DISCUSSION

The main goal of the present study was to detect the impact of genotype and human papillomavirus (HPV) on the risk of developing cervix cancer by evaluating genetic polymorphism in anti-inflammatory immunomodulatory cytokines, DNA repair, folate metabolic pathway (global hypomethylation), CpG island hypermethylation (tumour suppressor genes) and expression profile of pro-inflammatory cytokine genes with an aim to determine the host genotype susceptibility for cervical cancer.

5.1. EPIDEMIOLOGY

The study was carried out on 200 cervix cancer cases and an equal number of matching controls. 140 tissue samples of histopathologically confirmed cervical cancer patients were collected and analyzed for the presence of HPV types (16, 18). As many as 134 (95.7%) samples were found to be positive and 16 (11.4%) were negative for HPV. HPV 16 (63.5%) occurred most commonly among cervix cancer patients. HPV18 was present only in 6.4% of the patients with cervix cancer and 18.5% had both HPV16 and 18. Similar results were obtained by Nagpal et al. (2002) who carried out study on cervical cancer patients visiting A.H. Regional Cancer Research and Treatment Center, Cuttack, Orissa, India. Sobti et al. (2002) had also detected human papillomavirus (HPV) in 85 and 63.6% of patients with invasive cervical cancer and minor cervical abnormalities respectively and HPV16 was found to be dominant in both the groups of women. In the American Indian women of the Northern plains, these two genotypes (HPV 16 and HPV 18) were present in 48% of the oncogenic infections. The remaining 52% of women were infected
Discussion

with other oncogenic HPV genotypes, such as HPV 59, the second most predominant genotypes (Maria et al., 2007). In IARC multicenter case-control study, the pooled age and centre-adjusted odds ratio for the presence of 10 most common HPV types and cervical cancer was estimated the 83.3% (Bosch et al., 2002). In the present study, when the tumours were stratified histologically, HPV 16 was detected in 60.3% of cervical cancer patients with SCC. HPV18 along with HPV 16 was more prevalent (18.8 and 18.7% respectively) in patients with AC. Anderson et al. (2001) had also determined adenocarcinoma of cervix to be predominated by HPV18 as compared to the squamous cell carcinoma of cervix.

Cervical cancer predominantly affects women of the lower social classes. 80% of the cervical patients in the present study had low socio-economic status. The prevalence of cervical cancer was also noted to be elevated in women of lower educational status. 81% of the patients were illiterate in the present study. Smoking is associated with an increased risk of developing high grade squamous intraepithelial lesions in women who are infected with oncogenic HPV (Tolstrup et al., 2006). Also smoking maintains cervical HPV infection longer and decreases the potential of clearing an oncogenic infection (Giuliano et al., 2002). Cervical cancer persists as an international health problem and the global public health impact of smoking on cervical cancer incidence and related mortality is substantial. It is also well documented that cigarette smoking plays a role in increasing the risk of developing cervical cancer (Winkelstein et al., 1997; IARC, 2004). Kuceral et al. (1987) in a retrospective review of 1300 women with cervical carcinoma observed that those with stages III and IV of the disease had significant difference in the 5-year survival rate between smokers (20-30%) and non-smokers (33.9%), and significant difference in the 5 year survival rate for those with
stage I and II disease between smokers (53.9%) and non-smokers (69.4%).

Passive or secondary smoking acts as a risk factor for high-risk type HPV infection (Coker et al., 2007). Although less attention has been paid to the potential link between passive smoking and the development of cervical neoplasia, yet from the association between active smoking and cervical cancer, it can be deduced that passive exposure to cigarette smoke could plausibly contribute to cervical carcinogenesis. Cotinine, a nicotine metabolite, is present in measurable concentrations in the cervical mucus of the active cigarette smokers (McCann et al., 1992; Poppe et al., 1995). The results of several case-control and cross-sectional studies indicate that women married to smokers experience a higher risk of cervical neoplasia than those married to non-smokers (Tay et al., 2004).

Cornelia et al. (2005) investigated the associations of active and passive smoking with respect to invasive cervical carcinoma and cervical carcinoma in situ, to the extent that active and passive smoking are also associated with cervical intraepithelial neoplasia, not accounting for women who developed CIN II/III and who did not progress to in situ or invasive disease, could lead to relative risk (RR) estimates closer to the null value of 1 than may have otherwise been the case. Since most of the women in this region were not active smokers, the exposure to smoke was, therefore, passive and so passive smoking was found to increase the risk of cervix cancer. As 44% of cases and 28% of controls were passive smokers, for them increased risk of cervical cancer (OR=2.0) was observed. In the present study, use of oral contraceptive was suggested to be statistically significant decreased risk of developing cervix cancer (OR = 0.4, P = 0.00001).
The data reported overall 12% reduction in the risk of cancers (adjusted relative risk $RR = 0.88$, 95% CI = 0.83-0.94). Many results were not statistically significant and a more modest 3% reduction in cancer risk with oral contraceptive use was reported. These overall risk reductions are average effects among pill users. A 12% reduction equates to approximately one case of cancer for every 2200 women who had used the pill for a year and 3% equates to one fewer case of cancer for every 10 000 women (Hannaford et al., 2007). Smith et al. (2003) reviewed 21 studies, but did not discuss possible behavioural or biological routes of causation. Three causal routes could have been investigated with the collected studies:

1. Use of oral contraceptives (OCs) may be behaviourally related to increased risk of human papillomavirus (HPV) transmission. Compared to age-matched controls, women who use OCs may be more active sexually, more frequently screened for cervical cancer and less appropriate to use barrier methods. Increased exposure to and screening for HPV over time would lead to increased observed cancer rates among users. OCs themselves would be exonerated of any causal role in cervical cancer.

2. Use of hormonal contraception may increase the biological vulnerability of the cervix. Given the same exposure to HPV, hormone users may be at higher risk of transmission.

3. Use of OCs may increase the chances or speed with which HPV infection progresses to in situ or invasive cervical cancer.

Beral et al. (1998) and WHO (1993) have reported that the relative risks are statistically significant and their magnitude increases with time. Beral et al. (1998) have reported the stronger prospective cohort design, and WHO had a massive sample size (3848 cases and 13,644 controls). Several epidemiological studies
have reported an increased risk of invasive cervical carcinoma in relation to ever OCs use, and a stronger risk for a longer duration of use. The evidence of an association between OCs use and adenocarcinoma of the cervix is based on more limited data (IARC, 1999). The relative risk of cervical cancer is increased in current users of oral contraceptives and declines after use ceases. 10 years use of oral contraceptives from around age 20 to 30 years is estimated to increase the cumulative incidence of invasive cervical cancer by age 50 from 7.3 to 8.3 per 1000 in less developed countries and from 3.8 to 4.8 per 1000 in more developed countries (Appleby et al., 2007).

However an association emerged between long-term OC use and *in situ* adenocarcinoma. Smith *et al.* (2003) analyzed data from 28 studies that included 12,531 women with cervical cancer. It suggested that the risk of cervical cancer may decrease after OCs use was discontinued. The IARC has initiated a study to reanalyze all data related to use of OCs and cervical cancer risk (Franceschi *et al.*, 2005).

### 5.2. RELATIONSHIP BETWEEN CYTOKINE GENE POLYMORPHISMS AND RISK OF CERVIX CANCER

Cytokines act on target cells, generally within the haematopoietic system, by binding to specific receptors and initiating signal transduction and second messenger pathways within the target cells. Cytokines function as players in a highly complex and coordinated network in which they modulate their own synthesis as well as that of other cytokines and cytokine receptors. Many single nucleotide polymorphisms (SNPs) and a more limited number of microsatellite polymorphisms have been detected within cytokine gene sequences, particularly within the promoter regions of these genes (Bidwell *et al.*, 1999).
Many genetic studies trying to correlate these cytokine polymorphisms with immune mediated disease have been performed (Bidwell et al., 1999; Haukim et al., 2002). A number of studies have reported association between cytokine gene polymorphisms and particular cancers, including chronic lymphocytic leukemia (Demeter et al., 1997), non-Hodgkin’s lymphoma (Warzocha et al., 1998), and breast cancer (Mestiri et al., 2001). Cytokines play a significant role in the defense against HPV induced infections, modulating viral replication and polarizing the immune response to a Th1 (cellular) or Th2 (humoral) patterns (Hausen et al., 2002). Several studies have demonstrated that genetic polymorphisms in cytokine genes contribute to the variation in the levels of cytokines produced and this variation may influence the severity of several infectious diseases (Calhoum et al., 2002; Sibata et al., 2002; Wu et al., 2002).

