RESEARCH COMMUNICATION

# Role of Tobacco in the Development of Head and Neck Squamous Cell Carcinoma in an Eastern Indian Population

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## Abstract

**Head and neck squamous cell carcinoma (HNSCC) accounts for about 30-40% of all cancer types in India and the subcontinent in general. HNSCCs are primarily not hereditary, but rather a disease of older and middle aged adults. Many etiological factors like tobacco, alcohol and HPV infection are known to play important roles. Eastern India, particularly Kolkata, has a population heavily exposed to various types of smoked and smokeless tobacco, with only limited exposure to alcoholic beverages. Since there have been no previous epidemiological studies on tobacco as the main risk factor for head and neck carcinogenesis in Kolkata, we here carried out a hospital based case control study in the city and its adjoin regions. Data from 110 patients diagnosed with HNSCC and a similar number of matched control samples were analyzed using chi-square ($\chi 2$) test. Survival status of the patients was also analyzed using the Kaplan-Meier method. A tobacco habit was significantly correlated with the incidence of HNSCC and persons with current addiction had a 2.17 fold increased risk of cancer development. Dose-response relationships were seen for the frequency (p=0.01) and duration (p=0.02) of tobacco exposure with the risk. No significant difference in impact was found with smoked as opposed to smokeless tobacco in the development of the disease. Among HNSCC patients, significant poor survival in cases with tobacco habit than in those with no addiction and in cases with >10 years of addiction than in those with ≤ 10 years of addiction. Our data suggest that tobacco in both smoked and smokeless forms is the most important risk factor for both development and prognosis of HNSCCs and may be a major source of field cancerization on the head and neck epithelium in the eastern Indian population.**

**Key Words:** Head and neck squamous cell carcinoma - epidemiology - tobacco - Eastern India

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## Introduction

Head and neck squamous cell carcinoma (HNSCC) is an epithelial malignant disease arising from the mucosa of the upper aerodigestive tract (oral cavity, larynx, oropharynx and hypo pharynx) (Nagai, 1999). It is the fifth most common cancer worldwide (Parkin et al., 1993). In some parts of the world, these cancers represent the most common malignancies found in men. For example, in South-Central Asia (India, Pakistan, Bangladesh, Iran, Afghanistan, and the Central Asian Republics), that accounts for one fifth of the world's population, head and neck cancer accounted for approximately 1,55,400 new cases of cancer in 1990 (17% of all cancers and 25% of all cancers occurring in men) (Parkin et al., 1999). Although in most regions of the world, laryngeal and naso-pharyngeal cancers account for between one third and one half of all head and neck malignancies, in South-Central Asia, 80% of head and neck cancers are found in the oral cavity and oropharynx (Sankaranarayanan et al., 1998; Parkin et al., 2005). Cancers of the oral cavity accounted for 2,74,000 cases in 2002, with almost two-third of them in men (Parkin et al., 2005). In most regions of India, cancer of the oral cavity is the leading malignancy diagnosed in men, accounting for up to 20% of cancers in men, and oral cavity cancer is the third most common cancer in Indian women (Sen et al., 2002).

HNSCCs are primarily not hereditary, but a disease of older and middle aged adults with a long history of tobacco smoking (Cann CI et al., 1985). Tobacco smoking has long and consistently been identified as the major risk factor for HNSCC. Actually head and neck regions like oral cavity, larynx, oropharynx and hypopharynx, as directly exposed to tobacco smoke, have a relatively higher risk of developing cancer than other regions like the pancreas and urinary bladder, for example (Vineis et al., 2004).

A recent report found a 20 fold increased risk of oral and pharyngeal cancer below age 46 for heavy smokers, and a 5-fold increase for heavy drinkers; the combination of heavy smoking and drinking led to an almost 50 fold increased risk (Rodriguez et al., 2004). Oral cancer is also increased by tobacco chewing (Cullen et al., 1986; Chen et al., 1990) and is prevalent in communities such as India

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and Indonesia where chewing of betel quid – betel nut (Areca catechu) wrapped in betel leaf, sometimes mixed with lime - is prevalent (Muir & Kirk, 1960). Use of snuff also increases the risk of developing oral cancer (Winn et al., 1981).

