CHAPTER 5 DISCUSSION

Oral cavity cancer is one of the commonest cancers in India and other South Asian countries (Park, 2005). It is a major cause of cancer morbidity and mortality, and its poor prognosis ensues in full blown cancer (Pindborg, 1977; Saranath, 2000). Therefore an improvement in prevention and control is of critical importance. Researchers agree that the early diagnosis of oral carcinoma greatly increases the probability of cure with minimum impairment and deformity. In this regard, detection of mutations in tumor suppressor genes or oncogenes and interindividual variability in sensitivity to the carcinogens might facilitate identification of individuals who are at a high risk of developing cancer.

Keeping this in view, the purpose of the present study was to evaluate the expression of p53 (tumor suppressor gene) and Cyclin D1 (cell Cycle regulator gene) as well as association of DNA repair genes (hOGGI Ser$^{326}$Cys, XRCCI Arg$^{280}$His) and drug metabolizing gene (CYP2E1 Rsal and Dral) polymorphisms with risk of oral cancer in tobacco and betel quid chewers of northern India.

The present study included 100 patients and 150 healthy subjects as controls. Biopsy specimens were taken from 60 oral cancer patients and 10 controls. Blood samples were collected from all the human subjects. A thorough proper history with special emphasis on the areca nut & betel quid intake was taken. Patients having habit of smoking and/or alcohol intake were excluded from the present study.
Age Incidence

In this study the highest number was between 50-75 years of age group i.e. 62 individuals (62%) suffering from cancer. Peak age incidence in Wahi’s series (1958) was 50-54 years while in another study by Sankararanayanan et al., (1989), 45% of oral cancer cases were between 5th & 6th decade. In study by Chiang CP et al., (1999), 51.85% patients belonged to 50-70 age group. The maximum incidence, according to Kuo MY et al., (1999), is between 50-70 year age group (64.5%) while in another study by Huang et al., (2001), 60% of oral cancer cases were between 50-70 year age group.

There was no significant difference in age incidence between the age groups of patients of our series and that of other workers.

The average age in this study was 56.75 years in patients in comparison to 54.8 years in the study of A. Nandakumar et al., (1990) and 50.35 years in the study of D.N. Rao et al., (1994). The average age in study by Stefania Staibano et al., (1998) was 62.04 years while it was 50 years in study by Chang et al., (2002).

Sex Incidence

Oral cancer is more common in males as compared to females. Krihna et al., (1967) reported the incidence of males to be 77.6% while Mehrotra R. et al., (2003) from Motilal Medical College Allahabad have reported about 74% of oral cancer in males. In our series, also, males constituted about 75% of the total cases.
In Wahi’s series (1958), the male female ratio was 2:1. In case control study by Krishna et al., (1967), there were 552 males and 224 females i.e. 2.5:1 ratio. In Mehrotra series, (2003), the female ratio was 2.3:1. In our series, it was 3:1. Mean age of male and female cases in our series was 58 and 53 years respectively while the mean age was found to be 63.5 years for males and 60.6 years for females in study by Langdon JD et al., (1977).

Relation of tobacco and betel quid chewing with oral cancer

In our study we have observed that tobacco and betel quid chewing is significantly associated with development of oral cancer. In India, association of tobacco and betel quid chewing and smoking with oral cancer has been demonstrated in earlier studies (Jayant et al., 1977, Notani, 1988; Shankaranarayanan et al., 1989; Nandakumar et al., 1990). The observations made by all of them pointed that the habit of tobacco and betel quid chewing is important risk factor in development of oral cancer. Our findings are in accordance with observation made by earlier studies.

Habit of Paan chewing is common in India in both genders. Paan generally includes calcium hydroxide, areca nut (from the areca catechu tree) and betel leaf (from the piper betel vine). R. Sankaranarayanan et al., (1989) showed that paan-tobacco chewing is major risk factor for cancers of buccal and labial mucosa. Similarly Nandakumar et al., (1990) confirmed that paan tobacco chewing is a major risk factor in the occurrence of cancers of the oral cavity. In the study conducted by
Prabha Balaram *et al.*, (2002), 591 oral cancer cases (309 males and 282 women) with 584 hospital controls were taken. They found that most cases of oral cancer in both genders were attributed to habit of paan tobacco intake.

**Incidence of site of lesion**

In our series, maximum 45 cases (45%) were of cheek carcinoma (Buccal mucosa) followed by malignancy of tongue (22%) and the floor of mouth (17%).