There is a well-documented association between cytokine polymorphisms and the development of gastric adenocarcinoma subsequent to Helicobacter pylori infection, as originally reported by El-Omar and colleagues (El-Omar et al., 2000). IL-10 polymorphisms are of particular interest in relation to cancer, since IL-10 has both immunosuppressive (potentially cancer promoting) and anti-angiogenic (potentially cancer inhibiting) properties. The promoter polymorphisms of IL-10 have been subject to the most secreting, particularly with regard to possible influences upon gene transcription and protein production. For example, the IL-10 -1082 SNP and -819, -592 A haplotypes have been reported to be associated with differential IL-10 expression in vitro. The IL-10, -1082, -819 T, -592 A haplotypes are associated with decreased IL-10 expression when compared with the -1082 G, -819 C, -592 C haplotypes. Although several studies have confirmed that IL-10 production is related to the immune response.
against HPV-associated cervical cancers and other studies have reported that IL-10 production might be associated with IL-10 polymorphisms, a few studies have examined the association between genotype frequency of IL-10 and risk of cervical cancer (Turner et al., 1997). In the present study on IL-10 -592, the frequency of homozygous wild (AA) genotype was more in patients with cervix cancer (44.0%) as compared to that in controls (40.5%). The frequency of heterozygous mutant genotype (AC), however, was higher in controls (51%). Ju et al. (2002) have also shown similar genotype frequencies of IL-10 (-592) promoter polymorphism in cervical cancer and control groups. The present study has already suggested that frequency of genotype (AC) is more prevalent in patients with adenocarcinoma (56%) as compared with SCC (46.9%). IL-10 production has been implicated in the tumourigenesis of various types of cancers, such as ovarian, colon cancers and lymphoma (Pisa et al., 1992; Gastl et al., 1993; Levy, 1994), and may also protect tumours by inhibiting cytotoxic T lymphocyte (CTL)-mediated tumour specific cell lysis (Matsuda et al., 1994).

Abnormal IL-10 production has already been reported in patients with cervical precancerous lesions and invasive cancer. It has also been shown that peripheral blood mononuclear cells from patients with both precancerous lesions and invasive cancer produce higher levels of IL-10 following mitogenic stimulation, compared with control groups (Clerici et al., 1997). On the other hand, the present study on IL-10 (-592) has revealed no association between the genotypes and overall risk of cervix cancer. Roh et al. (2002) had also not found any significant increase in the total serum concentration of IL-10 with different genotypes in non cervical (8.15 ± 45.0 pg/ml) and invasive cervical (3.52 ± 12.8 pg/ml, p = 0.26) cancer in Korean women. Zoodsma et al. (2007) have suggested a possible role for the IL-10 gene in CIN.
and squamous cell carcinoma susceptibility in the Caucasian population.

Stanczuk et al. (2001) reported that the genetically acquired ability to produce higher levels of IL-10 in Zimbabwean women might be a significant factor in the development of cervical cancer, which is in contrast to the finding of the present study. This may be due to their small sample size (77 cervical cancer patients). The present findings are, however, based on a relatively large sample size consisting of 200 cervical cancer cases, which should provide more confident results. Furthermore, the present study on the interaction between IL-10 and passive smokers having AC genotype have shown 1.7 fold (95% CI = 0.90-3.34) increased risk of cervix cancer, but this association was not statistically significant (p = 0.1).

IL-4 is a highly pleiotropic cytokine that is able to influence the cell differentiation. Early secretion of IL-4 leads to polarization of Th cell differentiation towards Th2 like cells. Th2 type cells secrete their own IL-4, and subsequent autocrine production of IL-4 supports cells proliferation (Mosmann et al., 1996).

IL-4 has marked inhibitory effects on the expression and release of the pro-inflammatory cytokines. It is able to block or suppress the monocyte-derived cytokines, including IL-1, TNF-α, IL-6, IL-8 and macrophage inflammatory protein (MIP) (Paul et al., 1991). The immunologic effects of IL-4 in the presence of bacterial infection are complex and incompletely understood. The role of IL-4 in the presence of systemic infections is not adequately defined and will necessitate additional chemical investigations. IL-4 is able to affect a variety of structural cells. It can potentiate proliferation of vascular endothelium and skin fibroblasts decrease proliferation of adult human astrocytes and vascular
smooth muscle cells (Brown et al., 1997). Seno et al. (2007) have reported that variation in IL-4 is negatively associated with the risk of developing gastric cancer following H. pylori infection. In a study of 63 patients with stage IV non-small cell lung cancers, data on treatment with recombinant human IL-4 suggested a possible dose related response. IL-4 may act by stabilizing diseases and modifying tumour growth rates in addition to induce tumour shrinkage and cell death without causing side effects, suggesting a possible adjuvant role for IL-4 in the treatment of malignant diseases (Vokes et al., 1998).

Two polymorphisms for IL-4 including the IL-4 -590 promoter and IL-4 -intron 3 have been demonstrated. The polymorphism of IL-4 -intron 3 is composed of a variable number of tandem repeats (VNTRs) of a 70-bp sequence -intron 3 (Mout et al., 1991). The present study sought to identify the possible link between IL-4 (-intron 3) polymorphism and the risk of developing cervix cancer in north Indian women. In this study, the distribution of IL-4 genotypes (Rpl/Rpl and Rp2/Rp2) among cases and controls in cervix cancer was found to be not much different from controls (4.5 and 5.5% Rpl/Rpl and, 62.5%, 63.0% Rp2/Rp2 respectively). The frequency of Rpl/Rp2 genotype was more in cases as compared to controls (33.0 and 31.5% respectively). However, according to histological subtypes, Rp2/Rp2 genotype was more prevalent in cervix cancer patients with SCC (62.9%), while Rpl/Rp2 genotype was more in the patients with AC (40.0%). No association between Rp2/Rp2 genotype and risk of cervix cancer (OR = 1.2, 95% CI = 0.45-3.31) was found in the present study, whereas there was a marginal increase in the OR of patients with IL-4 Rpl/Rp2 genotype (1.3, 95% CI = 0.45-3.64). Fuu-Jen et al. (2005) have reported a significant difference in genotype distribution between the groups (p < 0.01) with the Rpl homozygote (86%) and controls (64%) in patient with transitional cell carcinoma of urinary
bladder and they suggested the $IL\cdot 4$ gene -intron 3 polymorphism is a potential genetic marker in screening for the possible cause of bladder cancer. On the other hand, the present study has observed statistically significant decreased risk of adeno-carcinoma for $Rp1/Rp2$ (OR = 0.2, 95% CI = 0.06-0.67, p = 0.004) and $Rp2/Rp2$ (OR = 0.2, 95% CI = 0.06-0.48, p = 0.005) genotypes. However, Kang et al. (2005) have shown significant differences in the $Rp1/Rp2$ genotype proportion (p = 0.04) and the $Rp2$ allelic frequency (p = 0.03) of $IL\cdot 4$ -intron 3 polymorphisms in children with acute and chronic immune thrombocytopenic purpura. In another study Yao-Yuan et al. (2002) have reported the $Rp1$ allele in -intron 3 to be present in higher frequency in rheumatoid arthritis patients. They have also observed an association between rheumatoid arthritis and the $IL\cdot 4$ -590 promoter.

Interleukin-18 (IL-18) is a novel cytokine that is mainly produced by activated macrophages and like interleukin-12 (IL-12) is able to induce interferon gamma and tumour necrosis factor-alpha (TNF-alpha) induction, as well as enhancing the cytotoxicity of NK cells and Fas expression (Kretowski et al., 2002). $IL\cdot 12$ and $IL\cdot 18$ act synergistically on T cells to induce IFN-$\gamma$ production. Thus together they may play an important role in both anti-tumour immunity and protective effects against infection of intracellular pathogens including viruses (Tanaka-Kataoka et al., 1999). Infection of HPV in cervical epithelial cells and cervical intra-epithelial neoplasia (CIN) can persist for decades before progression to the malignant carcinoma.

