SUMMARY AND CONCLUSION

Histopathologically confirmed cervix cancer patients were recruited for the present study. Epidemiological data collected from the patients showed that most of the study participants were from the state of Punjab (49%) followed by Haryana (20%), Himachal Pradesh (13.5%), Uttar Pradesh (13.5%) and Chandigarh (4.0%).

80% of them had low socio-economic status and 81% were illiterates. This clearly indicates that cervical cancer is predominantly a problem of people with poor socio-economic background. As compared with the controls, cases had young age at the time of marriage (16.36) and birth of first child (18.39) and had greater mean number of children (4.11).

44% of the cases and 28% of the controls were passive smokers and an increased OR (2.0) was observed amongst passive smokers. Usage of oral contraceptives was found to be statistically significant, but decreased the risk of cervix cancer (OR = 0.4, p = 0.00001). 88.57% of tissue samples were found to be HPV positive, while 11.43% were negative.

HPV 16 was the most common type occurring among cervix cancer patients. HPV 18 was present only in 64% of the patients. 16.6% of the patients had both HPV 16 and 18. When stratified according to tumour histology; HPV 16 was observed in 65.3% of SCC of cases as compared to only 50.0% of AC cases. However, HPV 18 alone or along with HPV 16 was more prevalent in patients with AC (18.8 and 18.7% respectively). Certain polymorphic forms of NBS1, IL-4 and IL-18 genes increased the risk of cervix cancer. However, APE-1, MS, MTHFR were found to decrease risk of cervix cancer significantly. The polymorphic forms of IL-10 did not show any association with the risk of cervix cancer.
With regard to tumour histology, the following points could be highlighted.

i) **APE-1** (Asp/Glu) genotype had significantly decreased risk of SCC of cervix.

ii) **NBS1** (Glu/Gln) and (Gln/Gln) genotypes had statistically insignificant increased risk of SCC of cervix.

iii) **MS** (AG) and (AG+GG) genotypes were associated with statistically significant decreased risk of SCC of cervix.

iv) **MTHFR** (CT) and (CT+CC) genotypes significantly decreased the risk of SCC of cervix.

v) **IL-18** (GC) and (GC+CC) genotypes had statistically significant increased risk of SCC of cervix.

vi) Increased risk for AC was observed in the carriers of **NBS1**, (Glu/Gln) and (Gln/Gln) genotypes.

vii) Significantly decreased risk for SCC was observed in the carriers of **IL-4** (Rpl/Rp1 and Rp2/Rp2), **MS** (AG), and these associations however, decreased risk of AC.

In passive smokers, heterozygous mutant genotypes of **NBS1** (Glu/Gln), **IL-4** (Rpl/Rp1 and Rp2/Rp2), **IL-10** (AC) and **IL-18** (GC), increased the risk of cervix cancer. However, polymorphic forms of **APE-1**, **MS**, **MTHFR**, and HPV were not associated with the risk of developing cervix cancer in passive smokers.

For users of oral contraceptives, statistically significant increased risk of cervix cancer was observed for **NBS1** (Glu/Gln) and HPV (16, 18, 16+18).

On studying gene-gene interactions, the following combinations were found to be associated with the risk of developing cervix cancer:
Summary and conclusion

1. Statistically significantly increased risk of cervix cancer was observed for the following genotypes:
   (i) APE·1 (Asp/Asp) and NBS1 (Glu/Gln+Gln/Gln)
   (ii) IL·10 (AA) and IL·18 (GC+CC)

2. Increased but statistically insignificant risk was observed for the following genotype:
   (i) NBS1 (Glu/Gln) and IL·4 (Rp1-Rp1).
   (ii) NBS1 (Glu/Gln) and IL·10 (AA)
   (iii) NBS1 (Gln/Gln) and MS (AA)
   (iv) NBS1 (Glu/Gln) and MTHFR (CC)
   (v) NBS1 (Glu/Gln) and IL·18 (GC+CC)
   (vi) IL·4 (Rp1/Rp2) and MTHFR (CC)
   (vii) IL·4 (Rp2/Rp2) and HPV (16+18)
   (viii) IL·10 (AC) and HPV (18)
   (ix) MS (GG) and IL·18 (GC+CC)
   (x) MS (AG) and HPV (18)
   (xi) MTHFR (CC) and IL·18 (GC+CC)