In Wahi's series (1958), cases of carcinoma cheek were maximum in the oral cancer patients (53.91%). In a study conducted by Rao *et al.*, (1994), the frequency of carcinoma cheek was maximum (44.4%). In study conducted by Agarwal *et al.*, (1999), buccal mucosa was predominant site in betel related oral cancer. Ranasinghe *et al.*, (1993) have also shown that buccal mucosa cancer is predominant site of oral cancer in tobacco and betel quid chewers while Chang *et al.*, (2002) found 57.8% cases of oral cancer to be cheek carcinomas in betel and tobacco related oral cancer. Our findings about incidence of site of lesion are in accordance with earlier studies.

**p53 Expression**

The immunohistochemical detection of p53 in biopsy specimen as a potential marker is of immense interest to researchers, as it is most commonly identified mutated gene in various human cancers like lung, breast, prostrate and oral cancer.
The gene coding for p53 protein i.e. TP53 is located on human chromosome 17 P 13:1 and encodes a 53 KDa nuclear phosphoprotein that plays an important role in regulation of normal cell proliferation (Raybaud-Diogene et al., 1996; Lane and Benchimol, 1990). The wild type p53 protein has a half life time of 6-20 minutes (Chiang et al., 2000), while the mutant form has a half life of several hours and can be detected immunohistochemically.

p53 mutations are often associated with the development and/or progression of malignant neoplasm (Allred et al., 1993). About 90% of mutation at p53 locus were found to be mis-sense mutations, within the region of exon 5 to 8 (Lane DP, 1998 and Lain et al., 1999). Immunohistochemical studies of p53 expression in SCC of oral mucosa has shown overexpression of p53 protein (Kaur et al., 1994; Kuttan et al., 1995; Baral et al., 1998; Chang et al., 1999; Chiang et al., 1999; Pande et al., 2002).

In the present study, immunohistochemical studies for p53 expression was done on formalin fixed and paraffin embedded tissue sections, using FL-393 antibody (Santa Cruz Biotechnology) on biopsy specimens of 60 oral SCC patients with tobacco and betel quid chewing habit and 10 controls. Only strong brown nuclear staining was considered positive and p53 scoring was done in accordance to Hall and Lane (1994) and Chiang et al., (2000). The scores were expressed as the percentage of positive cases in each category and the percentage of p53 positivity in each case.

In control specimens who were taken from normal individuals, there was no expression
of p53 protein. This can be attributed to fact that p53 in its wild form has very short life time (16-20 min) and can not be detected by immunohistochemistry. In present study, increased percentage of positive cases as well as mean p53 percent positivity was observed with increasing grade of differentiation in oral squamous cell carcinoma (oral SCC) patients with tobacco and betel quid chewing habit. Thirty nine oral SCC cases (65%) showed positive p53 expression and mean positivity was $26.46 \pm 23.90$.

Many previous studies have reported similar positivity in oral SCC patients with tobacco and betel quid chewing habit. Agarwal et al., (1999) reported that 65.3% of cases of oral SCC with tobacco and betel quid chewing habit showed p53 positivity while Pande et al., (2002) reported $65\% \left( \frac{69}{105} \right)$ positivity, Jie Xu et al., (1998) reported 59% $\left( \frac{20}{34} \right)$ positivity and Kaur et al., (1998) reported 70% $\left( \frac{102}{145} \right)$ positivity respectively in patients with tobacco and betel quid chewing habit. However lower values were observed by Kuttan et al., (1995), Baral et al., (1998), Thongsukai et al., (2001) who reported $\left( \frac{13}{23} \right)$ 56.5%, $\left( \frac{22}{48} \right)$ 45.8%, and $\left( \frac{132}{156} \right)$ 38.5% positivity respectively but all researchers reported increase in number of positive cells.

In our study, we further investigated the expression of p53 in various sites of oral cavity. p53 expression was more frequently seen in gingivia ($\frac{1}{1}$, 100%), floor of mouth
Studies which have correlated the expression of p53 with site of incidence in oral cavity. Kaur et al., (1998) found that there was no association between p53 expression and primary site of cancer (p=0.08). Similarly Claudia et al., (2006) found no association between p53 expression and different oral sites (p=0.53). In our study, expression of p53 was not found to be associated with site of oral cancer (p=0.964) thus supporting the earlier studies that p53 expression is independent of site of incidence of oral cancer.