Although it is generally accepted that IFN-$\gamma$ and cell-mediated immunity are important in determining the progression of HPV-related cervical lesion, including cervical cancer, there is no direct evidence that high risk HPV oncoproteins E6 and E7 can modulate the production of IFN-$\gamma$ by IL-18 (Clerici et al., 1997).
Three single nucleotide polymorphisms (SNPs) in the promoter of IL-18 gene at the position -656 G/T, -607 C/A and -137 G/C have been identified, and the two SNPs at position -607 C/A and -137 G/C in the promoter have been predicted to be nuclear factor binding sites for cAMP-responsive element binding protein and H4TF-1 nuclear factor, respectively. Wei et al. (2007) have suggested an association between IL-18 gene promoter -137 G/C polymorphism and oesophageal SCC in a Chinese population. On analyzing the frequency distribution of IL-18 (-137) in promoter region genotype in cervix cancer cases and controls, it was observed that 52.0% of cases and 37.0% of controls were the carriers of IL-18 (GC) genotype. With regard to tumour histology for IL-18 (GC) -137, no significant variation in the frequency of wild type genotype (GG) (44.0 and 44.6% respectively) was observed between cases and controls. The heterozygous mutant (GC) genotype was however, more pronounced in patients with adenocarcinoma (56.0%). Ping-An et al. (2005) have reported significant difference in the genotype distribution and in the allele frequency between the patients with chronic hepatitis B and the control subjects. The GG genotype at position -137 was present with significantly higher frequency in the patients with chronic hepatitis B as compared to that in the controls. The OR of the GG genotype for the comparison with that of the GC and the CC genotype was 1.8 (95% CI = 1.21-2.68, p = 0.01). The present findings have suggested marginal increased risk in OR of IL-18 (-137) (GC) and (GC/CC) genotypes (OR = 1.8, 95% CI = 1.17-2.76, p = 0.006) for GC and OR = 1.6, 95% CI = 1.09-2.50, p = 0.01 for GC/CC respectively). However, the real roles of IL-18 gene promoter polymorphisms in the pathogenesis of developing cervical cancer needs further investigations on large populations.
5.3. RELATIONSHIP BETWEEN DNA REPAIR GENES AND RISK OF CERVICAL CANCER

DNA damage may occur as a by-product of normal cellular metabolism as a result of exposure to environmental and biological mutagens. Unrepaired DNA damage leads to apoptosis or mutations, and the presence of the latter may facilitate carcinogenesis. Multiple systems, both at the cellular and molecular levels, are in place to respond to this damage (Hoeijma et al., 2001). DNA repair and maintenance is essential in protecting the genome of the cell from environmental hazards like tobacco smoking. Reduced DNA repair capacity (DRC) can render a high risk of developing many types of cancers, including cervical cancer (Benhamou et al., 2000; Berwick et al., 2002). Polymorphism in DNA repair genes may also contribute to the genetic instability and carcinogenesis (Goode et al., 2002; Hu et al., 2002). In both homologous recombination repair (HRR) and non-homologous end-joining, the initial step in repair is the recognition of double strand breaks (DSBs) by a complex of proteins that include Nijmegen breakage syndrome (NBS) meiotic recombination 11 homologue and human RAD 50 homologue (Mohrenweiser et al., 2003).

NBS1 is one of the key proteins participating in recognition and repair of double strand breaks (DSBs) that if unrepaired may lead to genomic instability and cancer (Lu et al., 2006). DNA double strand breaks (DSBs) are the most severe DNA lesions caused directly and indirectly by benzene metabolites. They may lead to chromosomal aberrations, apoptosis and haematopoietic progenitor cell suppression (Shen et al., 2006). The NBS1 polymorphism could affect the capacity of this complex to repair the damage caused by DNA adducts and increase the risk of cancer causing mutations (Hainant et al., 2000). Many population
studies on the \textit{NBS1-Glu185Gln} gene variant have been focused on breast cancer risk assessment (David-Beabes \textit{et al.}, 2001; Kuschel \textit{et al.}, 2002). Odilia \textit{et al.} (2006) have reported the frequency of different genotypes of \textit{NBS1} and found no association in patients with breast cancer in total population that was studied after cancer radiotherapy. The present data, however, supported a significant association of risk of cervix cancer with \textit{Glu/Gln} (OR = 7.5, 95\% CI = 3.59-15.86, p < 0.001) genotypes. Similarly \textit{NBS1} codon 185 \textit{Glu/Gln} has not been found to be associated with risk of breast cancer in northern Europe or Finland (Kuschel \textit{et al.}, 2002; Forsti \textit{et al.}, 2004). Lan \textit{et al.} (2005) have reported an association of \textit{NBS1} (34 \textit{A}, 185 \textit{Glu}) variants with an increased risk of lung cancer in individuals exposed to smoke of coal, especially those with \textit{p53} and in \textit{Gln/Gln} genotype (OR = 4.2, 95\% CI = 1.40-12.66, p = 0.01). According to Ryk \textit{et al.} (2006), the \textit{NBS1} 185\textit{Glu/Gln} genotype to be significantly associated with an increased risk of lung cancer among non-smoking and low dose smoking women. Also the alteration in DNA repair \textit{NBS1 Gln/Gln} and detoxification due to genetic polymorphism also influences that occurrence of \textit{P53} mutation in bladder cancer (Ryk \textit{et al.}, 2005). Matullo \textit{et al.} (2006) have reported the case-control distribution of genotypes for the different malignancies. In \textit{NBS1} gene, most of the malignancies analyzed did not show any significant difference in the distribution of genotype between cases and controls, except the present data which has shown statistically significant association and increased risk for developing cervical cancer in variant \textit{Glu/Gln} and \textit{Gln/Gln} (OR = 7.5, 95\% CI = 3.59-15.86, p < 0.001 and OR = 6.3, 95\% CI = 2.69-15.08, p = 0.000003 respectively) genotypes, in north Indian women. Therefore, the present findings suggest that the \textit{NBS1} double strand breaks (DSBs) repair pathway may be contributing to an increased risk for cervical cancer.
We investigated relation of *APE-1* gene polymorphism also with the risk of cervix cancer. The apurinic/apyrimidinic endonucleol (APE-1) and DNA repair enzyme play a central role in the DNA base excision repair (BER) pathway, which operates small lesions such as oxidized or reduced bases, fragmented or non-bulky adducts or those produced by methylating agents (Lu *et al.*, 2001; Vidal *et al.*, 2001). APE-1 also encodes a protein involved in both BER and regulation of gene expression as a redox co-activator. It has been shown that cells carrying the *APE-1* 148 Glu/Glu genotype have a prolonged mitotic delay when regulated by ionizing radiation as compared with the Asp/Glu and Asp/Asp genotypes. It suggests that *APE-1* polymorphism may have an association with hypersensitivity to ionizing radiation (Hu *et al.*, 2001). Hidemi *et al.* (2004) have reported that *APE-1* gene polymorphisms are not associated with statistically significant risk as values virtually remained unchanged after adjustment for age, sex, smoking and pack years of smoking (OR=1.0, 95% CI, 0.66-1.49) for Asp/Glu, and (OR = 1.3, 95% CI = 0.75-2.21) for Glu/Glu, in patients with lung cancer. However, the present findings have suggested that the *APE-1*148 Asp/Glu genotype is protective in the development of cervical cancer (OR = 0.5, 95% CI = 0.3-0.82, p = 0.03 for Asp/Glu and OR = 0.5, 95% CI = 0.33-0.78, p = 0.001 for combined genotypes Asp/Glu+Glu/Glu respectively). For *APE-1* codon 148 gene, the impact of the Glu/Glu genotype in current smokers appeared higher than that of Asp/Asp and Asp/Glu for the risk of lung cancer. ORs of current smokers with Asp/Asp, Asp/Glu and Glu/Glu genotypes were 3.0 (95% CI = 1.39-6.51, p = 0.005), 2.73 (95% CI = 1.29-5.77, p = 0.008) and 7.3 (95% CI = 2.93-18.3, p = 0.001), respectively when compared with non-smokers with Asp/Asp genotypes (Hidemi *et al.*, 2004). Thus the *APE-1* gene polymorphism appears to be playing an important role in modifying the direction and magnitude of the association.
between cigarette smoking exposure and lung cancer. Matullo et al. (2006) have found that the APE-1 148 Glu variant allele is significantly associated with a reduced risk of upper airo-digestive cancer (UADC), whereas the APE-1 gene in Asp/Glu genotypes has borderline association of risk of bladder cancer. Besides the essential role played by this protein in BER and other pathways, there is a belief that it is important to investigate APE-1 polymorphism in cervix cancer. To the best of our knowledge the relationship between APE-1 Asp/Glu genotype and cervix cancer susceptibility has not been investigated previously and this is the first study in this direction. The present findings have suggested that APE-1 gene might be responsible for reducing the risk of cervical cancer even when HPV (16, 18) are present. The result of this study on APE-1 148 Asp/Glu polymorphism in north Indian women have shown a decreased risk in variant genotypes, in cervix cancer as compared with controls.

