6. Discussion

With the completion of the Human Genome Project, rapid and ongoing advances in genomic profiling and basic biological insights have lead to a more complete and sophisticated understanding of interactions among genes, environment and affected tissues. As a result, increasingly comprehensive views of patients’ genetic endowment and transcriptional changes within organs and tissues affected by diseases are being derived.

Cancer has always been an area of concern for scientists and clinicians, contributing to approximately 12% of major global death causes. It is actual health burden throughout the world, causing 6.2 million deaths. Cancer is a fear for 22.4 million persons living with it (Parkin, 2001). The prevalence of prostate cancer is so high that it could be considered a normal age related phenomenon. One out of every 59 men in India is expected to contact prostate cancer during their life time (Sunny et al., 2004). In U.S. alone, 29% of all detected cases in 2005 were of prostate cancer.

Knowledge of genetic changes underlying the initiation, development and progression of prostate cancer is accumulating rapidly. A clinical application of this knowledge would be a radical treatment to ensure that the associated morbidity could be avoided in prostate cancer that is unlikely to progress. Risk of prostate cancer is influenced by environment, culture change, dietary habits etc. According to an estimate, 42% of the prostate cancer risk may be attributed to genetic influences and individual and combined effects of genes (Hsing and Chokklingam, 2006).

Demographic Factors and Risk of Prostate Cancer

Physical activity, ethnicity, occupation, alcohol consumption, smoking, obesity and fat rich diet are important risk factors and play a role in one way or the other in the development of prostate cancer.

Physical activity enhances blood circulation, maintains energy balance and improves the immunity level (Kujala et al., 1996). It also checks obesity, thereby preventing other diseases. In UK, physical activity was found to be inversely proportional to the risk of any type of particular cancer (Aronson et al., 1996) in men,
but Norman et al. (2002) had defied any such evidence. Some studies (Le Marchand et al., 1991; Ilic et al., 1996), however, have found significant association of physical activity and risk of prostate cancer.

Studies carried out in North America, Asia and Europe have found that physical activity in any form, occupation or on field or both leads to decreased risk of prostate cancer by 10-70% (Andersson et al., 1995). Nilsen et al. (2006) also reported that recreational physical exercise is associated with reduced risk of advanced prostate cancer and prostate cancer death.

In India, the manual workers are considered as highest physically active group in comparison to sedentary ones (Hartman et al., 1998). In the present study, frequency of manual workers was 50.9% in prostate cancer cases and 50% healthy controls. Frequency of sedentary subjects was 45.9% in prostate cancer cases and 47.1% in controls. On comparison with healthy controls (OR=1.05; 95% CI=0.67-1.63), no association was found between risk of prostate cancer and manual workers (Table 5.1). The results are compatible with the findings of Norman et al. (2002) who showed no such association in Swedish population.

A comparison was made between aged and young Americans by Lee and coworkers in 1992, showing 1.9 times risk of prostate cancer in former with lesser physical activity as compared to their counterparts and no significant association was observed in the latter.

Certain occupations and occupation exposures have been found to affect the risk of prostate cancer such as rubber industry workers, farmers, and persons working in metal industries (Krstev et al., 1998; Brown and Deizell, 2000). Frequency of manual industry workers was 3.2% in prostate cancer cases and 2.9% in healthy controls. As such there was no association found between risk of prostate cancer and industrial workers as compared to healthy group of controls (OR=1.11; 95%CI = 0.31-3.99). A study carried out by Zeegers et al. (2004) on Netherland population also did not find any association of prostate cancer risk in metal workers (OR=0.92).

Nilsen et al. (2005) had reported that divorced separated men had high incidence of prostate cancer as compared to married men, whereas Lightfoot et al.
(2004) found no such association. All the subjects of the present study were married, and as such no comparison could be made.

About 56% of prostate cancer cases and 58.8% of controls belonged to urban areas, whereas 44% of prostate cancer cases and 41.2% of controls belonged to rural areas of north India. No association was found between risk of prostate cancer and their residence in rural or urban areas. No significant co-relation could be established between education and risk of prostate cancer. Only marginal risk, 1.3 times, was found in highly educated cases as compared to illiterates (OR=1.38; 95% CI=0.76-2.56) that may be due to consumption of processed foods and lethargic life style. Results are compatible with those of study on Italian population by Bosetti et al. (2004).

Diet plays a very important role in prostate cancer. -methyl-coenzyme-M-reductase enzyme plays a key role in the oxidation of fatty acids. This enzyme is upregulated in prostate cancer, but not in healthy prostate. Milk, dairy products and red meats are major sources of fatty acids. Due to the oxidation of these fatty acids, hydrogen peroxide is produced, which might be the cause of oxidative damage to the prostate genome (Grönberg, 2003). The results of the present study showed marginal association between milk consumption and prostate cancer cases as compared to healthy controls (OR 1.68, 95% CI= 0.84-3.36).