The complete absence of p53 positivity in some squamous cell carcinomas was explained by Nylander et al., (2000), as the tumors completely lacking detectable p53 could either comprise of wild tumor protein or have a change in function in TP53 gene resulting in production of a truncated, non-functional and non detectable protein.

The relationship between p53 expression and tumor grade was also evaluated in our study. An increased positivity with increasing grade was observed in the present study. The difference was found to be significant between well differentiated (20.0 ± 19.32) and poorly differentiated (47.5 ± 31.38) oral SCC, p=.001. However no statistical significance (p=0.27) was observed in p53 positivity among poorly differentiated SCC (p53 positivity = 47.5 ± 31.38) and moderately differentiated SCC (p53 positivity = 29.33 ± 23.50), as well as between well differentiated SCC (p53 positivity = 20 ± 19.32) and moderately differentiated SCC (p53 positivity = 29.33 ± 23.50), p value =0.27.
Although most of the published data have shown no positive relationship between p53 expression and histological grading of oral SCC (Chiang et al., 1999; Saranath et al., 1999. Kerdpon et al., 2001). However, some studies have demonstrated a positive correlation between p53 expression and high grade of malignancy (Kaur et al., 1998). In our study, significant association was found between p53 positivity and degree of differentiation of tumors (p= .029). Similar to results in our study, Zariwala et al., (1994) and Shintani et al., (1995) found a tendency towards higher incidence of p53 positivity in poorly differentiated oral carcinomas.

Cyclin D1 Expression

Cyclin D1 gene encodes a protein that is a cell cycle regulator (Hunter et al., 1994). The Cyclin D1 gene (CCND1, bcl-1 or PRAD1) located on chromosome 11q 13 (Jaun Carlos et al., 2002) encodes a protein that forms a complex with Cyclin dependent Kinases, CDK4 and CDK6. Cyclin D-CDK4 and CDK6 complexes phosphorylate Rb (Retinoblastoma) protein during the G1-S transition which leads to their dissociation from the EF2 transcriptional factor and the initiation of DNA replication (Michalides RJAM et al., 1999; Kudo et al., 2000). Cyclin D1 overexpression, either by amplification or transcriptional upregulation, shows accelerated G1 progression and cell enters in the S phase, with lower cell dependence on growth factors for proliferation (Kuo MY et al., 1999).

Immunohistochemical studies of cyclin D1 expression in SCC of oral cavity has shown overexpression of cyclin D1 protein (Akerall JA et al., 1997; XU J et al., 1998; Lam KY
et al., 2000). In present study, immunohistochemical studies for cyclin D1 expression was done on formalin fixed and paraffin embedded tissue sections, using H-295 antibody (Santa Cruz Biotechnology) on biopsy specimens of 60 oral SCC patients with tobacco and betel quid chewing habit and 10 controls. Only strong brown nuclear staining was considered positive and the scores were expressed as the percentage of positive cases in each category and the percentage of Cyclin D1 positivity in each case.

In control specimens who were taken from normal individuals, there was no expression of Cyclin D1 protein. In present study, an increased percentage of positive cases as well as mean cyclin D1 positivity was observed in oral SCC patients with tobacco and betel quid chewing habit. Thirty five oral SCC (58.33%) cases showed positive Cyclin D1 expression and mean positivity was 22.16 ± 22.18.

Many previous studies have reported similar positivity in oral SCC patients. Arora et al., (2004) reported that 61% of cases of betel related oral SCC showed Cyclin D1 positivity while Lam et al., (2000) reported 63% positivity. Similarly Staibano et al., (1998) reported 60% positivity and Gimenez-conti IB et al., (1996) reported 61% positivity for Cyclin D1 in oral SCC patients. Angadi et al., (2007), Akervell et al., (2002), Kuo et al., (1999) and Van Oijen et al., (1998) have observed higher cyclin D1 positivity in oral SCC patients and have reported 70.7%, 78%, 83% and 69% positivity respectively. However lower values were observed by koontongkaew et al., 2000, Takes et al., (1998); Xu J et al., (1998); Akervall et al., (1997), Michalides et al., (1995) who
reported 39.62%, 29%, 38%, 43% and 33% positivity respectively for Cyclin D1 in oral SCC patients.