5.4. RELATIONSHIP BETWEEN FOLIC ACID METABOLIC PATHWAY GENES AND RISK OF CERVIX CARCINOGENESIS

Folate deficiency has been associated with neural tubal defects, cardiovascular diseases and anaemia (Eichholzer et al., 2001). It has been hypothesized that the folate may modulates cancer risk, notably risk of cervix and colorectal cancer, less well studied breast cancer and a rapidly growing other cancer of sites such as lung, pancreas, stomach, oesophagus, leukemia, skin and endometrium (Zhang et al., 1997; Van et al., 2000). Folic acid is an important nutrient required for DNA synthesis, and the related methionine metabolic pathway is necessary for DNA methylation. Folate is also essential for the synthesis of nucleotides, DNA excision and repair, and DNA hypomethylation (Everson et al.,
A clinical trial had shown improvement in dysplasia in patients treated with 3 months of daily oral folic acid supplementation compared to placebo (Butterworth et al., 1982). However, a follow-up study reported that low red blood cell folate levels have been shown not to be directly associated with an increased risk of cervical dysplasia, but instead enhanced HPV-16 infection (Butterworth et al., 1992). MTHFR is a key enzyme in folate metabolism. It catalyzes reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the predominant circulatory form of folate and a carbon donor for the remethylation of homocysteine to methionine. The two functional polymorphisms have been associated with protection for some cancers, and a higher risk of others. MTHFR genotypes particularly C677T frequently associated with a variety of conditions, such as vascular disease, neural tubal defects, spontaneous abortion and malignancy, and controversy exists as to the role of these polymorphisms in cervical cancer (Ma et al., 1997; Rady et al., 2002; Heijmans et al., 2003; Henao et al., 2005).

On analyzing the frequency distribution of MTHFR, the present results have shown the frequency of wild genotype (CC) to be more in patients (85.0%) with cervix cancer than in controls (62.5%). The frequencies of CT and TT genotypes were more pronounced in controls than cervix cancer patients (34.0 for CT and 3.5% for TT respectively). However, in histological subtypes, the frequency of CC genotype was more in patients with SCC (85.7%) and that of CT genotype in AC (20.0%). A case control study of MTHFR polymorphism in cervical carcinogenesis has been carried out in African-American population. The frequency of CC genotype (94%) was higher in cases as compared with controls (91%), and that of TT was more pronounced in controls (9%) than cervix cancer (6%) (Gautam et al., 2006). Similarly Matsuo et al. (2001) also reported the frequency of wild genotype (CC) to be
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more in patients (45.9%) with malignant lymphoma, than in controls (33.3%), but the frequencies of $CT$ and $TT$ genotypes were more pronounced in controls (51.9% and 14.8% respectively) than in patients with malignant lymphoma. Angeline et al. (2004), however, reported the frequency of $CC$ genotype to be more in controls (85.0%) than patients with myocardial infarction (78.85%), and the frequency of heterozygote mutant ($CT$) genotype was more pronounced in patients (19.2%) than in controls (15.0%). The $MTHFR$ 1298C allele increases the risk of cervical cancer strongly in women with multipregnancies and early age of intercourse, while $MTHFR$ C667T genotype has a lower risk without becoming a protection factor (Delgado-Enciso et al., 2006).

In the present results no association was observed in $MTHFR$ variant genotypes and overall risk of cervical cancer, but a statistically significant association with individual genotypes of $MTHFR$ was observed. Similarly in histological subtype no association was observed between individual genotypes and risk, but it was significant in $CT$ (OR = 0.3, 95% CI = 0.16-0.49, $p = 0.000002$) and combined $CT+TT$ genotypes (OR = 0.3, 95% CI = 0.16-0.48, $p = 0.0000007$) in patients with SCC of cervix. According to Kang et al. (2005) $MTHFR$ 677 C-T genotype is not associated with an increased risk of uterine cervical cancer. They suggested that there is a possible interaction between epigenetic and genetic factors in uterine cervical cancer. On the other hand Gautam et al. (2006) confirmed a significant difference in the $MTHFR$ (677 C→T) allele frequencies among racial groups, but there is no association of either the 677 C→T or 1298 A→C polymorphisms in cervical carcinogenesis.

Methionine synthase (MS) is a cobalamin dependent enzyme that catalyzes the methyl transfer from homocysteine to methionine, thus, playing a critical role in maintaining adequate
intracellular S-adenosyl-methionine (SAM) levels for DNA methylation having a cancer suppressor effect. The MS 2756 A→G polymorphism converts an aspartic acid to a glycine residue and has been predicted to alter enzyme activity that may affect DNA methylation process (Skibola et al., 2002). In the analysis of MS 2756 polymorphism, the frequency of AA genotype to be more pronounced in patients with cervix cancer than controls (90.5% and 59.0% respectively). However, the frequency of AG and GG genotypes was more pronounced in controls as compared to cases. According to histological classification, AA genotype was higher in patients with SCC than with AC (91.0% and 88.0% respectively). On studying the association of MS (2756) gene polymorphism with cervical cancer risk, MS (AG) genotype was found to be protective with respect to the risk of cervix cancer (OR = 0.1, 95% CI = 0.07-0.26, p < 0.001). Similarly, histological stratification of MS (AG) genotype was significant: associated both with the decrease in the risk of SCC (OR = 0.1, 95% CI = 026-0.25, p < 0.001) as well as AC (OR = 0.3, 95% CI = 0.08-0.87, p = 0.02). Kang et al. (2005) also reported the MS A275QG polymorphisms were not associated with an increased risk of uterine cervical cancer.

Emad et al. (2005) have reported a higher susceptibility to malignant lymphoma with mutant form of MS polymorphism. Similarly Skibola et al. (2004) have also reported that MS 2756 (AG/GG) genotypes are associated with an increased risk of non-Hodgkin lymphoma (NHL) with an OR of 1.3 (95% CI = 0.99-1.7). Contrary to that, lower susceptibility to colorectal cancer in patients harbouring the mutant allele has been reported by Ma et al. (1999). However, Sokbom et al. (2005) have suggested no significant association of MS gene and risk of cervical cancer.
5.5. EFFECT OF INTERACTION BETWEEN APE-1, NBS1, IL4, IL-10, MS, MTHFR, IL-18 AND HPV GENOTYPE AND PASSIVE SMOKING ON RISK OF CERVIX CANCER

Smoking experience of the partner has been confirmed to be a risk factor for cervical cancer (Settheetham-Ishida et al., 2004). The analyzed data has indicated that the risk of lung cancer in the U.S. increased approximately by 1.6 folds by environmental tobacco smoke (USAE Environmental Protection) and 10 year European study of 2000 adults found a small, but definitely elevated risk. Ferrera et al. (2000) had reported that HPV-positive women exposed to tobacco smoke had higher risk of developing cervix cancer. In the present study, the interaction between various genes and passive smoking and its effect on risk of developing cervix cancer has been analyzed.

5.5.1. Effect of interaction between APE-1, NBS1, gene polymorphism and passive smoking on the risk of cervix cancer

Genetic variations in DNA repair genes are thought to modulate DNA repair capacity and are suggested to be related to cancer risk (Rayjean et al., 2006). Lan et al. (2005) have suggested that heavy smoking i.e., more than 25-pack in a year, is weakly associated with NBS1 gene and an increased risk of lung cancer (OR = 1.6, 95% CI = 0.82-3.49) in the men, a finding which is consistent with previous studies in Xuan Wei, China (Mumford et al., 1987). The impact of Glu/Glu genotype of APE-1 148 in current smokers appeared higher than that of Asp/Asp and Asp/Glu genotypes, in lung cancer patients (Lan et al., 2002).

In the present study, in passive smokers a non significant reduced risk (OR = 0.8, 95% CI = 0.42-1.59 and OR = 1.1, 95% CI = 0.58-2.15) was observed in Asp/Glu and Glu/Glu genotypes of APE-1.
gene respectively. The \textit{NBS1} (Glu/Gln) genotype was found to cause 9.7 fold (90% CI = 3.23-29.16, \( p < 0.001 \)) increased risk and statistically highly significant in passive smokers. It appears that \textit{APE-1} and \textit{NBS1} genes do interact with tobacco smoke. This is the first study on the relationship of passive smoking and two DNA repair pathway genes such as \textit{NBS1} and \textit{APE-1} with risk of cervix cancer in north Indian population and a study with larger sample size is needed to be carried out to further analyze such interactions.