As far as consumption of tea is concerned, there are contradictory views regarding its role in risk of prostate cancer. Some studies have reported tea and coffee to be mutagenic in nature (Nagao et al., 1979), while Ren et al. (2000) found that components of tea rather interfere in the development of prostate cancer. Bolt et al. (1996) ruled out any such role. Many other studies have ruled out any such association (Jain et al., 1998; Villeneuve et al., 1999; Ellison, 2000). Results of the present study are in concordance with the latter studies, as no association was found between tea consumption and risk of prostate cancer when compared with healthy controls (OR=1.19; 95% CI=0.52-2.71) (Table 5.2).
Relationship of Metabolic Gene Polymorphism and Risk of Prostate Cancer

Polymorphism plays an important role in susceptibility to different types of cancers. More common DNA sequence variations may have substantial impact on individual’s susceptibility to cancer. Most of these variations consist of single nucleotide polymorphisms (SNPs) that occur when a single base pair in the genome differs among individuals. Although some SNPs are relatively rare, many have a more balanced frequency in certain populations. In fact SNP frequencies often vary between populations. An SNP common in one geographical or ethnic group may be rarer in another. So called non synonymous SNPs are variants in the coding sequence that affect the polypeptide sequence once a gene is transcribed and translated. However, SNPs in the non coding regions of genes can still affect gene splicing and transcription factor binding. Most importantly, variations in the DNA sequences can affect cancer occurrence and responses to pathogens, chemicals and drugs.

Glutathione S-transferase isozymes present in human tissues belong to at least four major families, which show remarkable similarities in their primary sequence within their respective classes. Any alteration in the structure, function, or expression of GSTP1 gene may alter the ability of the cell to inactivate carcinogens or mutagens and thus modify an individual’s risk to cancer (Lu et al., 2010). Despite only minor sequence differences, the functional properties of isoforms within a class are also often quite distinct. The heterogeneity of substrate specificity between isoforms in any given class may have significant physiologic and pathophysiologic importance in detoxification of endogenous and exogenous compounds. The catalytic efficiencies of these isoforms are likely to be significantly different not only in vitro, but also under conditions of competition between various enzymes for limited substrate pools, e.g. in a cell (Zimniak et al., 1994).

**GSTP1 (Exon 5) Genotype**

The population under study did demonstrate that GSTP1 was polymorphic at amino acid 105 by analysis of the individuals predicted to be ile/ile (homozygous wild), ile/val (heterozygous variant), val/val (homozygous mutant) genotype.
In a population of 170 healthy controls, the frequencies of ile/ile, ile/val, val/val genotypes were 42.9, 47 and 10% respectively. The frequencies of these genotypes in a population of 170 BPH cases were 45.8, 44 and 10% respectively. In order to establish whether the GSTP1 alleles were associated with cancer susceptibility, the frequency of each genotype was studied in prostate cancer cases. The frequencies were 38.8, 45.8 and 15.2% respectively. Surprisingly, the frequency of ile/val genotype was maximum in healthy controls followed by prostate cancer cases and BPH cases. The frequency of mutant genotype was maximum in prostate cancer cases as compared to healthy controls and BPH cases. The frequency of ile/ile (homozygous wild) genotype was minimum in prostate cancer cases (61% in cancer cases versus 73% in controls and 78% in BPH) (Table 5.4).

In the present study, the distribution of val/val genotype of GSTP1 (exon 5) had shown 1.68 times higher risk when compared with healthy controls (OR=1.68; 95%CI=0.83-3.43). The results are in concordance with a study on Japanese population reporting that the subjects with val/val genotype of GSTP1 gene were at significant risk of prostate cancer (OR=9.03; 95%CI=0.47-18.4; \(p=0.030\)) (Nakazato et al., 2003). Kote-Jarai et al. (2001) had revealed similar results showing ile/ile homozygotes to have the lowest risk and the risk increased with the number of 105 val alleles (ile/val: OR=1.30; 95%CI=0.99-1.69 and val/val: OR=1.80; 95%CI=1.11-2.91; \(p=0.026\)).

A study on prostate cancer showed a marked reduction in the frequency of val/val genotype of GSTP 1 gene although it was not statistically significant \(p=0.693\). The study also showed, significant reduction in ile/ile genotype \(p=0.008\) and a significant increase in the proportion of ile/val genotype \(p=0.003\) (Harries et al., 1997).

Mutant genotype when compared with BPH cases showed 1.80 times (OR=1.80; 95%CI=0.89-3.65) higher risk of prostate cancer in the present study (Table 5.4).
**GSTP1 (exon 6) Genotype**

Studies have suggested that polymorphisms of *GSTP1* (exon 6) (*ala*/*val*) have functional effects on the GST gene product resulting in reduced enzyme activity. The highest frequency of polymorphism of *GSTP1* (exon 6) was of heterozygous (*ala/val*) genotype, being 48.4 (OR=1.59; 95%CI=0.99-2.57) and 46.4% (OR=1.27; 95%CI=0.78-2.04) in cases and BPH respectively (Table 5.9). It was found to be 41.7% in healthy controls. The frequency of mutant genotype (*val/val*) was minimum i.e. 17.8% in prostate cancer cases and 12.9% in healthy controls (OR=1.87; 95%CI=0.96-3.61). It was observed to be 12.3% in BPH cases (OR=1.76; 95%CI=0.90-3.43). The frequency of wild type genotype (*ala/ala*) was observed to be 33.7, 45.8, and 41.1% in prostate cancer cases, healthy controls, and BPH cases respectively. The frequency of heterozygous genotype *ala/val* showed 1.59 times higher risk as compared to healthy controls and 1.27 times more risk in BPH cases (Table 5.9).

