs of XPC, two are commonly studied: Lys 939 Gln (A33512C, rs2228001) and Ala 499 Val (c21151T, rs2228000). XPC-PAT, a novel variant in intron 9 first reported by Khan et al. (2004) was found to be in linkage disequilibrium with an A to C substitution in exon 15 that gives risk to a Lys to Gln substitution at position 939 and this variant was later investigated for its functional relevance and its association in DNA repair capacity.

The knowledge of mutated XPC gene suggested that normal XPC gene is critical for the cells to complete excision repair of bulky DNA lesions (Berneberg and Lehmann, 2001). Several epidemiological studies have been conducted to explore the associations of XPC polymorphisms with cancer risk, but results are contradictory.
e.g., Vogel et al. (2005) found an increased risk of lung cancer associated with the 939 Gln allele in a Danish population. Sanyal et al. (2004) reported that carriers with C allele had an increased risk of bladder cancer in a Swedish population and Shen et al. (2005) found XPC variant 939G14 genotype to be associated with a borderline significant risk of lung cancer in a Chinese population. Many mutations in the XPC gene were reported by Chavanne et al. (2000). A R579 top variant was found in the XPC gene in a Xeroderma pigmentosum family in Turkey and Italy (Guzukara et al., 2001).

Studies have reported that extracts from XP patients showed delayed repair with a particularly strong decrease in the activity of XPC extracts for all lesions tested.

Hirata et al. (2007) reported an association between the polymorphism of XRCC1 Arg 399 Gln and risk of renal cell carcinoma. Van Gils et al. (2002) found no discernible difference between prostate cancer cases and controls for the XRCC1 codon 399 variants. They, however, found a remarkable risk for the combination of low dietary intake of vitamin E and the XRCC1 codon 399 Gln/Gln genotypes (Van Gils et al., 2002). Rybicki et al. (2004) and Ritchay et al. (2005) also investigated the XRCC1 codon 399 polymorphism in prostate cancer. Wang et al. (2004) found the XRCC7 gene polymorphism to be associated with glioma. Hirata et al. (2007) hypothesized that the polymorphisms of DNA repair genes could be risk factors for prostate cancer.

### 2.12 Polymorphisms of Metabolic Genes: GSTP1 (exon 5 and 6)

The GST pi class, most relevant in human cancers, is encoded by a single gene, mapped to chromosome 11q13 (Board et al., 1989; Islam et al., 1989). The GSTP1 protein catalyzes the glutathione conjugation of several anti-cancer agents (Ishikawa et al., 1993; Ishimoto et al., 2002). A random population analysis demonstrated GSTP1 to be polymorphic at amino acid 105. Polymorphism in GSTP1 is associated with drug resistance, failures of therapy and poor patient survival (Oguchi et al., 1994; Yasuno et al., 1999). Because of the functional importance and widespread nature of GSTP1, a number of studies have been carried out to investigate the role of polymorphisms in disease susceptibility, particularly in cancer. In gliomas, the nuclear localization of GSTP1 showing its high expression is considered as a determinant of poor survival (Ali-Osman et al., 1997).
It has been found that mothers with Ala 113 polymorphism of GSTP1A, had an increased risk of having children born with autistic disorder, suggesting its potential role in neurodevelopment (Williams et al., 2007). A highly significant increase in frequency of GSTP1b/ GSTP1b genotype was observed in a cohort of bladder cancer patients.

An increase in the proportion of individuals homozygous for the low activity of GSTP1b allele was observed in a COPD population (Palmer et al., 2006). A highly significant increase in the proportion of individuals homozygous for GSTP1b allele was found in teratoma and seminoma cancer samples. The study showed no significant increase in susceptibility associated with GSTP1b allele compared to controls in breast and colon cancers. The same study showed marked reduction in frequency of GSTP1b homozygotes, and a highly significant reduction in homozygotes for the GSTPIa alleles in prostate cancer. The observations indicate that polymorphism at the GSTP1 locus may be an important factor in susceptibility to different types of cancer.

A meta-analysis on 17 studies with 5281 cases and 7176 controls of colorectal cancer demonstrated that the GSTP1 polymorphism is unlikely to be a major risk for susceptibility to colorectal cancer (Gao et al., 2009) consistent with a previous meta-analysis of 2005 on four comparisons (Chan et al., 2005).

Four allelic variants have been described for the GSTP1-1 gene (A, B, C, D) leading to different amino acid substitutions in position 105 and 114 of the protein sequence. The proteins encoded by different alleles show different abilities to metabolize carcinogens and anticancer agents, suggesting an association between GSTP1 polymorphism and the risk for a variety of cancers as well as between said polymorphism and varying responses to cancer treatments.