5.5.2. Effect of interaction between \textit{IL-4}, \textit{IL-10} gene polymorphism and passive smoking on the risk of cervix cancer

Th2 cells produce IL-4 and IL-10 in addition to favoring humoral immunity (Mosmann \textit{et al.}, 1997). Polymorphisms in the promoter region of the \textit{IL-4} and \textit{IL-10} genes influence the extent of gene transcription and the amount of cytokine that is produced. The present study has suggested prominent risk (OR = 1.8, 95% CI = 0.80-4.03) of cervix cancer in case of passive smokers having \textit{IL-4 Rp1/Rp2} genotype. The \textit{IL-10 (AC)} genotype had 1.7 fold (95% CI = 0.90-3.34) increased risk of cervix cancer in passive smokers. It can, therefore, be interpreted that constituents of passive smoking cause an increased risk of cervix cancer in the presence of different polymorphic forms of \textit{IL-4} and \textit{IL-10}.

5.5.3. Effect of interaction between \textit{MS}, \textit{MTHFR} gene polymorphism and passive smoking on the risk of cervix cancer

In the present study, it was observed that passive smokers having \textit{MS (AG)} genotype had statistically significant decreased risk of cervix cancer (OR = 0.4, 95% CI = 0.20-0.76, \( p = 0.00009 \)). Decreased risk of developing cervix cancer was also observed in passive smokers carrying various genotypes of \textit{MTHFR}. Hongbing
et al. (2001) did not find any association between MTHFR genotype and risk of lung cancer among different subgroups for either C667T or the A1298C polymorphism when stratified for smoking and alcohol. To further complicate our understanding, two recent studies have suggested a protective effect of MTHFR C667T in invasive cervical cancer, but not CIN or in CIN with an additive effect in conjunction with polymorphism of MS in the same metabolic pathway (Henao et al., 2005; Zoodsma et al., 2005). The data collected in present investigations suggest significant decreased risk of cervical cancer with passive smokers.

5.5.4. Effect of interaction between IL-18, HPV variants and passive smoking on the risk of cervix cancer

For IL-18, 4.3 fold elevated risk of cervix cancer was observed in case of passive smokers with GC genotype (OR = 4.3, 95% CI = 2.13-8.99, p = 0.00001). In case of HPV (16,18) no significant association was observed between HPV(16,18) and development of cervix cancer in passive smokers. These associations were, however, statistically insignificant which may be due to small sample size, on may both interaction between IL-18 genotype and HPV (16,18) and risk of cervical cancer. A convincing evidence that smokers maintain cervical HPV infections significantly longer and have a lower probability of clearing an oncogenic infection than women who never smoked has been presented by Castle et al. (2002).

5.6. EFFECT OF INTERACTION BETWEEN APE-1, NBS1, IL-4, IL-10, MS, MTHFR, IL-18 AND HPV GENOTYPES AND USE OF ORAL CONTRACEPTIVES ON THE RISK OF CERVIX CARCINOMA

Use of oral contraceptives has been found to be associated
with cervical cancer in many, but not in all epidemiologic studies that were adjusted for HPV status. Choice of contraceptive method also affects the risk of cervical cancer. Barrier method of contraception lowers the risk, probably decreasing exposure to infectious agents. Hildesheim et al. (2001) had observed a 3.1 fold increased risk for users of 5 or more years as compared with never users, but this association was observed only among women who had two or fewer pregnancies, and the HSIL/cancer group included only 30 cases. IARC studies had reported the use of oral contraceptives to be moderately associated with the risk of cancer (OR = 1.4). No increase in the risk of cervical neoplasia was found for the duration of contraceptive use for upto 4 years. However, the use of oral contraceptives for longer than 5 years was significantly associated with the development of cervical neoplasia (OR = 3.4, 95% CI = 2.1·5.5) (Moreno et al., 2002). In the present study, the association of different gene polymorphisms in combination with use of oral contraceptive on the risk of cervix cancer was evaluated. This is the first case control study to analyze such an association in north Indian women.

5.6.1. Effect of interaction between APE-1, NBS1 gene polymorphism and use of oral contraceptives on the risk of cervix cancer

In the present study statistically significant association, but decreased risk (OR = 0.2, 95% CI = 0.13·0.48, p = 0.00001) of cervix cancer was observed in individuals of APE-1, Asp/Glu using oral contraceptives. The NBS1, Glu/Gln individuals had an increased risk of cervix cancer on the use of contraceptives (OR = 2.7, 95% CI = 1.2·5.79). No data are available for the association of APE-1 and NBS1 genotypes and risk of cervical cancer in those using oral contraceptives.
5.6.2. Effect of interaction between \textit{IL-4}, \textit{IL-10} gene polymorphism and use of oral contraceptives on the risk of cervix cancer

In case of \textit{IL-4}, there was marginal increase in OR (OR = 1.3, 95% CI = 0.05-3.22) for risk of cervical cancer in individuals with \textit{Rp1/Rp2} genotype. Statistically significant, but decreased risk of cervix cancer was observed for contraceptive users with \textit{IL-10} (AC) (OR = 0.4, 95% CI = 0.19-0.70, p = 0.001) genotype.

5.6.3. Effect of interaction between \textit{MS}, \textit{MTHFR} gene polymorphism and use of oral contraceptives on the risk of cervix cancer

Geometric mean serum and red blood cell plates, using either assay and adjusted for age, ethnicity and study site were not significantly different between women who had used oral contraceptives and who had not. In addition, although it had been hypothesized that oral contraceptive use would deplete cervical folate stores, no elevation in risk by folate status was observed among users of oral contraceptives (Butterworth \textit{et al.}, 1982). In the present study, there was decreased risk, but highly significant, with \textit{MS} (AG) genotype (OR = 0.2, 95% CI = 0.09-0.42, p < 0.001) in users of contraceptives. Similarly in case of \textit{MTHFR} statistically reduced risk in heterozygous mutant (CT) (OR = 0.2, 95% CI = 0.11-0.45, p < 0.001) using contraceptive was observed. It has, therefore, been suggested that \textit{MS} and \textit{MTHFR} have synergistic effect for reducing risk of developing cervix cancer in contraceptive users. Conner \textit{et al.} (2004), reported an inverse association of \textit{MTHFR} 677 TT genotype and breast cancer among hormone replacement therapy (HRT) users. Similarly, Loic \textit{et al.} (2004) have reported a stronger inverse association of \textit{MTHFR} TT genotype with breast cancer in women with hormone replacement therapy (HRT).
5.6.4. Effect of interaction between *IL-18* and HPV gene polymorphism and use of oral contraceptives on the risk of cervix cancer

No significant association was observed between different genotypes of *IL-18* on risk of developing cervix cancer in contraceptive users. Elevated risk (OR = 3.1, 95% CI = 0.8-11.8) of developing cervix cancer was observed for contraceptive users who harbored HPV type 16. In case of HPV (16+18), statistically significant elevated risk was also observed for contraceptive users (OR = 4.0, 95% CI = 1.03-15.46, p = 0.03). Hormone estrogen related mechanisms may influence the progression from pre-malignant to malignant cervical lesions by promoting integration of HPV DNA into host genome, which results in deregulation of *E6* and *E7* expression (IARC, 1999). Data from experimental studies have demonstrated a synergistic mechanism between long-term estrogen exposure and HPV 16 oncogenes that modulate squamous cell carcinoma of cervix (Arbeit *et al.*, 1996; Elson *et al.*, 2000).

5.7. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPES OF *APE-1*, WITH *NBS1*, *IL-4*, *IL-10*, *MS*, *MTHFR*, *IL-18* GENES AND HPV

Most malignancies have some genomic instability. These can be defect in base excision repair, or some form of chromosomal instability (Draviam *et al.*, 2004).

*APE-1* encodes a protein involved in both BER and regulation of gene expression as a redox co-activation of different transcription factors, such as *P53* and *APE-1* (Hu *et al.*, 2001). Polymorphism in this encoding gene is associated with altered DNA repair capacity and, thus, may have impact on the risk of cancer. Statistically significant increased risk (OR = 3.6, 95% CI = 1.72-7.46, p = 0.000001) of cervix cancer was observed for the
carrier of Asp/Asp (APE-1) along with NBS1 (Glu/Gln+Gln/Gln) genotype. On histological stratification, it was observed that the risk is significantly increased for SCC with APE-1 (Asp/Asp) and NBS1 (Glu/Gln+Gln/Gln) (OR = 4.8, 95% CI = 1.91-12.12, p = 0.000001) genotype. Ryk et al. (2006) have reported that genetic variation in XRCC1, XRCC3, APE-1 and NBS1 influence susceptibility to lung cancer among women and that combinations of risk alleles in the two homologous repair (HR) genes can enhance the effects. Evaluation of interaction between APE-1 and IL-4 in north Indian women has suggested no significant association between different polymorphic forms of APE-1 and IL-4 and overall risk of cervical cancer.