Studies on other cancers especially lung cancer have shown significantly increased risk for exon 6 variant genotypes (OR=2.17; 95%CI=1.41-3.33) (Wang et al., 2003). A Chinese study on leukomogenesis in children found no *ala114val* variant genotype in the population, instead it reported the presence of *asp147try* genotype (Yuan et al., 2003). A COPD study on Japanese population also did not find 114*val* allele in either group of subjects (Ishii et al., 1999). Still one study on patients with Stages III and IV NSCLC and having exon 6 variant genotype (*ala/val* or *val/val*) were reported to have significantly better survival as compared to patients having wild type genotype (*ala/ala*; *p*=0.037) depicting its protective association (Lu et al., 2006).

The present study depicted that individuals with *val/val* genotype have 1.87 times (OR=1.87; 95%CI=0.96-3.61) increased risk as compared to healthy controls and 1.76 times (OR=1.76; 95%CI=0.90-3.43) increased risk when stratified with BPH cases (Table 5.9).
Relationship of Repair Genes and Risk of Prostate Cancer

Genomic stability and integrity are important in maintaining accurate DNA replication. DNA gets damaged by a number of endogenous and exogenous factors such as UV light, smoking, dietary factors, reactive oxygen species and carcinogens leading to DNA mutations. These mutations, in the form of gene arrangements as translocations, amplifications, and deletions can in turn contribute to cancer development. DNA repair mechanisms defend against these assaults, correcting DNA damage as well as normal replication errors (Goode et al., 2002). A deficiency in repair capacity due to the defect in genes involved in DNA repair can lead to genomic instability and carcinogenesis. Individuals with a repair capacity below the population mean can be at an increased risk of developing different kinds of cancer. It is likely and has been shown in certain studies that single nucleotide polymorphisms in coding and regulatory sequences may result in subtle structural alterations in DNA repair enzymes modulating cancer susceptibility (Sturgis et al., 1999; Kumar et al., 2003).

Different pathways of DNA repair operate on specific types of damaged DNA and each pathway involves numerous molecules. Polymorphisms in DNA repair genes, such as the Xeroderma pigmentosum XPD and XPG, have been studied for their association with lung (Chen et al., 2002, Hou et al., 2002) and breast (Kumar et al., 2003) cancer.

Malignant transformation of prostate cells is accompanied by somatic genomic changes, including deletions, amplifications, and point mutations (Bova et al., 1996; Dong et al., 1996). In vitro studies of human prostate tissue have demonstrated that after exposure to environmental toxins, DNA forms adducts in prostate tissue (Wang et al., 1999; Nelson et al., 2001). Moreover, intake of antioxidants via the diet or as supplements may decrease prostate cancer risk through the inactivation of reactive oxygen species, thereby protecting the DNA from oxidative damage (Fleshner et al., 2001). This evidence suggests that DNA repair capacity may be playing an important role in prostate carcinogenesis, but not much is known about what direct effect DNA repair capacity has on prostate cancer risk.

The entire process leading to DNA damage and subsequent repair of the damage involves a host of enzymes. NER is the most important and complicated
repair process, involving the protein products of more than 30-40 genes (Wood et al., 2001). It removes the broadest spectrum of genomic damages, including UV-induced photoproducts, bulky mono-adducts, cross-links, and oxidative damage. Several known genetic defects in NER lead to xeroderma pigmentosum, which is associated with a 1000-fold increase in skin cancer as well as a 20-fold increase in other internal tumours (Cleaver, 2000).

The XPD and XPC genes code for a DNA helicase involved in transcription and nucleotide excision repair (Evans et al., 1997). To explore the tentative modulating effect of DNA repair polymorphisms, these biomarkers, that are assumed to be relevant to the onset of prostate cancer have been studied. In the present study, the potential links between genetic polymorphisms in genes coding DNA repair enzymes especially xeroderma pigmentosum complementation group D (XPD) and group C (XPC) involved in nucleotide excision repair (NER) has been investigated. The SNPs studied included xeroderma pigmentosum complementation group C (XPC) exon 15 (Lys939Gln, A/C) and XPD exon 23 (Lys751Gln, A/C). This is probably the first population based study between repair gene polymorphisms and risk of prostate cancer of North Indian origin.

XPC Genotype

In the present study, frequency of mutant genotype of XPC (CC) was found to be highest in cases (12.7%) and the frequency of wild type genotype (AA) was found to be maximum in healthy controls (46.5%). Also the frequency of heterozygous genotype (AC) was more in cases (46.5%) as compared to controls (44.7%) (Table 5.14).