Population based screening and early detection programs are rare or nonexistent and in spite of surgical advances, these cancers remain a disfiguring disease associated with a relatively low survival rate (Berrino et al., 1998; Forastiere et al., 2001). But despite the clear role of tobacco in the etiology of HNSCCs, this association has not been assessed clearly among the eastern Indian population. In the present study, we therefore explored the role and impact of tobacco in head and neck carcinogenesis and for this, data were analyzed in a large, hospital-based, case control study of head adn neck cancer. The aim was to establish the association of tobacco with development of HNSCCs, focusing on: a) the tobacco habit as a major risk factor; b) the contribution of various characteristics related to tobacco habit (i.e. dose and duration; smoking and chewing); c) the survival status.

## Materials and Methods

*Samples:*

We have categorized our samples into two broad groups. Case samples were patients, histologically diagnosed in the participating hospital with invasive cancer of head and neck region. A total of 110 cases were studied for our analysis. Equal numbers of control samples were identified from Kolkata matched with the cases by gender age and also the locality (out of total 465 HNSCC patients visited to the hospital for treatment between 1998 to 2006, 110 from the greater Kolkata region were only selected as case samples for our study). Individuals with an admission diagnosis related to tobacco consumption were not accepted as controls.

*Sources:*

The detailed clinical history of the case samples was collected from the record section of the Chittaranjan National Cancer Institute, Kolkata. The written consent of the hospital authority was taken prior to our study. The control samples were selected from both North and South Kolkata. Normal healthy individuals willing to participate in our study were selected randomly at the first round. Finally among the participating individuals, control samples were selected on the basis of sex, age group and other criteria.

*Methodology:*

Questionnaire: All the controls were interviewed properly to collect the necessary data for our study. The questionnaire elicited detailed information on demographic, educational and socioeconomic characteristics, on the characteristics of tobacco habit [i.e.- type of the tobacco habit (smoking/chewing/both smoking and chewing), duration of each habit and also age of starting, average number of cigarettes smoked / average number of chewing per day etc.], tobacco related illness and also the family history of cancer (If any). In our study, current smokers/chewers were defined as those having the habit at the time of interview, as well as those stopping the habit within the year before the date of the interview.

**Table 1. Clinicopathologic Characteristics (110 cases)**

| Characteristics | No. of patients | | Median age | Age range |
|---|---|---|---|---|
| Primary Site | | | | |
| Orofacial (28) | Maxilla | 15 | 49 | 32-76 |
| | Mandible | 12 | | |
| | Nasal cavity | 1 | | |
| Oralcavity (60) | BM | 23 | 48 | 30-74 |
| | Tongue | 16 | | |
| | Alveolus | 8 | | |
| | Tonsil | 6 | | |
| | Palate | 5 | | |
| | Lip | 2 | | |
| Larynx (15) | | 15 | 58 | 50-75 |
| Thyroid (7) | | 7 | 55 | 40-70 |
| Tumor Stage | Stage-I | 15 | 60 | 58-62 |
| | Stage-II | 28 | 46 | 40-76 |
| | Stage-III | 33 | 50 | 39-75 |
| | Stage-IV | 34 | 54 | 45-70 |
| Tumor Differentiation | | | | |
| Well Differentiated | | 45 | 52 | 40-77 |
| Moderately Differentiated | | 40 | 50 | 41-65 |
| Poorly Differentiated | | 25 | 44 | 35-75 |
| Lymph Node Involvement | | | | |
| | No | 60 | 50 | 40-70 |
| | Yes | 50 | 52 | 45-76 |
| Gender | Male | 80 | 50 | 38-68 |
| | Female | 30 | 45 | 40-65 |

Statistical analyses: 2x2 Chi-square analysis was performed to determine the association of tobacco habit and HNSCC development. Chi-square for trends was also performed to determine the significance of various parameters of tobacco habit like dose, duration etc. For analysis of the survival status of the case samples, Survival curves were calculated according to Kaplan-Meier method. Post-operative overall survival was measured from the date of surgery to the date of last follow-up or death (up to 5 years). Probability value (P-value) $\leq 0.05$ was considered statistically significant. All the statistical analysis was performed using statistical program SYSTAT-9.0(Binary Semantics).