The interaction between APE-1 and IL-10 indicated no significant association between various polymorphic forms of APE-1 and IL-10 and risk of developing cervix cancer. The combined interaction of APE-1, MS and APE-1, MTHFR has shown no significant association between different individual genotypes and risk of cervical cancer. However, in histological subtypes, statistically protective effect for SCC was observed for APE-1 and IL-10 (OR = 0.5, 95% CI = 0.24-0.92, p = 0.02). On studying the interaction between APE-1 and IL-18, there was marginal increase in risk of cervix cancer (OR = 1.3, 95% CI = 0.74-2.20) for APE-1 (Asp/Asp) and IL-18 (GC/CC) genotype. Thus, the present study suggested cross-link between single nucleotide polymorphism of the DNA-repair pathway with cytokine and folate metabolic pathway genes on the risk of cervix carcinoma.

5.8. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPES OF NBS1 WITH IL-4, IL-10, MS, MTHFR, IL-18 GENES AND HPV

Double-strand breaks can be produced by replication errors and by exogenous agents such as repair of ionizing radiation of
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double-strand breaks is intrinsically more difficult than other
types of DNA damage because no undamaged template is available
(Khanna et al., 2001). For carriers of heterozygous mutant
genotype of NBS1 i.e., Glu/Gln and Rp1/Rp1 genotype with IL-4,
there was statistically insignificant increased risk (OR = 3.0, 95%
CI = 0.51-18.71) of developing cervical cancer. Increased risk of
developing cervix cancer was observed for different combined
genotypes of NBS1 and IL-10. Similarly trend was there for SCC
alone. Thus, these results suggest synergistic effect of combined
genotypes of NBS1 and IL-10 on the risk of cervical cancer in
north Indian women.

Increased risk was observed for subjects having NBS1
(Gln/Gln) and MS (AA) (OR = 6.6, 95% CI = 2.43-18.50) genotype.
Likewise after stratifying histologically, it was found that NBS1
(Glu/Gln) and MS (AA) combined genotypes cause statistical
significant increased risk for SCC (OR = 7.9, 95% CI = 3.26-19.78,
p = 0.0000002). Furthermore, statistically significant increased
risk of developing AC was observed for NBS1 (Glu/Gln) and MS
(AA) (OR = 7.7, 95% CI = 1.07-55.34, p = 0.02) genotype. Statistically insignificant increased risk (OR = 8.5, 95% CI = 3.55-
21.28) for the development of cervix cancer was observed in the
carrier of NBS1 (Glu/Gln) and MTHFR (CC) was observed in the
development of cervical cancer. In addition statistically significant
increased risk of developing SCC was found for different genotypes
of NBS1 with combined genotype of MTHFR in patients with
cervical cancer. 6.9 fold (95% CI = 0.96-50.37, p = 0.03)
statistically significant increase in risk for AC was observed for
those carrying NBS1 (Glu/Gln) and MTHFR (CC) combined
genotypes. The combined genotype of NBS1 (Glu/Gln) with IL-18
(GC+CC) showed an increased risk (OR = 8.2, 95% CI = 2.75-
24.64) of cervical cancer. Correspondingly development of NBS1
(Gln/Gln) and IL-18 (GC+CC) increased the risk (OR = 8.2, 95% CI
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2.69-25.05) for development of cervix cancer. Similarly statistically significant increased risk with this combined genotype of NBS1 and IL-18 was observed for SCC (OR = 7.9, 95% CI = 2.59-24.31, p = 0.000003).

5.9. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPES IL-4, WITH IL-10, MS, MTHFR, IL-18, AND HPV

Two polymorphisms for IL-4 i.e., IL-4-590 promoter and IL-4-intron 3 have been demonstrated. They have been associated with various disease conditions or susceptibility to diseases like rheumatoid arthritis, asthma, rhinitis, atopic dermatitis, ‘graves’ disease, multiple sclerosis, cancer such as bladder etc. (Hunt et al., 2000). Yao et al. (2002) have reported that genotype and allelic frequencies of IL-4 intron-3 polymorphism not candidate genetic markers for susceptibility to endometriosis in Taiwan Chinese women.

In our study no significant association was observed between different polymorphic forms of IL-4 with IL-10 combined genotypes and overall risk of cervical cancer in north Indian women. The interaction between genotypes of IL-4 (Rp2/Rp2) combined with MS (AA) indicated marginal increase in OR (OR = 1.3, 95% CI = 0.76-2.29) for developing cervix cancer. When stratified into histological subtypes, 1.6 fold increase (OR = 1.6, 95% CI = 0.50-5.16) of SCC in the carrier of IL-4 (Rp2/Rp2) and MS (AA) genotypes. There was a marginal increase in OR (OR = 1.8, 95% CI = 0.60-5.05) for individual with IL-4 (Rpl/Rp2) and MTHFR (CC) genotypic combination. Also when stratified histologically, the risk was borderline for SCC (OR = 1.6, 95% CI = 0.51-4.89) for IL-4 (Rp1/Rp2) and MTHFR (CC) genotypes. For IL-4 (Rp1/Rp2) and IL-18 (GC+CC) genotypes, 1.4 fold increased risk
of cervical cancer was observed. Interaction between \( \text{IL}-4 \) (\( Rp2/Rp2 \)) and HPV (16+18) genotype showed 1.4 fold (95% CI: 0.34-5.67) increased risk of cervix cancer. No significant association was observed between genotypic combination of \( \text{IL}-4 \) and HPV and risk of cervical cancer in north Indian population.

5.10. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPE OF \( \text{IL}-10 \), WITH \( \text{MS} \), \( \text{MTHFR} \), \( \text{IL}-18 \), AND HPV

An analysis of the expression of IL-10 in the normal or abnormal uterine cervix demonstrated that the severity of squamous intraepithelial lesions correlates with an increased expression of IL-10 in the transformation zone of the uterine cervix (Clerici et al., 1998). Increased serum levels of IL-10 have been reported to be associated with both the presence and the progression of cervical cancer (Chu et al., 1999). It has been reported that IL-10 increased both Th1 cytokine production and cytotoxic potential in cultured HPV-specific CD8+CTL related to the tumourigenesis of cervical cancer (Santin et al., 2000).

Statistically highly significant decreased risk (OR = 0.2, 95% CI = 0.08-0.43, \( p < 0.001 \)) of developing cervix cancer was observed for the combined genotype of \( \text{IL}-10 \) (\( AC \)) and \( \text{MS} \) (\( AG+GG \)). Similarly according to histological classification, statistically highly significant decreased risk (OR = 0.2, 95% CI = 0.08-0.45, \( p < 0.001 \)) of SCC was observed between \( \text{IL}-10 \) (\( AC \)) with \( \text{MS} \) (\( AG+GG \)) genotypes.

Interaction between \( \text{IL}-10 \) (\( AC \)) with \( \text{MS} \) (\( AA \)) genotypes indicated marginal risk (OR = 1.3, 95% CI = 0.45-3.60) of AC of cervix. Statistically significant inverse association (OR = 0.3, 95% CI = 0.13-0.75, \( p = 0.006 \)) was observed for \( \text{IL}-10 \) (\( AA \)) with \( \text{MTHFR} \) (\( CT+TT \)) genotypes. Similarly, significant decreased risk
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(OR = 0.3, 95% CI = 0.14-0.58, p = 0.003) was observed IL-10 (AC) and MTHFR (CT+TT) genotypes. When stratified for tumour histology, the risk for SCC was marginally increased (OR = 1.3, 95% CI = 0.47-3.78) for IL-10 (CC) and MTHFR (CC) genotypes. Similarly, the risk was marginal for AC when IL-10 (AC) and MTHFR (CC) genotypes were combined (OR = 1.4, 95% CI = 0.48-4.02). Two fold (95% CI = 1.04-3.90, p = 0.03) statistically significant increased risk for developing cervix cancer was observed for IL-10 (AA) and IL-18 (GG+CC) genotype. Histologically, stratification also gave similar results for SCC with IL-10 (AA) and IL-18 (GC+CC) (OR = 2.0, 95% CI = 0.98-3.84) genotype. In case of AC also, an elevated risk for IL-10 (AA) and IL-18 (GC+CC) (OR = 2.4, 95% CI = 0.75-7.56) genotype was observed. There was a borderline increase in risk of developing cervix cancer (OR = 1.6, 95% CI = 0.58-4.23) for IL-10 and HPV (16+18) genotypic combination. An increased risk (OR = 2.5, 95% CI = 0.60-10.34) of cervix cancer was observed for the IL-10 (AC) and HPV (18) genotypic combination for SCC.