In our study, we further investigated the expression of Cyclin D1 expression in various sites of oral cavity. Cyclin D1 expression was more frequently expressed in hard palate, buccal mucosa and lip and less frequently in tongue and floor of mouth. There are only few studies that have described the expression of Cyclin D1 in various sites of oral cavity in oral SCC patients. Akervell et al., (1997) and Xu J et al., (1998) reported that expression of Cyclin D1 in oral SCC patients was more frequently seen in sites like tongue and retromolar region. In our study, Cyclin D1 expression was more frequently expressed in hard palate \( \left( \frac{3}{4} \cdot 75\% \right) \), buccal mucosa \( \left( \frac{21}{31} \cdot 67\% \right) \) and lip \( \left( \frac{3}{5} \cdot 60\% \right) \). The correlation between Cyclin D1 expression and primary site of oral cancer was also evaluated in our study. It was found that there was no significant association between Cyclin D1 expression and primary site of oral cancer \( (p=0.528) \). Similar results were reported by various studies \( (Kuo et al., 1999; Carlos et al., 2002) \) who found no association between Cyclin D1 expression and primary site of oral cancer.

The relationship between Cyclin D1 expression and tumor grade was also evaluated in our study. An increased positivity with increasing grade was observed in the present study. The difference was found to be significant between well differentiated SCC (Cyclin D1 positivity=16.61 ±17.89) and moderately differentiated SCC (Cyclin D1 positivity= 24.38±21.93, \( p=.002 \)) as well as between well differentiated SCC (Cyclin D1 positivity=16.61 ±17.89) and poorly differentiated SCC (Cyclin D1...
positivity=37.0±32.51, p=.043). Similarly statistically significant difference was observed between moderately differentiated SCC (Cyclin D1 positivity=24.38±21.93) and poorly differentiated SCC (Cyclin D1 positivity=37.0±32.51, p=.043). Although most of published data have shown no positive relationship between Cyclin D1 expression and histological grade of oral SCC (Kuo MY et al., 1999; Wu M et al., 2002; Neves Adac et al., 2004) but Angadi et al., (2007) have observed positive correlation between Cyclin D1 expression and histological grade of oral SCC. In our study, we found no significant association between Cyclin D1 positivity and degree of differentiation of tumor (p=0.138) in oral cancer patients with tobacco and betel quid chewing habit. Further in our study, we found a tendency towards higher incidence of Cyclin D1 positivity with high grade of differentiation of tumors. Similar results were reported by Lam KY et al., (2000) who found that Cyclin D1 expression was more positive in high grade lesions.

hOGGI Genotyping

Molecular cloning of a human counterpart of yeast OGGI paved way for possible application of hOGGI variants as genetic markers for individual susceptibility to various cancers (Aburatani et al., 1997; Radicella et al., 1997). The hOGGI gene maps to 3p25 chromosomal region and encodes a DNA glycosylase/AP-lyase that catalyzes removal of 8-OH-dG adducts as part of the base excision repair pathway (Boiteux et al., 2000; Sunaga et al., 2001). 8-OH-dG is one of major forms of DNA adducts induced by oxidative damage and increased 8-OH-dG formation in DNA is likely to be involved in mutagenesis and carcinogenesis (Cheng et al., 1992; Kamiya et al., 1992). The hOGGI
gene is expressed as 12 alternatively spliced isoforms with only the $\alpha$-form containing a nuclear localization signal (Shinmura et al., 2000) while $\beta$-hOGGI is targeted to mitochondria. Although no differences in catalytic activities were observed between the 326 Cys and 326 Ser variants in one study (Dherin et al., 1999), the hOGGI protein encoded by wild type Ser326 allele exhibited substantially higher DNA repair activity than the 326Ser variant in an in vitro E.coli complementation activity assay (Kohno et al., 1998).

At least 10 polymorphism of hOGGI have been identified, one of which is C→G at bp (C1245G) in the $\alpha$ specific exon 7 that causes an amino acid substitution from Ser to Cys in codon 326 (Ser 326 Cys), potentially resulting in functional alteration (Kohno et al., 1998). In the present study, the association between Ser$^{326}$ Cys polymorphism and oral cancer risk was evaluated in tobacco and betel quid chewers. The study consisted of 100 oral cancer patients with tobacco and betel quid chewing habit and 150 controls. In this study we have evaluated the risk of combined variant genotype (Ser/Cys+Cys/Cys) versus wild type homozygote (Ser/Ser) genotype to evaluate the association between Ser$^{326}$ Cys polymorphism and oral cancer risk.