An increased overall risk of cancer for variant homozygotes of lys939gln (OR=1.16; 95%CI=1.05-1.28) was reported in a metaanalysis of 16 studies (Qiu et al., 2008). A particular study in prostate cancer cases found frequency of 939gln variant at XPC lys939gln to be significantly low in prostate cancer cases (OR=0.39; p=0.016) (Hirata et al., 2007). The study concluded that XPC lys939gln variant may be a risk factor for prostate cancer in Japanese.
The present study has revealed statistically non significant higher risk of prostate cancer for homozygous (CC) and heterozygous (AC) variants of lys939gln of XPC gene. The overall results revealed by many epidemiological studies on the association of XPC polymorphism with cancer risk are contradictory e.g. Vogel et al. (2005) suggested increased lung cancer risk with 939gln allele in a Danish population.

The XPC 939Gln allele has been reported to be associated with an excessive risk of bladder (Sanyal et al., 2004) and lung (Vogel et al., 2005) cancer but Shen et al. (2005) found a border line significant risk of lung cancer in Chinese population. However, a negative association was noted for lung cancer (Lee et al., 2005).

XPD Genotype

The xeroderma pigmentosum group D (XPD) protein is a well-characterized DNA helicase necessary for the nucleotide excision repair of bulky DNA lesions, such as those induced by cigarette smoking. Polymorphisms in several exons of the XPD gene have been identified; two of them, asp312asn and lys751gln, are common and result in an amino acid change. Most of the reported data indicate higher levels of DNA adducts in people carrying variant asn or gln alleles, which suggests that these persons have lower repair efficiency (Benhamou and Sarasin, 2005). Mutations in the XPD gene can completely prevent DNA opening and dual incision, steps that lead to the repair of DNA adducts (Evans et al., 1997). Several common single base pair substitution polymorphisms in the XPD gene have been identified. Lunn et al. (2000) showed that individuals with the XPD codon 751 Lys/Lys genotype had a 7-fold increased risk of suboptimal DNA repair. The XPD codon 312 polymorphism has been associated with increased adduct levels in breast tumour tissue (Tang et al., 2002).

In the present study, the frequency of heterozygous genotype (AC) was highest in prostate cancer cases (53.5%) followed by homozygous wild (AA) (40.8%) and homozygous mutant (CC) (5.7%) genotypes. The frequency of mutant genotype (CC) was more in cases (5.7%) as compared to healthy controls (3.0%). Also the wild type frequency (AA) was maximum in healthy controls (44.7%) as compared to cases (40.8%) (Table 5.15).
Comparison of these results with other studies revealed variations in frequencies in different cancers and also in different populations. Approximately fifty percent of the subjects carried heterozygous (AC) genotype and ten to fifteen percent carried homozygous variant (CC) genotype as per a study carried in European and North American population. The variant allele depicted less frequency in African American population also (5.6%) (David-Beabes et al., 2001). Liang et al. (2003) found this allele to be quite uncommon in Chinese population. Similarly Park et al. (2002) carried out a study on South Korean population and Hamajima et al., (2002) analyzed Japanese population. All of them reported 90% of the subjects to be carrying homozygous wild (AA) genotype, while the homozygous variant (CC) was rarely observed. Still another study on Chinese population reported 18% of the subjects to be carrying homozygous variant (CC) genotype (Chen et al., 2002).

The present study is first of its kind in the North Indian population and has revealed that individuals carrying the heterozygous variant (AC) genotype have significantly increased risk of developing prostate cancer ($p=0.02$) (Table 5.16).

Previous molecular epidemiological studies have reported that XPD 751Gln allele is associated with an increased risk for head and neck (Sturgis et al., 2000), melanoma skin (Tomestu et al., 2001) and lung (Hou et al., 2002; Xing et al., 2002) cancer. However, inconsistent findings have also been reported, including absence of any association with lung cancer (Misra et al., 2003; Park et al., 2002), and paradoxical inverse associations with basal cell carcinoma (Dybdahl et al., 1999). Previous reports on the association between XPD 312Asn allele and cancer risk are also controversial including either positive (Hou et al., 2002; Liang et al., 2003) or null results (Winsey et al., 2000; Spitz et al., 2001). Reasons for the previous inconsistent findings may include small sample sizes, inappropriate study design and may be different Linkage Disequilibrium with other SNPs in different populations.

**Relationship of Methylation and Risk of Prostate Cancer**

A great emphasis is being laid on the development of tumour biomarkers to aid the management of cancer. For this DNA alterations have to be assessed from a primary tumour sample. A less invasive and patient friendly option would be the detection of these changes within the patient’s blood. The correlation of alterations in
tumour DNA with circulating tumour DNA would allow potential development of clinically relevant biomarker blood tests for early detection of cancer, prediction of a likely treatment effect and assessment of tumour response to therapy (Board et al., 2008). Detection of genetic and epigenetic alterations in the tumour DNA offer a potential source of development of prognostic and predictive biomarkers for cancer. DNA methylation of the promoter region of tumour suppressor genes is one such alteration which causes downregulation of tumour suppressor gene expression, a frequent event in carcinogenesis.