The ile105val allele has been shown to influence the risk of Barret’s oesophagus and oesophageal carcinoma (Van Licshout et al., 1999). The polymorphisms have also shown to modulate the response to chemotherapy in patients with metastatic colorectal cancer (Stoehlmacher et al., 2002) and multiple myeloma (Dasgupta et al., 2003). The ile105val allele modulates the risk of chemotherapy related acute myeloid leukemia in patients treated for breast cancer and other cancers (Allan et al., 2001). Latest meta analysis on 30 published case control studies including 15901 cases and 18757 controls performed in 2010 suggests that
GSTP1 ile/val polymorphism may increase susceptibility to breast cancer in Asian population (Lu et al., 2010).

Several studies have shown that these GSTP1 polymorphisms may be linked to susceptibility to inflammatory diseases such as asthma (Palmer et al., 2006), allergies (Gilliland et al., 2004) and systemic sclerosis (Palmer et al., 2003). Variations in GSTs have also been associated with liver diseases. Homozygosity for the GSTP1 Ile105Val allele is increased in cystic fibrosis patients with significant liver disease (Henrion-Caude et al., 2002). GST expression is also altered in the liver of patients with alcohol liver disease (ALD) (Harrison et al., 1990). This disease has a degree of inter-individual variation of reactive oxygen species (ROS) and their toxic metabolites due to raised oxidative stress.

Not much work has been done on the association of exon 6 in prostate cancer, however, a few studies in other cancers have been done. Transition of cytosine to thymidine in exon 6 changes ala114 to val at the protein level. Wang et al. (2003) performed a study of 582 Caucasian lung cancer cases and reported that GSTP1 exon 6 polymorphism was associated with lung cancer. A study on COPD in Japanese population did not report 114 val allele in either group of subjects showing that the allele was not a significant contributor to the development of COPD in the population (Ishii et al., 1999). One of the studies has also reported a significantly better survival in patients who had exon 6 variant genotype (ala/val or val/val) as compared to patients who had the wild type genotype (ala/ala; p=0.037), thus concluding that GSTP1 exon 6 variant genotypes may be associated with improved survival among patients with stage III and IV Non-Small Cell Lung Carcinoma (NSCLC), the protective association being found in younger patients (<62 years) and in males (Lu et al., 2006).

2.13 Methylation

Cancer arises through a series of not only genetic, but also epigenetic alterations. Hypermethylation of the DNA plays a key role in gene silencing. DNA hypermethylation patterns are frequently altered in human cancers. Changes in methylation pattern have a central role in tumorigenesis, particularly the methylation of CpG islands has been shown to be important in transcriptional repression of numerous genes which function to prevent tumor growth or development. The first tumor suppressor gene found to be silenced through promoter hypermethylation was Rb1 (Sakai et al., 1991). Almost half of the tumour suppressor genes implicated in
familial cancer are known to be inactivated by DNA hypermethylation and it seems that this is as frequent an event as mutations occurring within the coding region of genes. These methylation changes include genome wide hypomethylation as well as regional hypermethylation (Ehrlich, 2002). Aberrant hypermethylation in cancer cells occurs at CpG islands that are generally protected from methylation in normal tissues. Each tumour has its own characteristic set of genes with an increased propensity to become methylated. Besides this, an individual tumour within a single patient has unique epigenetic fingerprint which reflects/visualizes the gradual formation (evolution) of tumour as compared to the tumour of the same type in different patient population. Development of cancer is closely related to DNA methylation and its presence or absence affects its prognosis. Studies have shown several methylated genes to be closely related to the prognosis of cancer. As per one study, p16 promotor methylation was detected in 42% of tumours (Yi et al., 2001). Hence, due to above mentioned characteristics, CpG methylation provides important information to study changes as well as can also be invaluable tool in cancer diagnosis and prognosis.

Mutations in individual genes have outlined critical aspects of tumourigenesis. Global genome screens have provided important information about molecular events occurring in tumourigenesis, but do not provide any universal markers. On the other hand, single type of DNA alteration i.e. aberrant methylation of gene promoter can point to the pathway disrupted in every type of cancer and can provide marker for sensitive detection.