## Results and Discussion

*Subjects' Characteristics:*

The detail clinical histories of the case samples are presented in Table 1. Out of total 110 cases, 60 were from oral cavity (55%), 15 from larynx (14%), 28 from orofacial region (25%) and 7(6%) from thyroid. Among oral cavity, buccal mucosa seems to be the most commonly affected site (39%; 23/60) followed by tongue (26%; 16/60), alveolus (13%; 8/60), tonsil (10%; 6/60), palate (8%;5/60) and lip (3%;2/60). Among orofacial regions, maxillae and mandibles are the most common (53%; 15/28 and 43%; 12/28), however one nasal cavity tumor was also reported. All the tumors are of invasive category, no dysplasia has been recorded among the case samples. The tumors were clinically staged (I, II, III & IV) according to UICC TNM classification and the histopathological

**Table 2. Subject Details**

| | Cases | Controls | $\chi^2$ p-Value |
|---|---|---|---|
| Age Group | | | |
| 35-44 Years | 15 (14%) | 35 (32%) | 0.2 |
| 45-54 years | 49 (44%) | 30 (27%) | |
| 55-64 years | 35 (32%) | 30 (27%) | |
| ≥65 years | 11 (10%) | 15 (14%) | |
| Sex | | | |
| Male | 80 (73%) | 70 (64%) | 0.147 |
| Female | 30 (27%) | 40 (36%) | |
| Education | | | |
| Illiterate | 5 (5%) | 1 (1%) | 0.0001 |
| Primary | 15 (14%) | 5 (5%) | |
| Secondary | 50 (45%) | 30 (27%) | |
| ≥Higher | 40 (36%) | 74 (67% | |
| Religion | | | |
| Hindu | 55 (50%) | 60 (55%) | 0.6 |
| Muslim | 45 (41%) | 30 (27%) | |
| Christian | 10 (9%) | 20 (18%) | |
| Occupation | | | |
| Manual | 45 (41%) | 40 (36%) | 0.4 |
| Teacher/office worker | 15 (14%) | 25 | 23% |
| Business | 10 (9%) | 18 (16%) | |
| Retired | 30 (27%) | 20 | 18% |
| Others | 10 (9%) | 7 (7%) | |
| Tobacco History | | | |
| Nonaddicted | 58 (53%) | 76 (69%) | 0.027 |
| Addicted | 52 (47%) | 36 (31%) | |

**Table 3. Cancer Risk Relative to Smoking History**

| Variables | Cases | Controls | OR (95%CI) |
|---|---|---|---|
| Status of the tobacco habit | | | |
| Never addicted | 40 | 60 | 1 |
| Ex- addiction | 18 | 14 | 1.93 |
| Current addiction | 52 | 36 | 2.17 |
| p value for trend test | | | 0.0087 |
| Type of tobacco habit | | | |
| Smoked tobacco | 30 | 20 | 1 |
| Non smoked tobacco | 13 | 10 | 0.87 |
| Mixed | 9 | 6 | 1 |
| p value for trend test | | | 0.92 |
| Type of smoked tobacco habit | | | |
| Cigarette | 19 | 15 | 1 |
| Bidi | 20 | 10 | 1.58 |
| Cigar | 0 | 1 | 0 |
| p value for trend test | | | 0.7 |
| Type of Non smoked tobacco habit | | | |
| Khaini | 11 | 8 | 1 |
| Snuff | 8 | 6 | 0.97 |
| Gutkha | 3 | 2 | 1.02 |
| p value for trend test | | | 0.96 |
| Amount of tobacco intake (Avg. no of smoking/chewing/day) | | | |
| 1-10 | 22 | 22 | 1 |
| 11-20 | 16 | 12 | 1.33 |
| ≥21 | 14 | 2 | 7 |
| p value for trend test | | | 0.01 |
| Duration of tobacco intake (Avg. year of smoking/chewing) | | | |
| 1-10 | 19 | 20 | 1 |
| 11-20 | 22 | 14 | 1.65 |
| ≥21 | 11 | 2 | 5.79 |
| p value for trend test | | | 0.02 |

grades (WDSCC, MDSCC & PDSCC) were also assessed.

Table 2 shows the distribution of study participants according to their case control status, selected socio-demographic characteristics and main descriptive statistics of tobacco habit. Chi-square ($\chi 2$) tests were performed to determine whether the distribution of these factors was related to disease status. The cases were more likely to be in the 45-54 year age group while the controls were more likely to be in the youngest age group of 35-44 years. Males were the predominating sex among cases and controls. The level of education was higher among the controls than the cases: the highest percentages of cases were in the secondary education category. We did not find any correlation (Neither positive nor negative) between education and tobacco habit; however the chewers and bidi (raw tobacco) smokers mostly belonged to the no education and illiterate category (Data not shown). Most subjects were Hindu among both cases and controls but the percentage of Muslims was higher among cases and the percentage of Christians was higher among controls. The majority of the cases and controls held a manual occupation. Controls had a higher percentage of teachers and office workers.