Epigenetic changes such as DNA methylation and histone modifications have an essential role to play in cellular and molecular alterations related to the development and progression of prostate cancer (Li et al., 2005; Schulz and Hatima, 2006). Identification of epigenetic alterations is a promising strategy to develop assays for the early detection of prostate cancer. Methylation of the cytosine nucleotide residues located within the dinucleotide 5’- CpG-3’ is the most frequent epigenetic alteration in humans. These CpG dinucleotides are not randomly distributed in the genome. Instead, there are ‘CpG islands’, frequently associated with the 5’ regulatory regions of genes, including the promoter, untranslated region and exon (Bird et al., 1986). Methylation of these CpG islands provides an effective means of regulating gene expression and pathological events such as carcinogenesis (Herman and Baylin, 2003). Normally, these CpG islands remain in the unmethylated state leaving aside a few which seem to acquire age-related methylation in particular genes (Issa et al., 1994; Waki et al., 2003). This equilibrium gets imbalanced in malignant cells. In certain promoter regions, the CpG islands become heavily methylated shutting down the expression of that particular gene (Estellar, 2000).

Prostate cancer manifests different clinical and morphological characteristics. It has been described to be heterogeneous and multifocal. In general it is indolent but 25 - 30% of tumours become clinically aggressive (Coffey, 1993; Greenlee et al., 2001). This cancer is normally an androgen-dependent tumour which becomes androgen independent and highly invasive. In its advanced stage, the tumour spreads locally and then metastasizes to pelvic lymph nodes and then to bones. After metastasis, it becomes incurable (Zetter, 1990; Arnold and Isaacs, 2002). BPH and prostate cancer are believed to be derived from prostatic intraepithelial neoplasia.
(PIN) lesions (Untergasser et al., 2005; De Marzo et al., 2007). Prostate carcinoma mainly occurs in peripheral zone, BPH in transition zone and central zone is believed to be relatively resistant to diseases and carcinoma (McNeal, 1969; McNeal, 1988).

**GSTP1 Hypermethylation**

Results of the present study showed the frequency of methylation among prostate cancer cases was maximum (92%) in the age group of 71-80 years and lowest (50%) in the age group of 81-90 years. In the other age groups, the methylation frequency varied from 82-88%. In healthy controls, methylation frequency was maximum (27%) in the age group of 71-80 years. It was zero percent in the age group of 31-40 years and showed a steady increase with age i.e. 13% in the age group of 41-50 years, 24% in the age group of 51-60 years and 26% in the age group of 61-70 years (Fig. 5.9).

The frequency of methylation in BPH cases was low (0-32%) as compared to prostate cancer cases. In the age group of 81-90 years, the frequency was maximum (32%) and negligible in the age group of 41-50 years (Fig. 5.5).

Several studies revealing the methylation pattern of GSTP1 gene have been carried out. Majority of them have reported methylation frequency to be from 70% - 100% in prostate cancer cases. Two reports have revealed methylation in plasma with detection rates of 72% (Geossel et al., 2000) and 36% (Jeronimo et al., 2002) in prostate cancer cases. Methylation in urine samples of prostate cancer cases has been reported in several studies with detection rates varying from 27% - 78% (Geossel et al., 2001; Gonzalgo et al., 2003; Cairns et al., 2001). In the, tissue samples taken by biopsies or during surgery, methylation was detected in 70% of cases, some detection rates being more than 90% (Jeronimo et al., 2001; Chu et al., 2002). The current study is in confirmation with the above mentioned studies as the overall methylation frequency was 80.1% in prostate cancer cases (Fig. 5.1).

As far as methylation of GSTP1 promotor in normal prostate tissues and BPH tissues is concerned, it is rarely found to be methylated (Nakayama et al., 2004) but Jeronimo et al. (2001) have reported it in 6.9% of BPH cases. The present study also shows 20.1% of the healthy controls to be methylated, the frequency being 0% in age
groups of 31-40 years, increasing gradually to 27% in the age group of 71-90 years (Fig. 5.9) and 17% in BPH cases, the frequency being 0% in age groups of 41-50 years (Fig. 5.5).

The inconsistency in levels of methylation in prostate cancer cases, 36% (Maruyama et al., 2002) and 88% (Yamanaka et al., 2003), may be attributed to several factors like difference in samples used, variation in analytical methods, the pretreatment of DNA, PCR conditions and methods adopted.

**MGMT Hypermethylation**

Decrease of *MGMT* expression has been studied in some tumor tissues and lack of activity in some cell lines (Soejima et al., 2005). However, not much has been reported in prostate cancer. Some studies have even reported a lack of significant *MGMT* methylation in prostate tumors (Maruyama et al., 2002; Yamanaka et al., 2003; Yegnasubramanian et al., 2004). However, in the present study, frequency of methylation was maximum in prostate cancer cases in the age group of 41-50 years (>60%). It was 60% in the age group of 51-60 years, 58% in the age group of 61-70 years and 52% in the age groups of 71-80 years. Cases in the age group of 81-90 years showed lowest frequency i.e. 30% (Fig. 5.13). In case of BPH patients maximum frequency was revealed in the age group of 61-70 years (22%). It was 20% in the age group of 31-40 years and just 5% in the age group of 51-60 years. However, there was no methylation observed in the age group of 41-50 years and 71-80 years (Fig. 5.17). Amongst the healthy controls maximum frequency (42%) was revealed in the age group of 71-80 years, 39% in the age group of 61-70 years, 36% in the age group of 51-60 years and 22% in the age group of 41-50 years. The frequency was zero percent in the age group of 31-40 years (Fig. 5.21). Moderate to high levels of *MGMT* methylation were also detected by Kang et al. (2004) and Konishi et al. (2002).

