7. Summary and Conclusions

In the past several decades, the epidemiological and screening studies have shown concerns about the pathogenesis of prostate cancer but definitive causes have not been established. A number of genetic and environmental factors are known to influence individual susceptibility towards the formation of prostatic tumours. Unlike genetics, epigenetic mechanisms such as DNA methylation confer different functional status to the same genes under different environmental conditions. Knowledge of such gene-environment interaction may lead to established biomarkers that will perform better than the standards of PSA, Gleason score and TNM staging. The present study is an endeavour to unravel the polymorphic genes implicated in the etiology of prostate cancer, prevalence of aberrant promoter methylation in MGMT and GSTP1 genes along with their environment interactions. This study was conducted on North Indian population. Polymorphism study was accomplished on 497 subjects (157 prostate cancer cases, 170 BPH cases, 170 healthy controls) and methylation on 250 subjects (100 prostate cancer cases, 50 BPH cases, 100 healthy controls). The following observations were made:

- Mutant allele val/val of GSTP1 (exon 5) had revealed increased risk of prostate cancer as compared to BPH and healthy controls.
- In smokers, significantly increased risk of prostate cancer was found in BPH subjects carrying a copy of heterozygous genotype ile/val in combination with mutant genotype ile/val/val/val.
- Mutant allele val/val of GSTP1 (exon 5) had also shown increased risk of prostate cancer in smokers and drinkers in comparison with BPH cases.
- About 2.30 fold elevated risk of prostate cancer was depicted in tobacco chewers with mutant genotype val/val of GSTP1 (exon 5) as compared to healthy controls. Similarly 2.65 fold of elevated risk was observed in tobacco chewers with mutant genotype val/val of GSTP1 (exon 5) as compared to BPH cases.
- Combined genotype ile/val/val/val of tobacco chewers depicted 1.99 times increased risk when compared with BPH cases and the risk was observed to be 1.9 times higher in tobacco chewers with heterozygous genotype ile/val in GSTP1 (exon 5) gene.
• Comparison with BPH cases showed 2.58 fold higher risk of prostate cancer in non-vegetarians with mutant genotype val/val; 1.97 fold higher risk in non-vegetarians with combined genotype ile/val/val/val and 1.88 fold higher risk in non-vegetarians with heterozygous genotype ile/val of GSTP1 (exon 5) gene.

• Frequency of mutant genotype (cc) of XPC gene was highest in prostate cancer cases (12.7%) as compared to controls (8.8%) whereas frequency of wild type genotype (aa) was highest in healthy controls (46.5%) as compared as compared to cases (40.8%).

• Heterozygous genotype (ac) of XPC gene depicted higher frequency (46.5%) as compared to controls (44.7%).

• Frequency of heterozygous genotype (ac) of XPD gene was highest in prostate cancer cases (53.5%) as compared to homozygous wild genotype (aa) which was 40.8%.

• Frequency of homozygous mutant genotype (cc) was more in prostate cancer cases (5.7%) as compared to healthy controls (3.0%).

• ac and cc genotypes of XPC gene revealed 1.15 and 2.2 fold increased risk of prostate cancer respectively in individuals taking non-vegetarian diet.

• AC genotype of XPD gene revealed 2.61 fold (p=0.02) significant increased risk of prostate cancer as compared to healthy controls and a 4 fold non-significant increased risk was revealed by CC genotype in individuals taking non-vegetarian diet.

• Analysis of AC and CC genotypes of XPD gene showed 1.73 and 3.24 fold non-significant increased risk of prostate cancer respectively in smokers.

• In drinkers, 1.74 and 2.46 times non-significant increased risk of prostate cancer was observed in cases with AC and CC genotypes of XPD gene respectively.

• Weak association was observed with CC genotype of XPC gene in smokers.

• CC genotype of XPC gene showed 2.02 fold non-significant increased risk of prostate cancer in individuals taking alcohol.

• Association of mutant alleles of XPD (AC+CC) and mutant genotype of XPC (CC) in individual revealed 3.4 times non-significant increased risk of prostate cancer.