*Effect of tobacco habit:*

The tobacco habit and its' related characteristics were stratified as the main potential risk factor by the disease status in Table-3. The overall prevalence of tobacco habit was higher among case subjects than among the controls. We explored the relationship of all the parameters of tobacco habit (dose or amount of intake per day, duration of the habit, type of tobacco etc.) and cancer risk. An increased risk for head and neck cancer was detected among the tobacco-addicted individuals compared to those with no addiction. Risk for HNSCC also increased with both dose and duration of the habit. No associations were found with age at start or age at quitting tobacco habit (Data not shown). Smokers of cigarettes with filters had the same risk as smokers of bidis and also the chewers.

Tobacco products contain a diverse array of chemical carcinogens that cause cancers of various types (Hecht, 2003). More than 60 known carcinogens have been detected in cigarette smoke and 16 in smokeless tobacco. Among these, tobacco specific nitrose amines (NNK, NNN etc), polycyclic aromatic hydrocarbons (benzo[a]pyrene), and aromatic amines seem to have important role as causes of cancer. Most carcinogens in tobacco undergo metabolic activation process initiated by cytochrome p450 enzymes (Part of normal mammalian systems designed to respond to the foreign compounds).

Metabolic activation makes the carcinogens electrophilic that now react with DNA to form DNA adducts. Cellular repair system remove DNA adducts and return DNA structure to its normal state, but if the adducts persist and escape repair, mutation arise. It has been established conclusively that DNA adducts of tobacco cause miscoding – most frequently G-T and G-A mutations. If these permanent mutations occur in crucial regions of oncogene like RAS and MYC or in tumor suppressor gene p53, CDKN2A, pRB, FHIT etc; this resulting disruption of cell cycle check points leading to loss of normal cellular growth control mechanism and development of cancer.

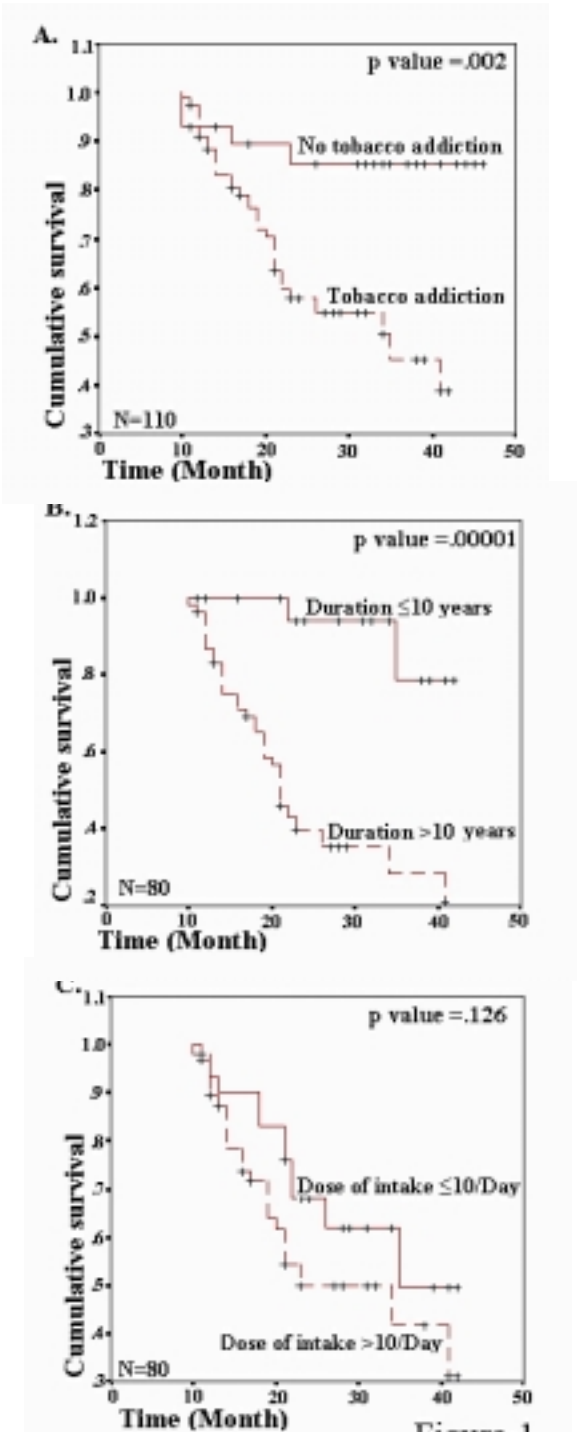


**Figure 1. Survival Curves Relative to Tobacco Exposure**

*Survival status of the case samples:*

The overall disease-free survival of the HNSCC patients was analyzed i.e. Patients died due to the disease or patients with recurrence of the disease at same or different site were considered as same category and designated by the value-"1"; while patients reported to be alive in the hospital follow-up record with no recurrence of the disease were designated by "0". Two approaches were taken to determine the association of tobacco (if any) with the poor survival of the HNSCC patients and also the bad prognosis of the disease.

