Summary & Conclusion
The following points can be drawn from the foregoing account:

1- The subjects for the present study were recruited from the major long term studies of Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, being referral centre for the patients from the states like Punjab, Haryana, Chandigarh, Himachal Pardesh and Uttar Pardesh. The majority of the patients were from Punjab and Haryana.

2- Most of cases were from rural areas with occupation as farmers or labours. It is therefore possible that exposure to agricultural chemicals might have something to do with the bladder cancer. The apparent reason is lack of awareness, about harms of smoking, alcohol drinking and life style and sanitation (like cooking and the source of drinking water) as most of cases were illiterate and below matric. This point also became evident that some factors like smoking and the existence of chemicals in occupation or residential place, effectively, and some factors like alcohol drinking and chewing tobacco, synergically, have role in risk of bladder cancer. No correlation, however, could be found between the urban occupations and the bladder cancer, probably because of the small sample size. Most of patients were vegetarians or occasionally had meat.

3- The majority of patients were above 50 years of age and male, it is evident that uro-epithelial cancer is disease of middle and old age. It can be concluded that this cancer is age and gender dependent.

4- There was no apparent association between LIGI (exon-6 A>C) and developing bladder cancer. The genotypes APE-I (Asp148Glu, IL-1β (-31, C? T) showed weak association with increased risk of bladder cancer. NBS (-185,Gln/Gln) and IFN-γ (+874, A/A) and he presence of C and D alleles in intron 3 of IL-1Ra were statistically associated with bladder cancer. The presence of at least one copy of Rpl allele in intron 3 of IL-4 showed strong association with increased risk of bladder cancer. The genotype IL-18 (-137, C/C) and FAS (-670 A/A) showed significant protective effect against of bladder cancer. FAS (-670, G/G) showed increased risk of uro-epithelial cancer.

5- The association APE (Glu/Glu), IFN-γ (AA), IL-1Ra (CC), IL-4(Rpl/Rpl) with uro-epithelial cancer was more strong in smokers (especially in active smokers
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with pack-years more than 50.1), those with risky job or living risky zone. Increased risk of bladder cancer in those with NBS (Gln/Gln) was not so much under effect of risk factors. The protective effect of IL-18 (CC) was decreased in smokers, drinkers and those with risky job or living in risky zone. It was observed that the effect of A or G allele of FAS is decreasing or increasing the risk of bladder cancer, respectively, was independent of studied risk factors. It meant that the effect of FAS alleles on developing bladder cancer in individuals with risk factors was almost similar in those without those risk factors.

6- Carriers of APE (Glu/Glu), IFN-γ (AA), IL-1Ra (CC) and IL-4(Rp1/Rp1) with some habit of chewing tobacco, drinking alcohol (especially those regularly consumed more than 26.8 alcohol-units per week), intake of liquid lesser than 2 liters per day (especially natural or direct tap water without any kind of treatment process) and hard physical job (continuously working on sun-light more than 3 hr/day) were more prone to the risk of bladder cancer.

7- No significant association between genotype of studied genes and usage of hair-dye, food-regime (on the basis of eating meat) and heredity with developing of uro-epithelial cancer was observed.

8- No association of LIG-I, APE and IL-1β genotypes and histopathological grade was observed. NBS (Gln/Gln) and FAS (GG) showed association with the development of bladder cancer in low and moderate grades (Gl and GII) and low stages (Ta+T1), conversely, the protective effect of FAS (AA) genotype was also observed in lower stages and low grades of cancer. The protective effect of IL-18 (CC) and FAS (AA) against of bladder cancer also appeared in low and moderate grades (Gl and GII) and low stages (Ta+T1) of cancer. The presence of at least one copy of A allele in IFN-γ genotype and C allele in IL-1Ra showed association with development of uro-epithelial cancer in moderate and high grades (GII and GIII) and high stages (T2+T3) of cancer. The role of Rp1 allele in IL-4 in developing bladder cancer was seen in all grades and stages.

