ABSTRACT

Cancer is one of the most dreaded diseases of mankind that causes alarming mortality and morbidity in humans. It has long been evident that cancer has a multi-factorial etiology and is a multi-stepped process involving initiation, promotion and tumor progression. Chemical carcinogens, physical agents, ionizing radiation, viruses and other agents have all been implicated, and clearly host factors are also involved, mainly via an immunological and/or genetic basis. Cancer-predisposing genes may act not only via immune surveillance systems affecting the host's ability to recognize and eliminate incipient tumors, but also may affect the ability to repair damage to DNA or might affect the rate of metabolism of pre-carcinogens or carcinogens.

Oral cancer is sixth most common cancer worldwide and third most common cancer in developing countries accounting for about up to 40% of all cancers. Incidence of oral cancer is increasing day by day due to more intake of various forms of tobacco and alcohol drinking, which are considered to be the two most important etiological factors in the development of oral cancer. It is estimated that 75-90% of all head and neck cancers are caused due to the tobacco use and tobacco users are between 20-40 times more likely to develop head and neck cancer than non consumers, depending upon the amount of use as well as the age, sex and race of the user. Tobacco may be taken in various ways like smoking, and chewing. The most common form of tobacco chewing in India is betel quid. The 'quid' for chewing consists of areca nut and pieces of unripe betel fruit or areca nut wrapped in a piece of betel leaf together
with white or red lime. Betel quid chewing has a strong association with oral cancer which arises predominantly from surface epithelium with evolution from early premalignant lesions. Oral SCC arise as a consequence of multiple molecular events induced by the effects of various carcinogens from habits such as areca nut and betel quid chewing, influenced by environmental factors, possibly viruses in some instances, against a background of inheritable resistance or susceptibility. An individual difference in the susceptibility to chemical carcinogens is one of the most important factors in the estimate of risk of human cancer as some patients appear susceptible because of inherited trait(s) in their ability or inability to metabolize carcinogens or pro-carcinogens, possibly along with an impaired ability to repair DNA damage. Oral carcinogenesis is a multi-step process in which 6-10 genetic events lead to the disruption of the normal regulatory pathways that control basic cellular functions. In recent years, several alterations in the expression of tumor suppressor genes and oncogenes in the development of Oral Squamous Cell Carcinoma (OSCC) have been described. Keeping in view above facts, the present study was done to investigate the expression of p53 (product of tumor suppressor gene) and cyclin D1 (product of cell cycle regulator gene) as well as to determine the frequency of polymorphism in DNA repair enzymes hOGG1, XRCCI and xenobiotic metabolizing enzyme CYP2E1 in oral carcinoma patients with tobacco and betel quid chewing habit.

The present study comprised of 250 human subjects with 100 oral cancer patients and 150 controls. Biopsy specimens were taken from 60 patients and 10 controls for
p53 and Cyclin D1 expression studies. Blood samples were collected from all the subjects for gene polymorphism studies. Immunohistochemistry was done to study the protein expression studies. For gene polymorphism studies, DNA was isolated from blood samples and quantified by agarose gel electrophoresis. Genotyping of DNA repair genes (hOGGI Ser^{326}Cys and XRCCI Arg^{280}His) and xenobiotic metabolizing gene (CYP2E1 Rsal and Dral sites) was performed using PCR-RFLP technique. For statistical analysis, computer programme SPSS (version 13) was used.

In the present study, out of the total 100 patients studied, 75 patients (75%) were males and majority of patients were in 50-75 age group and complained of dysphagia. The most common site of incidence of oral cancer in tobacco and betel quid chewers was buccal mucosa.

It was found that there was no p53 or Cyclin D1 expression in normal tissues while in oral SCC patients with tobacco and betel quid chewing habit, the percentage of positive cases as well as p53 or Cyclin D1 positivity showed an increase with increasing grade of SCC. The expression of p53 was significantly associated with histological grade in oral cancer in tobacco and betel quid chewers while no such association was found between Cyclin D1 expression and histological grade. Statistically significant difference was observed in Cyclin D1 positivity between well differentiated SCC and moderately differentiated SCC as well as between well differentiated SCC and poorly differentiated SCC. Similarly significant difference in Cyclin D1 positivity was observed between moderately differentiated SCC and
poorly differentiated SCC but in case of p53 expression, statistically significant
difference in p53 positivity was observed only on comparing well differentiated
SCC with poorly differentiated SCC.

Expression of oncoproteins was not similar in different sites of oral cavity. p53
expression was more frequently seen in gingivia, floor of mouth, tongue, and buccal
mucosa while Cyclin D1 expression was more frequently seen in hard palate, buccal
mucosa and lip.

The polymorphism studies of DNA repair genes (XRCCI Arg^{280}His and hOGGI
Ser^{326}Cys) and xenobiotic metabolizing gene (CYP2E1 Dral and Rsal sites) revealed that
these polymorphisms were significantly associated with risk of oral cancer in tobacco and
betel quid chewers. The individuals with variant XRCCI Arg^{280}His and hOGGI Ser^{326}Cys
genotypes were at increased risk of oral cancer as compared to individuals having wild
type homozygous genotypes. Thus XRCCI Arg^{280}His and hOGGI Ser^{326}Cys
polymorphisms lower DNA repair ability in tobacco and betel quid chewers which results
in increase in risk of oral cancer in this epidemiologically distinct population. The
individuals with the variant genotype of xenobiotic metabolizing gene (CYP2E1 Rsal
and Dra1 sites) were at increased risk of oral cancer as compared to individuals having
wild type genotypes thus supporting the hypothesis that environmental exposure to the
carcinogens plays an important role in the etiology of oral cancer.
In conclusion, it was found that there was over expression of tumor suppressor gene product, p53 and cell cycle regulator gene product, cyclin D1 in oral SCC patients from northern India with tobacco and betel quid chewing habit. It was also observed that XRCC1 Arg^{280}His, hOGGI Ser^{326}Cys, CYP2E1 Dral and CYP2E1 Rsal polymorphisms were closely associated with high risk of oral cancer in this epidemiologically distinct population.