**Impact of Environmental Factors and Risk of Prostate Cancer**

Genetic and environmental factors have an important role to play in the determination of cancer risk. More than 80% of the cancer cases are due to environmental factors and only 2% reveal the role of genetic factors in its development (Doll and Peto, 1981). Comprehensive knowledge about the
implications of environmental risk factors would widen the understanding of the underlying mechanism in prostate carcinogenesis. Studies have reported that the influence of environmental factors is very important in the development of prostate cancer. Factors like poverty, lack of education, health insurance, occupation, diet, physical activities, socio-economic status etc are important factors in the cause of cancer because they activate the risk and affect the prognosis and diagnosis. It is, thus, absolutely necessary that these factors are identified and their risk in the development of prostate cancer along with the polymorphism of the gene implicated be understood. In the present study, the effect of smoking and tobacco chewing and consumption of alcohol and non-vegetarian diet on metabolic and repair genes were studied along with their effect on the hypermethylation patterns of $GSTP1$ and $MGMT$ genes in the north Indian population.

**Smoking, Tobacco Chewing and Risk of Prostate Cancer**

The exact mechanism of how smoking and tobacco chewing are associated with prostate cancer is not clear as yet. Smoking is believed to cause hormonal imbalance such as lowering the levels of estrogen and sex hormone binding globulins and raising the level of testosterone (Gann et al., 1996). A number of studies have shown the cigarette smoke to be a risk factor for the development of prostate cancer (Fincham et al., 1990; Sobti et al., 2009, 2010; Thakur et al., 2010). Relative risk of 1.8 and 2.1 for cigarette smoking and tobacco chewing was reported in 1990 (Hsing et al., 1990). High risk in smokers has been attributed to the presence of cadmium which is a trace element present in cigarette smoke and alkaline batteries. Tobacco consumption is quite high worldwide, most of the cancers occur due to its use. Commonly used tobacco products include Pan and Gutka. Chemicals, that are found to be carcinogen are released from tobacco and get deposited in prostate cells via circulatory system (Smith and Hagopian, 1981).

In the current study, about 68.2% cases of cancer were non smokers and 31.8% were smokers. The frequency of smokers in controls was 27.7 (healthy) and 25.9% (BPH). The frequency of non-smokers in healthy and BPH controls were 72.3 and 74.1% respectively (Table 5.3). Tobacco smoke include chemical like benzopyrene and poly aromatic hydrocarbons which are metabolized by cellular enzymes (Phase1 and 2 enzymes). The chemical carcinogens are activated to produce
DNA adducts by phase-1 enzymes and the Phase 2 enzymes conjugate metabolic intermediates and convert them into soluble form. Glutathione-S transferase (GSTP1) metabolizes numerous carcinogenic compounds including benzopyrene. In the present study, two polymorphic forms of GSTP1 genes were studied. The mutant genotypes of both the polymorphisms of exon 5 and exon 6 depicted a significant impact on the risk of prostate cancer. The mutant genotype (val/val) when compared with BPH cases, revealed 1.7 times higher risk, but it was found to be non-significantly higher in individuals with combined genotype (ile/val/val/val) (OR=1.56) (Table 5.5). The variant genotype depicted significantly increased risk in smokers with heterozygous genotype (ala/val) (OR=3.02; p<0.001) and in subjects with combined genotype (ala/val/val/val) (OR=3.24; p<0.0004) (Table 5.10) of GSTP1 (exon 6) gene.

Besides prostate cancer, other cancers have also revealed similar results in exon 6 showing it to be associated with an elevated risk in lung cancer among ever smokers (OR= 1.58) (Wang et al., 2003), but the study did not depict ile105val polymorphism to be associated with lung cancer risk. Another study gave contradictory views depicting ile/ile genotype to be strongly associated with an increased risk of prostate cancer in smokers and indicated the variant allele to be protective (Mao et al., 2004).

In the current study non significant increased risk of 2.65 times was observed in tobacco chewers with mutant genotype val/val of GSTP1 (exon5) gene (OR=2.65) (Table 5.7). Similarly non significant increased risk of 2.51 and 5.67 times was observed in tobacco chewers with mutant genotype val/val of GSTP1 (exon6) gene as compared to healthy controls and BPH cases respectively. The results in this study have indicated that smoking has a significant effect on GSTP1 (exon6) ala114val polymorphism as compared to healthy control and increased risk was also observed in GSTP1 (exon5) ile 105 val polymorphism in comparison to BPH cases. Still a large sample size is required as the number of consumers was less in the present study.