The epidemiological studies that have evaluated the association between polymorphism at codon 326 of hOGGI and cancer risk have reported conflicting results. Elahi et al., (2002), Marchand et al., (2002), Xu et al., (2002), Cho et al., (2003) and Jiao et al., (2007) have reported that Ser$^{326}$Cys polymorphism is associated with risk of oropharyngeal and laryngeal, lung, prostrate, nasopharyngeal and gallbladder cancers.
respectively. While Choi et al., (2003), Zhang et al., (2004), Monteiro et al., (2005), Poplawski et al., (2006) and Park et al., (2007) have reported that there is no association of ser$^{326}$Cys polymorphism with breast, head & neck, laryngeal, gastric and colorectal cancers respectively.

Sugimura et al., (1999) have reported that there is no association of hOGGI Ser$^{326}$Cys polymorphism with lung cancer susceptibility when different types of lung cancer cases were taken together but hOGGI Ser$^{326}$Cys polymorphism is independently associated with increased risk of lung squamous cell carcinoma (OR=3.01, 95% CI=1.33-6.83) and non-adenocarcinoma (OR=2.18, 95% CI=1.05-4.54).

Kim et al., (2003) and Hashimoto et al., (2006) have reported that there is no association of Ser$^{326}$Cys hOGGI polymorphism with colon and head and neck carcinomas respectively but positive association is found between Ser$^{326}$Cys polymorphism in heavy smokers, in both colon (OR=2.75, 95% CI=1.07-7.53) and head and neck (OR=8.1, 95% CI=1.06-61.73) carcinomas. Similarly Takezaki et al., (2002) have reported that there is no association of Ser$^{326}$Cys polymorphism with stomach cancer but the association is significant in alcohol drinkers. These findings suggest that hOGGI ser$^{326}$Cys polymorphism may alter the impact of some environmental factors on cancer development. In our study, we have observed that Ser$^{326}$Cys polymorphism is significantly (p=0.00) associated with oral cancer risk in tobacco and betel quid chewers.

Most of the studies have reported that Ser$^{326}$Cys polymorphism increases the risk of cancer while one study has reported decrease in cancer risk. Increase in lung cancer risk
due to hOGGl ser^{26}Cys polymorphism has been reported by Wikman et al., (2000) [OR=2.2, 95% CI=0.4-11.8] and Le Marchand et al., (2002) [OR=2.1, 95% CI=1.2-3.7] respectively. Similarly Cho et al., (2003), Chen et al., (2003) and Xing et al., (2001) have reported increase in nasopharyngeal [OR=3.0, 95% CI=1.0-8.8], prostate cancer [OR=2.1, 95% CI=1.2-3.8] and esophageal cancer [OR=1.9, 95% CI=1.3-2.6] respectively. But Hansen et al., (2005) have reported that hOGGl ser^{26}Cys polymorphism is associated with lower risk of colorectal cancer (OR=0.56, 95% CI=0.33-0.95). In our study, we observed that hOGGl Ser^{26}Cys polymorphism increases the risk of oral cancer in tobacco and betel quid chewers of northern India (OR=2.3, 95% CI=1.5-3.61). Thus in our study we have observed positive correlation between Ser^{26}Cys polymorphism and oral cancer risk. We have found that Ser^{26}Cys polymorphisms lowers DNA repair ability in tobacco and betel quid chewers which results in increase in risk of oral cancer in this epidemiologically distinct population.

XRCCI Genotyping

Human cancer can be initiated by DNA damage caused by ultraviolet rays, ionizing radiation and environmental chemical agents. To safeguard the integrity of genome, humans have developed a set of complex DNA repair systems. Among the five main DNA maintenance mechanisms operating in mammals, base excision repair is the primary guardian against damage where non bulky base adducts produced by methylation, oxidation, reduction or fragmentation of bases by ionizing reduction or oxidative damage are removed (Yu et al., 1999). Therefore base excision repair is a universal event in the cells and is relevant for preventing mutagenesis.
XRCCI, one of more than 20 genes that participate in base excision repair pathway encodes a scaffolding protein that functions in the repair of single strand breaks, the most common lesion in cellular DNA (Caldecott et al., 1995). Both biological and biochemical evidence indicate a direct role for XRCCI in base excision repair because it interacts with complex of DNA repair proteins, including poly (ADP-ribose) polymerase DNA ligase III, and DNA polymerase β.