5.11. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPES MS, MTHFR, IL-18, AND HPV

The MS 2756 GG polymorphism was initially thought to be associated with lower enzyme activity than MS 2756 AA genotype (Leclerc et al., 1996). However, other investigators have indicated that the variant genotypes (2756AG 2756 GG) may be associated with lower homocysteine and/or higher methionine levels than the wild type genotype, implying a more effective enzyme activity (Chen et al., 2001; Silaste et al., 2001). In the present study, the combined effect of the interaction of MS with other genes on the risk of cervix cancer was analyzed. Statistically significant decreased risk of cervix cancer in different combined genotypes of
MS and MTHFR was observed. The present study suggested that these genotypes exert a protective effect on the risk of cervix cancer (OR = 0.2, 95% CI = 0.14-0.44, p = 0.0000005 for MS (AA) and MTHFR (CT+TT) and OR = 0.1, 95% CI = 0.03-0.41, p < 0.001 for MS (AG) and MTHFR CT+TT genotypes). Likewise after stratifying into histological subtypes, significant decreased risk (OR = 0.3, 95% CI = 0.14-0.49, p = 0.000006) of SCC was observed for MS (AA) and MTHFR (CT+TT) genotypic combination. Similar results were obtained for MS (AG) and MTHFR (CC) (OR = 0.1, 95% CI = 0.02-0.79, p = 0.009) genotype in patients with AC of cervix. The relationship between combined genotypes of MS and IL-18 showed statistically significant decrease in the risk of developing cervix cancer (OR = 0.1, 95% CI = 0.06-0.42, p = 0.0000001) for MS (AG) and IL-18 (GG) genotypes. According to histological subtypes, there was statistically significant decreased risk for developing SCC of uterine cervix (OR = 0.2, 95% CI = 0.07-0.44, p = 0.000006) for MS (AG) and IL-18 (GG) genotypes. The combination of MS (AG) and HPV 18 indicated marginal increase (OR = 1.4, 95% CI = 0.32-6.41) for development of cervix cancer. No significant association was observed between various MS and HPV genotypic combinations and developing of SCC or AC of cervix cancer in north Indian women.

5.12. RELATIONSHIP OF CERVIX CANCER WITH COMBINED GENOTYPES MTHFR, IL-18 AND HPV

The impact of folate metabolism on cervical dysplasia and invasive cancer is not well understood. Controversy continues to exist regarding the role of the MTHFR functional polymorphisms 677 C→T and 1298A→C as true modified genes in the process of cervical carcinogenesis (Gautam et al., 2006). Gerhard et al. (2003) were unable to confirm any strong association of MTHFR
polymorphisms and invasive cervical cancer (ICC), using family-based controls and a transmission/disequilibrium test. Conversely, Sull et al. (2004) have shown that polymorphism of MTHFR is associated with a higher risk of developing cervical cancer, and in particular for an early onset of cervical carcinogenesis. Statistically significant decreased risk (OR = 0.2, 95% CI = 0.11-0.53, p = 0.0001) of cervix cancer was observed for the MTHFR (CT) and IL-18 (GG). However, for the MTHFR (CC) and IL-18 (GC+CC), the risk of developing cervix cancer was marginally increased (OR = 1.4, 95% CI = 0.88-2.33). The present results suggested reduction in the risk of cervical cancer with SCC. Furthermore comparable results were also observed for MTHFR (CC) and IL-18 (GC+CC) genotypes in patient with AC of uterine cervix. No significant association was observed between MTHFR and HPV genotypic combination and risk of cervical cancer.

5.13. HYPERMETHYLATION CpG ISLAND P16INK4A AND P14ARF (TUMOUR SUPPRESSOR GENES) AND RISK OF CERVIX CANCER

Methylation plays an important role in tumourigenesis. In particular, aberrant methylation of normally unmethylated CpG islands for many tumour suppressor genes is associated with transcriptional inactivation and hence loss of expression (Jones et al., 1999). Studies have shown that epigenetics, plays an important role in cancer biology (Robertson et al., 2002), viral infection (Baylin et al., 1997), activity of mobile element (Costello et al., 2001), somatic gene therapy, cloning, transgenic technologies, genomic imprinting, developmental abnormalities, mental health and X-inactivation (Amir et al., 1999; Laird et al., 2003). The P16 protein plays a key role in controlling cell growth by inhibiting of cycline-dependent kinase-4 and preventing
phosphorylation of the retinoblastoma protein, which maintains the G1 checkpoint (Sherr et al., 1994; Weinberg et al., 1995). Inactivation of the P16 has been reported in different human malignancies, and the predominant mode of inactivation appears to be by homozygous deletion and methylation, but mutations of this gene appear to be less common.

The molecular inactivation of P16 is not clear, however, the fact is that it is not an early event in cervical carcinogenesis, but is more frequently methylated in advanced tumours (Wong et al., 1999). Studies on a rat model for lung carcinogenesis (Belinsky et al., 1998), and of precursor lesions to human oesophageal tumour and squamous cell lung cancers (Belinsky et al., 1998) have also suggested that hypermethylation of P16 may be an early event in tumourigenesis. In normal cell, most CpG island is associated with active genes or genes capable of active transcription, but, in cancer, methylation of CpG promoter regions is associated with inappropriate transcriptional repression and gene inactivation. Significantly, many of the inactivated genes are tumour suppressor genes (Herman et al., 2003; Li et al., 2004).

Inhibition of tumour suppressor gene by methylation is implicated in cancer initiation, development, and progression. Therefore, it is important mechanism for inactivating of tumour suppressor gene as an alternative to gene mutation or deletion in tumourigenesis (Herman et al., 2003). However the target genes that are associated by CpG methylation are different for each cancer type (Esteller et al., 2001). In this study, the promoter methylation frequency of P16 gene in cervical cancer was 43.8% (35/80). The frequency is in concordance with the results (Yang et al., 2004). The p16INK4a tumour suppressor gene was hypermethylated at a low frequency (7%) in the bladder tumours (Essel et al., 2004). Our result did not show any relation (p > 0.05)
between \textit{P16} CpG hypermethylation and clinicopathologic parameters of cervix cancer.

Recent studies have further been concentrated on silencing of tumour suppression genes by promoter hypermethylation. For example with \textit{P14ARF}, \textit{P15INK4b}, \textit{P16INK4a}, \textit{GSTP}, and \textit{MGMT} as a common feature in human neoplasm including head and neck cancers (Loughran et al., 1996). Progressive methylation of the \textit{P16} gene has been reported in early lesions of squamous carcinomas of the lung (Belinsky et al., 1998) and in 31% of cervical cancers (Wang et al., 1998). Arvind et al. (2001) found a 42% incidence of methylation for \textit{P16} in cervical cancers, and in 24% of high-grade dysplasia. Methylation was rare (3%) in non-dysplasia/low-grade CIN specimens. 82 cervix cancer samples was investigated for CpG island hypermethylation of \textit{p16}, 19.5% of which were positive (Sokom et al., 2005). On the other hand, the study of promoter methylation frequency of \textit{P16} gene in cervical cancer has been reported to be 57.0% (45/78) in Korean women (Dae et al., 2005). Seung et al. (2001) have detected the promoter hypermethylation of \textit{p16} in 30% (16/53) of cases with cervical cancer and it was more common in cases with SCC than in those with AC. In another study, the aberrant methylation of the promoter region of \textit{P16} has been reported to be 20% in non-Japanese patients having endometrial cancer (Wong et al., 1999). The differences in the frequencies of aberrant DNA in methylation of this gene in different types of cancers suggests that the mechanism of the induction of aberrant DNA methylation may widely differ between organs and tissues.