9- The study of gene-gene interaction revealed that the presence of allele (A) in exon-6 of LIG-I, allele Gln in position -185 of NBS-I, allele Glu in position +148 of APE-I, (G) allele -670 of FAS, (T) allele -31 of IL-1β, (A) allele in position of +874 of IFN-γ; Rp1 (183 bp) allele in minisatellite located in intron-3 of IL-4,
and (C) allele (510bp) in minisatellite located in intron-2 of IL-1Ra in combination with other genes, increased the risk of the bladder cancer. Conversely, (A) allele at position -670 of FAS and (C) allele of -137 of IL-18 in combination with other genes decreased the susceptibility or moderated risk against of uro-epithelial cancer.

10- No significant difference in mRNA expression of IFN-? by PBMCs between patients with uro-epithelial cancer and healthy controls was found, but scenario was different and the amount of IFN-? protein was significantly more in sera of patients. It has been concluded that source of IFN-? in sera of patients is not just PBMCs. The up-expression of IFN-? in both mRNA and protein level was more in higher stages of tumor.

11- Protein and mRNA expression in carriers of T allele in genotype of IFN-? (+874) was significantly higher than those with IFN-? (AA) genotype.

12- Three minisatellite alleles of 12, 13 and 14 CA repeat were observed (alleles #2, 3 and 4) in patients with bladder cancer. Alleles #2 (12CA repeat) was mostly common, but no significant difference was observed between patients and controls on the basis of presence of allele #2.

13- There was an absolute correlation between presence of IFN-? (+874 T/A) polymorphism and the number of CA repeats in the first intron of IFN-? gene. Individuals homozygous for allele #2 [(CA)12+/CA12+] were homozygote for T allele in +874 position, and those heterozygous for allele #2 [(CA)12+/CA12−] were heterozygous for this SNP (TA), while allele #2 negative individuals [(CA)12−/CA12−] were homozygous for A allele (AA).

14- It was seen that the homozygotes for allele #2 [(CA)12+/CA12+] had highest range of mRNA and protein expression and those who was not carrying even one allele #2, had lowest amount of mRNA and protein expression.

15- No significant difference in mRNA expression of IL-18 by PBMCs between patients with uro-epithelial cancer and healthy controls was found, but the amount of IL-18 protein was significantly more in sera of patients. It has been concluded that source of IL-18 in sera of patients is not just PBMCs and IL-18 might be secreted by tumour infiltrating inflammatory cells and gains access to the
circulation. The up-expression of IL-18 in both mRNA and protein level was more in higher stages of tumor.

16- Significant difference in protein and mRNA expression between carriers of C allele in genotype of IL-18 and IL-18 (CC) genotype was observed.

17- Significant difference in mRNA and protein expression of IL-13 between patients with bladder cancer and controls was observed. The increased up-expression was mostly observed in lower stages (Ta+T1) of cancer.

18- Age, smoking and alcohol drinking was not significantly had effect on expression of IL-13, IL-18 and IFN-7.

19- Significant difference between patients and controls regarding the methylation in promoter of Rbl and CASP-8 genes was observed and especially when both genes methylated. Methylation of CASP-8 was mostly observed in patients with age>60 years whereas methylation of Rbl was observed in both the age-groups. Methylation of both genes in higher stages (T2 and T3) of cancer showed increased risk of developing bladder cancer. Smoking showed a high significant effect on methylation pattern especially in those with pack-years more than 44.7. The risk of bladder cancer was marginally associated with drinking when both the genes were methylated, especially in those patients who consumed alcohol units>30 per week.

20- Significant reduction in expression was detected in patients with methylated Rbl and CASP-8. These results have suggested that age, smoking and drinking increase the probability of methylation of these genes and consequently increased the risk of development of bladder cancer to higher stages of disease.

21- It was found that methylation by itself reduced the expression of Rbl, but decrease in the CASP-8 expression was caused by methylation and risk factors together. Hence, methylation of Rbl can be considered as one of prognosis indicators for progression and development bladder cancer.