Multiple key cancer genes have been studied to obtain a map of this alteration in malignant transformation. Clusters of CpG sites found dispersed in the genome are called CpG islands, DNA stretches ranging from 0.5 to 5 kb with GC content of at least 50% (Cross and Bird, 1995). Most of these CpG islands are unmethylated in the promoter region as compared to those present in introns and repetitive sequences where they are heavily methylated and inactivated (Fig.2.3). As methylation occurs early and can be detected in body fluids, it may be of potential use in early detection of tumours and for determining the prognosis. Because DNA methylation is reversible, drugs like 5’ azacitidine (Jackson et al., 1997; Villar-Garea et al., 2003), decitabine and histone deacetylase inhibitors are being used to treat a variety of tumours. Novel demethylating agents such as antisense DNA methyltransferases and small interference RNAs are being developed, making the field of DNA methylation wider and more exciting.
Analysis of candidate gene can be seen as only a partial picture of the methylation changes in cancer. Completion of human genomes sequencing and discovery of new techniques to study new genes like methylation sensitive arbitrarily primed PCR, methylated CpG island amplification, restriction landmark genomic scanning and differential methylation hybridization will be extremely useful.

In cancer, CpG island cytosine hypermethylation has been observed in more than 60 genes, including known tumour suppressor genes. The factor underlying CpG island hypermethylation are not known, however, evidences have suggested the existence of a CpG island methylator phenotype (CIMP) involving the silencing and inactivating of multiple genes by promoter hypermethylation (Shen et al., 2002), possibly through upregulation of DNMT1 (Kanai et al., 2001) which is a maintenance methyltransferase (Mtase) and exhibits its effects on hemi-methylated DNA. Colorectal carcinogenesis is frequently characterized by CIMP positivity. Even premalignant adenomas (Rashid et al., 2001) and serrated adenomas (Chan et al.,
2002) exhibit CIMP positivity, suggesting this phenotype to be an early event in colon carcinogenesis (Toyota et al., 1999).

Hypermethylation at CpG sites can also predispose to mutations because 5-methyl cytosine (5-MeC) can spontaneously undergo hydrolytic deamination, causing C to T transitions. Increased mutation rates, such as those observed at CpG sites in the \( p53 \) gene (Robertson et al., 1997) have been associated with endogenous and exogenous exposures to mutagen. Methylation of \( CDH13 \) by itself or in combination with \( ASC \) is related to recurrence in patients who have undergone radical prostatectomy. Methylation in CpG dinucleotides in the promoter region of human \( RTVP-1 \) is largely responsible for the downregulation of human RTVP-1 (Ren et al., 2004). In fact methylation resistance is inversely correlated to the expression of Msh2, the initial mismatch repair factor.

\( O^\beta \)-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in the defence against alkylating agents that generate \( O^\beta \)-alkylguanine in DNA, a major trigger of genotoxicity and apoptosis Therefore, screening the individuals’ MGMT expression levels in tumours and normal tissue should predict efficacy of methylation based cancer therapies.

2.13.1 Methylation in \( GSTP1 \) Gene

Serum Prostate antigen (PSA) level, digital rectal examination (DRE) and transrectal ultrasonography in combination have increased the ability to detect prostate cancer in its initial stage (Brawer et al., 2000). Thus it accounts for the decreased mortality rate related to prostate cancer (Jemal et al., 2004). However, the limitations of PSA level as a screening tool has limited diagnostic value as its sensitivity and specificity is at the most 75% (Neal and Donovan, 2000). The test is unable to distinguish between malignant and benign lesions accounting for unnecessary biopsies (Catalona et al., 2000).

Similarly the value of DRE and imaging techniques are also limited in early disease detection. Thus none of the procedures, above or in conjunction have been able to diagnose prostate cancer. To establish a small set of reliable diagnostic investigation in prostate cancer, effort has been made to quantitatively assess the methylation status of CpG islands in regulating region of \( GSTP1 \) gene.

CpG island methylation of \( GSTP1 \) has been detected in several cancer types, including breast and hepatocellular, but only in prostate cancer has this abnormality been constantly detected with a prevalence of more than 90% as quoted in some
reviews. This is the most frequent epigenetic alteration reported in prostate cancer as also in high-grade prostate intraepithelial neoplasia (HGPIN), a prostate cancer precursor lesion (Jeronimo et al., 2001, Virmani et al., 2002, Lin et al., 2001a,b; Jeronimo et al., 2002; Brooks et al., 1998). Since induced GSTP1 expression in prostate cancer cell lines did not suppress cell growth, GSTP1 is not recognized as a tumour suppressor gene (TSG), it instead was proposed to act as a ‘caretaker gene’ (Nelson et al., 2001). This suggests that prostate cells devoid of GSTP1 expression are more susceptible to endure DNA damage induced by oxidants and electrophiles whether originated endogenously or from dietary intake (Nelson et al., 2001). CpG island methylation of GSTP1 is an attractive biomarker for prostate cancer screening for several reasons. First that GSTP1 promoter methylation is rarely found in non cancerous prostatic tissue, potentially giving the test a high specificity. Secondly GSTP1 hypermethylation is considerably less frequent in other genitourinary malignancies as bladder and renal cancer (Esteller et al., 1998; Maruyama et al., 2001; Chan et al., 2002), although it has been identified in many breast and liver carcinomas (Tchou et al., 2000; Estellar et al., 2001).