\textit{P14ARF} has been ascribed a role in modulating the cellular amounts of \textit{p53} through direct interaction with \textit{MDM-2} and \textit{P14ARF} inhibits MDM-2, tags \textit{p53} for degradation through the proteosome pathway (Kamijo et al., 1997). Homozygous deletion of the \textit{P14ARF} locus has been reported in a variety of cancers (Xing et
al 1999), and gene knockout of \( P14^{ARF} \) to be correlated with tumourgenesis. Esteller et al. (2001) have demonstrated that \( P14^{ARF} \) hypermethylation occurs frequently in sporadic colorectal cancer. The present results show that the methylation frequency for \( P14 \) gene in cervical cancer to be 10.0% (8/80) as compared to 2.5% (2/80) in controls. This study has suggested no methylation frequency for the \( P14 \) gene as compared with control groups observed. On the other hand clinicopathological parameter such as smoking have shown significant association between \( p14 \) and risk of cervix cancer (\( p = 0.006 \)). But the major limitation of our work in this area was a less number of current smokers in women with cervix cancer; therefore our study has been based on few cases with active smokers.

Essel et al. (2004) have also observed hypermethylation of \( P14 \) in 16 of 45 (35%) bladder tumours by methylation specific-PCR. Masayoshi et al. (2003) have suggested significant correlation between loss of \( P14 \) expression as detected by immunohistochemistry and homozygous deletion /promoter methylation (\( p = 0.02 \)). They have found a significantly higher frequency of \( P14 \) gene alteration than that in \( P16 \) gene (\( p = 0.02 \)).

5.14. RELATIONSHIP BETWEEN IL-18 AND IFN-\( \gamma \) GENE EXPRESSION IN RESPONSE TO CERVIX CARCINOGENESIS

IL-18 was initially described as IFN-\( \gamma \)-inducing factor in 1989, which up-regulates several cytokines, such as IFN-\( \gamma \), TNF-\( \alpha \), IL-1\( \beta \), and promotes Th1 cell differentiation. IL-18, mainly produced by dendritic cells and macrophages, has structural homology with IL-1, and provides a synergistic antitumour immunity in combination with IL-12. Most of the biological activities of IL-18 overlap with those of IL-12, but these two
interleukins seem to act through separate mechanisms (Cao et al., 1999; Hikosaka et al., 2004). Zheng-bao et al. (2007), have reported IL-18 expression only in 13 (26%) out of 50 cases, and not to be associated with pathologic differentiation, depth of invasion, lymph node metastasis, TNM stage and prognosis. But IL-18 was presented in 70% of gastric cancer with distant metastasis (M1), significantly higher than M 0-stage group (15%) (p = 0.001) (Zhang-bao et al., 2007).

Merendino et al. (2001) have reported serum IL-18 to be significantly higher in breast cancer patients with liver or bone metastases than in those without metastasis or healthy individuals. This implied that IL-18 could be regarded as a metastatic marker for breast cancer, irrespective of its biological activities. The pre-operative serum IL-18 level may represent a significant postoperative prognostic determinant in patients with gastric carcinoma. It was found that patients with IL-18 levels over 310 pg/mL experience a significantly lower survival rate after surgery than under 310 pg/mL (Kawabata et al., 2001). IL-18 could significantly increase MMP-9 production at both mRNA and protein levels (Zhang-bao et al., 2004), thus, enhancing the aggressiveness of malignant tumours, facilitating cancer metastasis through the induction of vascular cell adhesion molecule-1 (VCAM-1), and promoting cancer cell adhesion and liver metastasis in vivo (Vidal-Vanaclocha et al., 2000).

Liver metastasis frequently appears in late stage gastric cancer, and most of the M1-stage patients presented liver involvement which accounted for the phenomenon of high expression of IL-18 in M1-stage gastric cancer. Riedel et al. (2004) have observed an elevated serum level of IL-18 in the majority of head and neck squamous cell carcinoma (HNSCC) cancer patients, irrespective of its biological activity, thus suggesting that serum IL-18 might be a candidate for a new marker for HNSCC. The
pathways for IL-18 production and its mechanisms of action in patients with HNSCC remain to be determined. Understanding of the immunological pathways might offer new therapeutic options in head and neck cancer in the future. It would also be very pertinent in future to assess the expression pattern (up- and down-regulation) of different cytokines and other candidate genes in patients suffering from cervix carcinoma to understand the involvement of different genes/regulatory factors and dynamics of disease progression.

The precise relationship between the altered expression of these genes and cervical tumourigenesis is a matter of great importance. This will also help in elucidating the mechanism of cytokine network that potentially affects the immunological systems during cervix cancer. Apart from mRNA level expression, efforts should also be made to assess the IL-18 and IFN-γ levels in serum for cervix carcinoma patients so as to correlate with their tissue level expression. In many cancer types like lymphoblastic leukemia, chronic myeloid leukemia, breast cancers, head and neck squamous cell carcinoma, IL-18 serum levels have been found to be significantly elevated in comparison to their healthy counterparts. Also immunohistochemical and immunoblotting analysis of affected tissue with cervix carcinoma vis-à-vis normal tissue section will be prudent to ascertain the concentration of tumour-infiltrating immune cells and protein level expression of this cytokine (Riedel et al., 2004). Interferon gamma (IFN-γ) is a cytokine produced by activated T cells and natural killer (NK) cells that enhances cellular immune responses by increasing T-cell cytotoxicity and NK cell activity (Min et al., 1996). The striking correspondence between the various biologic activities of IFN-γ and the immunologic modifications observed in cervical carcinomas prompted us to measure IFN-γ messenger RNA
(mRNA) at the tumour site by means of a semi-quantitative polymerase chain reaction (PCR) assay.

Thus the present semi-quantitative analysis reflected the similar level of mRNA expression of both IL-18 and IFN-γ genes in patients suffering from cervix carcinoma and healthy controls. Tartour et al. (1998) have reported an association between intra-tumoural IFN-γ mRNA levels (as assessed by a quantitative PCR assay) and clinical outcome in patients with primary invasive cervical carcinoma. Cervical cancer patients who exhibited fewer than 1000 IFN-γ mRNA copies were identified only in the population with high risk of recurrence or low survival. Several studies have indicated a switch in the pattern of cytokine from Th1 (IL-2, IFN-γ) to Th2 (IL-4, IL-5, IL-10) groups in cancer patients compared with healthy control subjects, but the clinical prognostic value of this finding has not been determined (Ghosh et al., 1995; Nakagomi et al., 1995). Li et al. (2007) have suggested that the major role of IFN-γ in lung cells is to direct a pro-inflammatory gene expression program rather than having major effects on cell growth or survival or both. Pao et al. (1996) have shown that IFN-γ mRNA levels are significantly reduced in cervical intraepithelial neoplasia and cervical cancer tissue compared with normal cervix.

A defect in IFN-γ expression at the tumour site may favour tumour progression by various mechanisms. Since the decrease in IFN-γ mRNA expression in a group of cervical cancer patients may not be simply related to the number of infiltrating T or NK cells, other factors could also play a role in this phenomenon. It was shown that a decrease in IL-12 production by macrophages caused by tumour-derived factors such as prostaglandin E2 or phosphatidyl serine resulted in impaired IFN-γ production in mammary tumour-bearing mice (Handel-Fernandez et al., 1997).
An increase in prostaglandin E2 secreted by peripheral blood mononuclear cells has also been reported during the progression of cervical carcinoma (Pao et al., 1996). HPV did not seem to play a role in IFN-γ gene expression (Tartour et al., 1998).

This study has suggested that IFN-γ production at the tumour site may not be directly involved in the poor outcome of cervical cancer patients. However, results of the current study are the initial indicators and in order to validate these results, real time analysis should further be carried out to find out the mRNA copy numbers in absolute terms during different stages of cervix cancer and its comparison with healthy controls. Additionally, apart from these two genes, expression analysis of more genes known to be immunologically associated in several kinds of cancers, should also be investigated. Considering the fact that IL-18 plays an important role in the interactions among T cells, NK cells and macrophages and induces the IFN-γ production, efforts should also be made to understand the clinical impact of IL-18 cytokine in patients with solid malignancies, as not much study have been conducted in cervix carcinoma. Such analysis will help to answer one important hypothesis that whether the serum levels of IL-18 or IFN-γ or other cytokines is increased in patients with cervix carcinoma or not. This may lead us to find out a marker for cervix carcinoma and would provide good platform to understand the immunological pathways to develop new immunotherapeutic options for cervix cancer.

Another important approach to understand molecular mechanism of cervix cancer is to go for genome-wide gene expression profiling using microarray technology. A global analysis of gene expression profiles of tissue affected with cervical cancers will help us to find out most differentially expressed genes that can further be utilized to distinguish normal cervix from
cancer. This will also give an insight of the pathways that how different genes are coordinately and differentially regulated in normal vs. cervix cancer tissue. Such a comprehensive approach may reveal highly attractive candidate molecular markers/ targets in future for cervical cancer diagnosis, prognosis and therapy.