• Frequency of heterozygous variants ala/val of GSTP1 (exon 6) was highest in prostate cancer cases (48.4%) as compared to BPH cases and healthy controls.
- Frequency of mutant genotype (val/val) of GSTP1 (exon 6) gene was also highest in prostate cancer cases (17%) whereas wild type genotype (ala/ala) was highest in healthy controls and BPH cases as compared to prostate cancer cases.
- Mutant genotype (val/val) of GSTP1 (exon 6) depicted 1.87 times non significant increased risk in comparison with healthy controls and 1.76 times non significant increased risk in comparison with BPH cases.
- Heterozygous genotype (ala/val) of GSTP1 (exon 6) gene revealed 1.59 times non significant increased risk as compared to healthy controls.
- Significantly increased risk of 5.11 fold (p<0.004) and 3.83 fold (p<0.0001) was observed in non vegetarians carrying val/val and ala/val genotypes respectively as compared to BPH cases.
- Significantly increased risk of 4.0 fold was also observed in non vegetarians carrying combined genotypes of ala/val/val/val (p<0.0001) as compared to BPH cases.
- The valine variant of GSTP1 (exon 6) gene showed protective association in drinkers as compared to BPH and healthy controls.
- Non significant higher risk of prostate cancer was observed in tobacco chewers with valine variants of GSTP1 (exon 6) as compared to BPH and healthy controls.
- When compared with healthy controls, significantly increased risk of prostate cancer was observed in smokers carrying ala/val genotype and combined genotypes (ala/val/val/val) of GSTP1 (exon 6) gene (p<0.001 and p<0.0004) respectively.
- Non significant higher risk of 1.69 times was observed in smokers with mutant genotype val/val of GSTP1 (exon 6) amongst the BPH cases.
- GSTP1 gene exhibited highest frequency of methylation (79.6 %) in prostate cancer cases as compared to MGMT gene (52.4%).
- Age groups of 71-80 years exhibited maximum frequency of methylation (92%) in GSTP1 gene in prostate cancer cases.
- Age groups of 81-90 years exhibited maximum frequency of methylation (32%) in BPH cases whereas in the age groups 41-50 years the frequency of methylation was observed to be zero.
- In prostate cancer cases, maximum frequency (62%) of methylation in MGMT gene was observed in age group of 41-50 years.
• In healthy controls, the frequency of methylation in MGMT gene was maximum (42%) in age groups of 71-80 years whereas it was zero in age groups of 31-40 years.

• In prostate cancer cases, methylation in GSTP1 gene was 99% in smokers and 66% in non smokers while methylation in MGMT gene was 73% and 30% respectively.

• In prostate cancer cases, methylation in GSTP1 gene was 90% in drinkers and 64% in non drinkers while methylation in MGMT gene was 58% and 42% respectively.

• In prostate cancer cases, methylation in GSTP1 gene was 94% in non vegetarians and 51% in vegetarians while methylation in MGMT gene was 60% and 20% respectively.

• In BPH cases, methylation in GSTP1 gene was 25% in smokers and 8% in non smokers while methylation in MGMT gene was 37% and 5% respectively.

• In BPH cases, methylation in GSTP1 gene was 0% in drinkers and 11% in non drinkers while methylation in MGMT gene was 16% and 0% respectively.

• In BPH cases, methylation in GSTP1 gene was 19% in non vegetarians and 9% in vegetarians while methylation in MGMT gene was 18% and 10% respectively.

• In healthy controls, methylation in GSTP1 gene was 24% in smokers and 10% in non smokers while methylation in MGMT gene was 42% and 17% respectively.

• In healthy controls, methylation in GSTP1 gene was 44% in drinkers and 5% in non drinkers while methylation in MGMT gene was 63% and 18% respectively.

• In healthy controls, methylation in GSTP1 gene was 24% in non vegetarians and 2% in vegetarians while methylation in MGMT gene was 49% and 7% respectively.

The present study concludes that the risk of developing prostate cancer is significantly high in population with heterozygous (ala/val) and combined (ala/val/val/val) genotypes of exon 6 of GSTP1 gene in smokers as compared to healthy individuals. Consumption of non vegetarian diet poses a significantly high risk of developing prostate cancer in BPH subjects with three genotypes i.e. heterozygous (ala/val), mutant (val/val) and combined (ala/val/val/val). Non vegetarian with AC genotype of XPD gene also have a significantly higher risk of
developing prostate cancer. The study also finds that smoking and non vegetarian diet have a significant role to play in increasing the frequency of methylation in \textit{GSTP1} and \textit{MGMT} genes and their subsequent silencing leading to development of prostate cancer.