The function of XPD is critical to repair of genetic damage caused by tobacco and other carcinogens (Moncolin et al., 2001). Numerous studies have shown that most DNA lesions caused by tobacco- smoke carcinogens are repaired by XPC and XPD genes (Hoeijmakers, 1993; Sancar, 1996; Wang et al, 1996).
The C allele of *XPD* repair gene showed 3.24 times non significant increased risk of prostate cancer in smokers (Table 5.16), whereas a weak association was observed with *CC* genotype of *XPC* gene (OR=1.73) (Table 5.17). The results are similar to other studies. No association of *XPC* lys939gln genotype and smoking was found by Hirata *et al.* (2007) in prostate cancer.

As far as the methylation status is concerned, the *GSTP1* gene revealed a methylation frequency of 99% in smokers and 66% in non smokers amongst the prostate cancer cases (Fig. 5.3). In BPH cases overall methylation frequency was low. Methylation frequency of 25% was observed in smokers as compared to non smokers revealing only 8% (Fig. 5.6). In the healthy controls it was 24% in smokers and just 10% in non smokers (Fig. 5.10). The results indicated a significant effect on the methylation pattern of *GSTP1* gene in smokers.

The *MGMT* gene which is a repair gene and the product of this gene removes mutagenic and cytogenic adducts from O^6^-guanine in DNA also revealed significant change in its methylation frequency in smokers as compared to non smokers. Cadmium is an active inhibitor of the methyltransferase as shown in studies on cultured cells and chronic cadmium exposure causes hypomethylation of genomic DNA, which is later followed by hypermethylation of the genomic DNA (Takiguchi *et al.*, 2003). The methylation frequency was 53% in smokers and 40% in non smokers in prostate cancer cases (Fig. 5.14). It was 42 and 17% in smokers and non smokers amongst healthy controls respectively (Fig.5.22) and it was 37% in smokers and 5% in non smokers as compared to BPH (Fig. 5.18).

**Non Vegetarian Diet and Risk of Prostate Cancer**

Epidemiological studies on migrants have revealed the influence of diet on prostate cancer risk. The best example of such kind of influence has been of Japanese men who had low incidence of prostate cancer in their native country and who had migrated to the United States in younger age started reflecting the local incidence and mortality rates (Shimizu *et al.*, 1991; Whittemore *et al.*, 1995; Cook *et al.*, 1999). The reason may be high intake of fat, meat and dairy products. Non vegetarian diet has been associated with prostate cancer risk (Veierod *et al.*, 1997; Sobti *et al.*, 2009).
Cooking meat at high temperature produces heterocyclic amines and some carcinogens that increase the risk of certain malignancies (Shirai et al., 2002).

The present study comprised of 54.8% non vegetarians amongst prostate cancer cases, 49.4% amongst healthy controls and 45.9% amongst BPH cases (Table 5.2).

Comparison of prostate cancer cases with BPH cases showed 2.58 fold non significant increased risk (OR=2.58; 95% CI=0.76-8.75) in non vegetarians carrying mutant genotype (val/val), 1.97 fold non significant increased risk in non vegetarians with combined genotypes ile/val/val/val (OR=1.97; 95% CI=1.03-3.76) and 1.88 fold non significant increased risk in subjects with heterozygous genotype ile/val (OR=1.88; 95% CI=0.97-3.65) of GSTP1(exon5) gene. No such association was observed in the healthy controls (OR=1.23; 95%CI=0.40-3.72) (Table 5.8). Significantly increased risk was observed in non vegetarians carrying ala/val genotype (OR=3.83; 95%CI=1.89-7.75); val/val genotype (OR=5.11; 95%CI=1.53-17.03) and ala/val/val/val genotype (OR=4.006; 95%CI=2.02-7.93) of GSTP1 (exon6) gene when compared with BPH subjects (p<0.001; p<0.004; p<0.0001 respectively) (Table 5.13).

Significantly high risk of prostate cancer was observed in Chinese population consuming preserved meat (Jian et al., 2004), but the risk was quite low in Japanese population also consuming non vegetarian diet (Sonoda et al., 2004). The reason may be high intake of fish by Japanese people, which is known to contain selenium, an essential trace element which is protective against prostate cancer (Leitzmann et al., 2003; Klein, 2004).

The AC genotype of XPD gene showed 2.61 times significantly increased risk of prostate cancer amongst non vegetarians (OR=2.61; 95%CI=1.03-6.60; p<0.02) (Table 5.16). The risk was found to be 4 fold non significantly higher with the CC genotype of XPD gene (OR=4.0; 95%CI=0.91-17.51). The CC genotype of XPC gene had shown 2.02 (OR=2.02; 95%CI=0.77-5.31) fold non significant increased risk of prostate cancer in non vegetarians (Table 5.17).
In prostate cancer cases, the frequency of methylation in \textit{GSTP1} gene in relation to non vegetarian diet was 94% as compared to vegetarians in which it was 51% (Fig.5.4). The frequency of methylation was 19 and 9% in non vegetarians and vegetarians respectively amongst BPH cases (Fig.5.8). In healthy controls, the frequency of methylation was 24 and 2% in non vegetarians and vegetarians respectively (Fig. 5.12)

The methylation frequencies in \textit{MGMT} gene showed 60 and 50% respectively in non vegetarians and vegetarians having prostate cancer (Fig. 5.16); 48.2 and 7.1% in non vegetarians and vegetarians amongst healthy controls (Fig. 5.24) and it was 17 and 9% in non vegetarians and vegetarians with BPH respectively (Fig.5.20). The data revealed non vegetarian diet to be associated with hypermethylation of \textit{GSTP1} and \textit{MGMT} genes and as such is proposed to increase the risk of prostate cancer.