3. Statistically significant decreased risk of cervix cancer was observed for the following genotypes:
   (i) IL·4 (Rp2/Rp2) and MS (AG+GG)
   (ii) IL·10 (AC) and MS (AG+GG)
   (iii) IL·10 (AA) and MTHFR (CT+TT)
   (iv) MS (AG) and MTHFR (CT+TT)
   (v) MS (AG) and IL·18 (GG)
   (vi) MTHFR (CT) and IL·18 (GG)
4. On studying gene-gene interactions, following combinations of genotypes were not found to be associated with risk of cervix cancer:

(i) APE-1 with IL-4, APE-1 and IL-10
(ii) APE-1 with MS, APE-1 and MTHFR
(iii) APE-1 with HPV, IL-4 and IL-10
(iv) IL-10 with MTHFR, MTHFR and HPV
(v) IL-18 with HPV

When stratified according to histology, the following conclusions were drawn:

A. The combinations which increased the risk of SCC of cervix cancer:

(i) APE-1 (Asp/Asp) and NBS1 (Glu/Gln+Gln/Gln)
(ii) NBS1 (Glu/Gln) and IL-10 (AA)
(iii) NBS1 (Glu/Gln) and MS (AA)
(iv) NBS1 (Gln/Gln) and MTHFR (CC)
(v) NBS1 (Glu/Gln) and IL-18 (GC+CC).

B. The combinations that increased the risk of AC of cervix cancer:

(vi) NBS1 (Glu/Gln) and MS (AA)
(vii) NBS1 (Glu/Gln) and MTHFR (CC).

5. On studying promoter hypermethylation of P16 and P14 tumour suppressor genes, the following results were found:

(i) Promoter methylation frequency for P16 gene in cervical cancer was 43.8% (35/80), while in control non-neoplastic cervix it, was 8.75% (7/80).
(ii) No significant association correlation was observed between methylation frequency of \textit{P16} gene and clinicopathological parameters.

(iii) Methylation frequency for \textit{P14} gene in cervical cancer and control groups were 10.0 \( (8/80) \) and 2.5\% \( (2/80) \) respectively.

(iv) Statistically significant association between \textit{P14} gene and smoking status \( (p<0.006) \) was observed.


No significant difference between expression of \textit{IL-18} and \textit{IFN-\gamma} gene in cervix carcinogenesis and healthy controls was observed.

It is obvious that the genetic polymorphism in cytokine, DNA repair, folic acid metabolic pathway (global hypomethylation), HPV as well as tumour suppressor hypermethylation gene and cytokine gene expressions may be playing an important role in the development of cervix cancer and may act as co-factors in HPV associated carcinogenesis.

India has a high incidence of HPV associated cancer with women having poor socio-economic conditions. They are often exposed to a wide range of carcinogens including those derived from tobacco use, prolonged inhalation of smoke from kitchen firewood and use of contraceptive pills. An additional study on epigenetic gene silencing study and cytokine gene expression will be necessary to further improve the understanding of individual susceptibility to DNA damage, gene mutations and development of cervix cancer. Therefore CpG island hypermethylation has demonstrated its great versatility for the molecular monitoring of cancer patients, and is a likely target for future and smarter therapeutic approaches for cervical cancer. Finally a global
analysis of gene expression profiles of tissue affected with cervical cancer will help us to find out most differentially expressed genes that can further be utilized to distinguish normal from the carcionogenic cervix. This will also help in approaching the pathways that will throw some light on how different genes are coordinately and differentially regulated in normal vs. cervical cancer tissue. Such a comprehensive approach may reveal highly prominent candidate molecular markers in future for cervical cancer diagnosis, prognosis and molecular therapy.