Mouse XRCCI -1- Knockout mutation is lethal and mutation in XRCCI results in an increased sensitivity to these agents and decreased genetic stability, including increased frequencies of spontaneously or induced chromosome translocations or deletions (Thompson et al., 2000). There are a total of eight non synonymous coding single nucleotide polymorphism in XRCCI, three of which are common and lead to amino acid substitutions in XRCCI at codon 194 (Exon 6, base C to T, amino acid Arg to Trp), codon 280 (Exon 9, base G to A, amino acid Arg to His) and codon 399 (Exon 10, base G to A, amino acid Arg to Gln).These three polymorphism occur at residues that are identical in humans, hamster and mouse suggesting that these amino acid are evolutionary conserved (Shen et al., 1998; Lamerdin et al., 1995). Arg 399 Gln polymorphism is located in the region of BRCT-1 interaction domain of XRCCI within a poly (ADP-ribose) polymerase binding region and has been extensively studied as 399 Gln variant allele is most frequently found. XRCCI Arg\textsuperscript{194}Trp and Arg\textsuperscript{280}His variants occur in the newly identified proliferating cell nuclear antigen binding region (Fan et al., 2004).
There are relatively few studies conducted to examine the association between Arg\textsuperscript{280}His variant and cancer risk and only one study evaluated the association of Arg\textsuperscript{280}His polymorphism and altered DNA adducts (Zhibin HU et al., 2005). In the present study the association between Arg\textsuperscript{280}His polymorphism and oral cancer risk in tobacco and betel quid chewers was evaluated. The study consisted of 100 oral cancer patients with tobacco and betel quid chewing habit and 150 controls. Because of the rare variant allele frequencies of Arg\textsuperscript{280}His polymorphism, we evaluated only the risk of combined variant genotypes (His/His+Arg/His) versus wild type homozygote (Arg/Arg).

The epidemiological studies that have evaluated the association between polymorphism at codon 280 of XRCCI and cancer risk have reported conflicting results. Ratnasingha et al., (2001), Carla et al., (2002), Moullan et al., (2003), Cho et al., (2003), and Hao et al., (2004) have shown that Arg\textsuperscript{280}His polymorphism is associated with risk of lung, prostrate, breast, nasopharyngeal, and esophageal cancers respectively. Other studies have shown that there is no association of Arg\textsuperscript{280}His polymorphism with risk of oral cancer (Ramachandran et al., 2006; Majumder et al., 2007) breast cancer (Metsola et al., 2005), lung cancer (Mishra et al., 2003; Scheneider et al., 2005) and esophageal cancer (Lee et al., 2001) respectively. In a meta-analysis of 38 case control studies about association of XRCCI polymorphisms with cancer risk, Hu et al., (2005) observed that Arg\textsuperscript{280}His polymorphism is associated with cancer risk and individuals with the variant genotypes (His/His+His/Arg) had a borderline significantly increased cancer risk, compared with the individuals with wild type genotype (OR=1.19; 95% CI=1.00-1.42).
our study, we have observed that XRCC1 Arg280His polymorphism is associated with oral cancer risk in tobacco and betel quid chewers (p=.015).

Whether the Arg280His polymorphism increases or decreases the cancer risk, there are conflicting results. Some studies have reported that Arg280His polymorphism increases the risk of cancer while others studies have reported negative association between Arg280His polymorphism and cancer risk. Ratnasingha et al., (2001) have observed that XRCC1 Arg280His polymorphism increases the risk of lung cancer (OR=1.8, 95% CI=1.0-3.4) while Moullan et al., (2003) reported that XRCC1 Arg280His polymorphism increases the risk of breast cancer (OR=1.8, 95% CI=1.07-3.05). Similar increase in prostrate cancer risk was observed by Carla et al., 2002 (OR=1.5, 95% CI=0.7-3.5). But Cho et al., (2003) have reported that Arg280His polymorphism decreases the risk of nasopharyngeal cancer (OR=0.64, 95% CI=0.43-0.96). Similarly Hao et al., (2004) have reported that Arg280His polymorphism decreases the risk of esophageal squamous cell carcinoma (OR=0.79, 95% CI=0.56-1.11). In our study, we observed that Arg280His polymorphism increases the risk of oral cancer in tobacco and betel quid chewers of northern India (OR=1.63, 95% CI=1.1-2.413). Thus in our study we have observed positive correlation between Arg280His polymorphism and oral cancer risk. We have found that Arg280His polymorphism may result in lower DNA repair ability which results in increase in oral cancer risk in tobacco and betel quid chewers.
CYP2E1 Genotyping