Goessl et al. (2000) using a non-quantitative fluorogenic MSP assay, detected GSTP1 hypermethylation in 72% of plasma samples, 50% of ejaculates and 36% of voided urine samples from prostate cancer patients with benign prostatic hyperplasia (BPH). Not only limited to body fluids, the role of GSTP1 hypermethylation is now firmly established for tissue samples also. The alteration has subsequently been reported in non-cancerous prostate tissue (Brooks et al., 1998).

A study on frozen tissue samples of 69 patients with clinically localized prostate cancer, 28 paired high grade prostatic intraepithelial neoplasia (HGPIN) lesions and 31 patients with BPH screened for GSTP1 methylation using quantitative assay revealed methylation in 29% of BPH tissue samples and 91.3% of prostate cancer tissue samples (Jeronimo et al., 2001).

Maruyama and coworkers (2002) have reported GSTP1 methylation frequency of 36% in prostate carcinoma whereas Yamanaka et al. (2003) have reported a methylation frequency of 88% for the same gene. The latter value being in range with the reports by previous researchers (Lee et al., 1994; Brooks et al., 1998; Jeronimo et al., 2001; Lin et al., 2001; Jeronimo et al., 2002; Harden et al., 2003; Woodson et al., 2003). The discrepant results may be attributed to different methodologies as also to population differences.
All studies have reported a consistent link between GSTP1 methylation and prostate cancer. Detection of this methylation marker in urine or serum samples can be a valuable screening tool and its analysis might increase the accuracy of diagnosis of the malignancy in prostate biopsies. It can also help stratify patients with initial morphologically negative biopsies into high and low risk groups, thus improving patient care and reducing follow-up cost. Although the strength of the association has varied in tissues samples, it has been detected in 70 to 90% with sample numbers varying from 8 to 105 patients.

2.13.2 Methylation in MGMT Gene

O\(^6\)-methylguanine DNA methyltransferase (MGMT) is a 21 kDa protein which removes alkyl adducts from the \(O^6\) position of guanine and as such is involved in DNA damage repair. MGMT was initially found to act in the repair of \(O^6\) methyl guanine in \(E. coli\) (Foote et al., 1980) and DNA repair in mammalian species including human beings involves the activity of MGMT (Gerson et al., 1986; Woodhead et al., 1986). Methylation of cysteine in the MGMT gene and promoter region may correlate with the expression of the MGMT gene product (Ayi et al., 1992). MGMT expression is decreased in some tumour tissues, and lack of activity has been observed in some cell lines (Soejima et al., 2005), however, prostate cancer data are not clear. Indeed some studies have reported a lack of significant MGMT methylation in prostate tumors (Maruyama et al., 2002; Yamanaka et al., 2003; Yegnasubramanian et al., 2004), whereas others have detected moderate to high levels (Konishi et al., 2002; Kang et al., 2004). Loss of expression is rarely due to deletion, mutation, or rearrangement of the MGMT gene, but methylation of discrete regions of the CpG islands of MGMT has been associated with the silencing of the gene in cell lines. Among the more than 500 primary tumours studied, MGMT hypermethylation has been reported in a subset of specific type of cancer. In gliomas and colorectal carcinomas, aberrant methylation has been detected in 40% of the tumours, whereas in non-small cell lung carcinomas, lymphomas, and head and neck carcinomas, this alteration has been found in 25% of the tumours. MGMT methylation has not been reported in other carcinomas. It has been suggested that epigenetic inactivation of MGMT plays an important role in primary human neoplasia (Esteller et al., 1999). In another study on bronchial epithelium and sputum from current and former smokers, methylation of MGMT gene has been reported from 9 control samples (Belinsky et al., 2002).
India has multiethnic populations and the present study was undertaken in north Indian population. This study, which is first of its kind, is a step towards identifying new molecular markers for prostate cancer. An effort has been made to look into the polymorphic forms of repair and metabolic genes and correlate them to the susceptibility of prostate cancer. Also epigenetic mechanisms such as DNA methylation are frequently involved in controlling gene functions during tumorigenesis. If properly validated and explored in large number of individuals, this will further aid in revealing risk factors, individual susceptibility, early diagnosis and prognosis, and follow up treatment of the disease. These markers could also serve as potential therapeutic targets in drug development.