\textbf{Alcohol Consumption and Risk of Prostate Cancer}

There are many ill effects of alcohol consumption. They range from causing imbalance of hormone metabolism, androgen and estrogen, to suppression of testicular production in non-alcoholic men with repeated high doses. Increase in circulating estrogen levels also leads to development of feminine characters (Purohit, 2000). Ethanol is converted to acetaldehyde by alcohol dehydrogenase which is further converted to acetate by acetaldehyde dehydrogenase. Enhancement of the solubility and absorption of mutagens and inhibiting cytochrome P-450 detoxification enzymes may influence the risk of prostate cancer.

In the present study, the frequency of drinkers in prostate cancer cases and BPH was 41.4 and 41.2% respectively and in controls it was 40% (Table 5.3). The mutant genotype (val/val) of \textit{GSTP1} (exon5) gene depicted 1.9 times non significant increased risk in drinkers as compared to BPH (OR=1.9; 95%CI=0.53-6.81) while marginal risk of 1.28 times was depicted by drinkers carrying a copy of heterozygous and mutant genotypes each in combination (OR=1.28; 95%CI=0.67-2.43) (Table 5.6).

Polymorphism of metabolic genes is associated with consumption of alcohol especially in the Asian population (Chen \textit{et al.}, 1996; Konishi and Ishii, 2007). However, in the present study, the variant allele \textit{ala114val} of \textit{GSTP1} (exon6) gene
showed a protective association in drinkers when compared with BPH cases carrying *ala/val* genotype (OR=0.74; 95%CI=0.37-1.44) and those carrying combined genotypes (*ala/val/val/val*) (OR=0.78; 95%CI=0.41-1.48). On comparison with healthy controls also, the variant allele depicted protective association in drinkers carrying *ala/val* (OR=0.99; 95%CI=0.45-1.72), *val/val* (OR=0.99; 95%CI=0.30-3.19) and *ala/val/val/val* (OR=0.90; 95%CI=0.47-1.69) genotypes (Table 5.11).

Wine has been shown to reduce cell proliferation because of the polyphenols present in it, but the flavonoids in it block the production of PSA (Knowles *et al.*, 2000).

When polymorphisms in repair genes were studied in relation to drinking, 2.46 (OR=2.46; 95%CI=0.81-7.50) and 1.74 (OR=1.74; 95%CI=0.90-3.35) times non significantly increased risk of prostate cancer was observed in cases with *AC* and *CC* genotypes of *XPD* gene respectively (Table 5.16). The *CC* genotype of *XPC* gene also showed 2.02 (OR=2.02; 95%CI=0.77-5.31) times non significant increased risk of prostate cancer cases (Table 5.17).

Dennis (2000) found no association between prostate cancer risk and alcohol consumption. Moderately increased risk of prostate and bladder cancer with specific types of alcohol was reported by certain cohort studies (Sommer *et al.*, 2004).

Acetaldehyde is also known to inhibit enzymes in the DNA methylation pathway. Excessive alcohol consumption has been shown to alter DNA methylation in several tissues including liver, oesophagus, colon and uterus (Kouros *et al.*, 1983; Fiala *et al.*, 1987; Murdoch and Edwards, 1992).

Increased frequency of methylation was seen in the *GSTP1* gene of prostate cancer cases who consumed alcohol (90%) as compared to those who did not do so (64%) (Fig. 5.2).

In BPH cases it was quite unusual being 0% in drinkers and 11% in non drinkers (Fig. 5.7) and in healthy controls, the drinkers depicted 44% methylation frequency as compared to 5% in non drinkers (Fig. 5.11).
The frequency of methylation in \textit{MGMT} gene was 58 and 42\% in drinkers and non drinkers amongst prostate cancer cases (Fig. 5.15), it was 16 and 0\% in drinkers and non drinkers amongst BPH cases (Fig. 5.19) and 63 and 18\% in healthy controls (Fig.5.23). In both the genes, the frequency was on the higher side in drinkers as compared to non drinkers especially in the prostate cancer cases.

Not much literature is available on the interaction of this factor and risk of prostate cancer till date. It is concluded that except the variant allele ala114val of \textit{GSTP1} (exon6) gene, rest of the polymorphisms depicted a non significantly increased risk of prostate cancer. The methylation frequency of \textit{GSTP1} gene was on the higher side in drinkers except the subjects with BPH. The \textit{MGMT} gene also showed higher frequency of methylation in drinkers. It is also concluded that the drinking, smoking and excessive intake of non vegetarian diet significantly increase the risk of prostate cancer.