Both genetic and environmental factors are involved in the development of cancer. The environment-gene interaction on carcinogenesis has been well demonstrated by phase-1 & phase-II enzymes that are involved in the metabolism of carcinogens. An individual difference in the susceptibility to chemical carcinogens is one of the most important factors in the estimate of risk of cancer. Most chemical carcinogens require metabolic activation by phase I enzymes (cytochrome p-450) and detoxification by conjugation via the various phase II enzymes (epoxide hydrolase, N-acetyl transferase etc.) Thus, the coordinate expression and regulation of phase I and phase II drug metabolizing enzymes and their metabolic balance may be an important host factor in determining whether exposure to carcinogens results in cancer or not. The phase I enzymes, CYP, activate many environmental procarcinogens by adding or exposing their functional groups(Kawajiri et al., 1991)

CYP2E1 is ethanol induced phase 1 enzyme which has received much attention because of potentially important toxicological roles of this enzyme. Human CYP2E1 gene is highly conserved compared with other human p450 genes with products active in metabolism of xenobiotics. This enzyme is localized mainly in the liver but is also expressed and induced in the brain after ethanol treatment or ischemia and is also expressed at significant levels in human esophagus and other extrahepatic tissues (Nakajima et al., 1996; Lechevrel et al., 1999).
The physiological role of this enzyme seems to be connected mainly with the conversion of acetone to gluconeogenetic precursors. Among more than 70 different substrates specifically metabolized by this enzyme are most of the organic solvents, drugs (paracetomol, chlorzoxazone) and are several potential carcinogens (nitrosamines, benzene, aniline) which are transferred to their active forms. In addition, CYP2E1 causes oxidative stress and the oxygen radicals generated by this enzyme are able to initiate NADPH-dependent lipid peroxidation with the concomitant production of cytotoxic aldehydes (Hu et al., 1997). Although certain chemicals and physiological status can induce the activity of CYP2E1, considerable inter-individual variation has been observed before and after induction, suggesting that the variation may be determined by genetic factors in the locus. Thus any functional polymorphism of this enzyme might be an important factor in determining the relative risk of alcohol mediated hepatotoxicity, any form of cancer or susceptibility for drug toxicity.

Human CYP2E1 gene is polymorphic at several sites, the most important being PstI site, RsaI site and DraI site. PstI and RsaI site are present in the 5' flanking region while DraI site is present in intron 6. Phenotypic studies using the drug chlorzoxazone as a metabolic probe have shown that individuals with the variant RsaI allele have a lower CYP2E1 activity and that enzyme activity is less inducible by ethanol (Lucas et al., 1995). A polymorphism of CYP2E1 detected by RsaI restriction enzyme may be functionally important because it is located in a putative binding site for the transcriptional factor HNF 1 and has been associated with higher levels of CYP2E1 transcription (London et al., 1996).
In the present study, the association of Rsal polymorphism and Dral polymorphisms of the CYP2E1 gene with oral cancer risk in tobacco and betel quid chewers was evaluated. The study consisted of 100 oral cancer patients with tobacco and betel quid chewing habit and 150 controls. To find any association between these CYP2E1 Polymorphisms and oral cancer risk, we evaluated the risk of combined variant genotypes 

\[
\left( \frac{A}{C} \text{ for Rsal}, \frac{D}{C} \text{ for Dral} \right) \quad \text{versus} \quad \text{wild type homozygote}
\]

\[
\left( \frac{A}{A} \text{ for Rsal}, \frac{D}{D} \text{ for Dral} \right)
\]

genotypes.

Rsal polymorphism present in the 5' flanking region of CYP2E1 is most extensively studied polymorphism of CYP2E1 but the studies that have evaluated association of Rsal polymorphism of CYP2E1 and cancer risk have reported conflicting results. Hildesheim et al., (1995), Ladero et al., (1996), Marchand et al., (1998), Tan et al., (2000) and Sugimura et al., (2006) have reported that CYP2E1 Rsal polymorphism is associated with nasopharyngeal, liver, lung, esophageal and oral carcinomas respectively while Matthias et al., (1998), Watanabe et al., (1995), Ferreira et al., (2003), and Choi et al., (2003) have observed that there is no association of Rsal polymorphism with head and neck, lung, prostrate and breast carcinomas respectively. Choi et al., (2003) have reported that although there is no association between breast cancer risk and Rsal polymorphism but increased cancer risk is found in ever drinking women (OR=1.9, 95%, CI= 0.99-3.83) with C1 C2 or C2 C2 genotype compared to non-drinkers with CYP2E1 C1/C1 genotype.
Similarly Bouchardy et al., (2000) have reported that Rsal polymorphism is associated with risk of head and neck carcinoma (OR= 2.0, 95% CI= 1.0-3.5) but the risk is increased in drinkers (OR=5.8, 95% CI= 1.9-18.2). These findings suggest that gene-environment interactions play an important role in oral cancer. In our study, we have observed that CYP2E1 Rsal polymorphism is significantly (p=.001) associated with risk of oral cancer in tobacco and betel quid chewers.

Most of studies that have investigated association of Rsal polymorphism with cancer risk have observed increase in the risk of cancer while one study has reported decrease in cancer risk. Hildesheim et al., (1995) reported that individuals who have C2/C2 genotype were at increased risk for nasopharyngeal carcinoma (OR= 7.7, 95% CI= 0.87-6.8) compared to those who had C1/C1 genotype while Bouchardy et al., (2000) reported that subjects with C1C2 or C2C2 genotype have increased risk of oral cavity / pharyngeal cancer (OR= 2.6, 95% CI= 1.0-6.6) but Marchand et al., (1998) reported that Rsal homozygous variant genotype (C2/C2) was associated with 10 fold (OR= 0.1, 95% CI= 0.0-0.5) decrease in risk of overall lung cancer. We observed positive correlation between CYP2E1 Rsal polymorphism and oral cancer risk. Our findings support the hypothesis that environmental exposure to the carcinogens plays an important role in the etiology of oral cancer. In our study, we observed that Rsal polymorphism of CYP2E1 gene increases the risk of oral cancer in tobacco and betel quid chewers. The carriers of C variant allele (A/C or C/C) were at increased risk of oral cancer (OR=2.0, 95%CI=1.3-2.9).
The epidemiological studies that have evaluated the association of CYP2E1 Dra1 polymorphism with cancer risk have reported results which are inconclusive. Some studies have reported the association of CYP2E1 Dra1 polymorphism with cancer risk, while other studies have found no association. Hildesheim et al., (1995) Ferreira et al., (2003) and Sugimura et al., (2006) have reported that CYP2E1 Dra1 site polymorphism is associated with risk of nasopharyngeal, prostrate and oral cancers respectively. While Kato et al., (1994) and Matthias et al., (1998) have reported that there is no association of Dra1 polymorphism with lung and head and neck carcinomas respectively. Bouchardy et al., (2000) have further reported that risk of oropharyngeal cancer was increased in individuals with CC or CD variant genotypes as compared to other individuals (OR= 2.0, 95% CI= 1.0-3.9) but the risk was more increased in drinkers (OR= 5.8, CI= 1.0-3.5) suggesting that gene-environment interactions play a significant role in oral cancer. In our study, we have observed that CYP2E1 Dra1 polymorphism is significantly (p=.049) associated with risk of oral cancer in tobacco and betel quid chewers.

Most of earlier investigations that have reported association of CYP2E1 Dra1 polymorphism with cancer risk have observed increase in the risk of cancer while some studies have reported decrease in cancer risk. Hildesheim et al., (1995) have reported that subjects with CC variant genotypes were at increased risk of nasopharyngeal carcinoma (OR= 5.0, 95% CI= 0.95-1.6) while Ferreira et al., (2003) have reported that CYP2E1 Dra1 polymorphism is associated with increased risk of prostrate cancer (OR= 2.12, 95% CI= 1.11-4.05). However, Marchand et al., (1998) have reported that CC homozygous variant Dra1 genotypes was associated with 5 fold decrease in overall lung
cancer (OR= 0.2, 95% CI= 0.1-0.7) compared to corresponding homozygous wild type. In our study, we observed that Dral site polymorphism of CYP2E1 gene increases the risk of oral cancer in tobacco and betel quid chewers. The carriers of C variant allele (CC or CD) were at increased risk of oral cancer (OR=1.37; 95% CI=0.9-1.89). Thus in our study, we have observed positive correlation between CYP2E1 Dral polymorphism and oral cancer risk.