A). Survival status of the patients not addicted to tobacco (designated by "0") Vs. those addicted to the tobacco (designated by "1"), (Supplement-1). As evident from the figure-1A, the tobacco addicted HNSCC patients showed significant poor survival/ bad prognosis of the disease compared to tobacco non-addicted patients.

B). Survival status among the tobacco addicted patients i.e. -a. Tobacco addicted HNSCC patients were grouped on the basis of duration of the habit (Patients addicted to tobacco for 10 years or less were designated by "0", while those for more than 10 years were designated by "1"), (Supplement-2A). Figure-2B clearly showed that duration of the tobacco habit was positively correlated with the poor patient survival and also the bad prognosis of the disease.

The addicted HNSCC patients were also grouped on the basis of daily tobacco intake (Patients having 10 or less bidi/cigarette/chewing habit were designated by "0" and those having more than 10, were designated by 1), (Supplement-2B). But surprisingly, the dose of the daily tobacco intake was shown to have no significant impact upon the survival status of the patients (Figure-1C).

At CNCI, the cancer patients receive a quality treatment. They visit to the outdoor first for the diagnosis of the disease. Spot admission on emergency bed depends upon the severity of the disease. In case of HNSCC, the disease is diagnosed by punch biopsy (in case of oral cavity), endoscopy based biopsy (in case of esophagus and larynx) or by FNAC /Fine Niddle Aspirate Cytology (in case of salivary gland, thyroid etc.). If the disease was diagnosed previously, slides are crosschecked in pathology department and the test is repeated if necessary. After that a medical board is constructed including the attending onco-surgon, radiotherapist and also chemotherapist to determine the proper treatment procedure. If the tumor remains confined to the primary site, then it's surgical removal followed by radiotherapy (by cobalt or Linac Accelerator) / chemotherapy is recommended. In general, surgery removes 70-80% of the tumor cells and the rests destroyed by ray / chemotherapeutic agents. For this the disease free survival of this category of patients should be higher compared to those in which the tumor cells get metastasizes from the primary site. But surprisingly we found poor survival/ recurrence in some patients instead of surgical removal of their tumor followed by chemo/ radiotherapy. About 90% of these patients were also addicted to tobacco for a long duration of time (>10 years). Actually x-rays/chemotherapeutic agents impose genotoxic stress upon the cell by creating irreversible DNA damage. This damage is sensed by some master genomic element like p53 that induces the cell to commit apoptosis. Tobacco containing carcinogens form DNA adducts and thus inducing mutation and other types of alterations of p53 and other crucial genetic elements during malignant transformation of a cell. As a result, these cells become resistant to ray / chemotherapeutic agents resulting recurrence of disease and poor survival of the patients.

*Limitations of the study:*

Case control studies have some important limitations and are subject to bias, and our study is no exception. Selection bias might be an issue but this potential bias is

somehow minimized by the fact that: i) Controls were selected from the same sex and age groups as the patients; ii) Control individuals with a history of hospitalization for diagnoses related to tobacco consumption were carefully excluded and iii) The public hospital (Chittaranjan National Cancer Institute) from where the patients were selected, provides quality cancer care.

*Concluding remarks:*

Out of 110 cases, no dysplastic/ premalignant lesions of head and neck were included. It indicates that the common people are not aware about the early symptoms of the HNSCC. So in most of the cases, the disease was diagnosed at an advanced stage and therefore the probability of complete cure of the disease and disease free survival of the patients got reduced.

∑ Education has no positive impact against the tobacco habit. Therefore a greater part of the mass is addicted to tobacco after being acknowledged completely about its' harmful effects.

∑ Both smoked & nonsmoked tobacco and both dose & the duration of the exposure of tobacco seems to be equally important in the development of this disease.

∑ The duration of exposure of body tissue to the tobacco carcinogens seems to be more important over the dose in the survival of patients. Therefore long duration of exposure may have some positive impact on the process of development of drug resistance by tumor cells.

From our findings, it may conclude that, tobacco in all forms is a potential risk factor for both HNSCC development and also for the development of drug resistance of malignant cells; common people in majority are unaware about the early symptoms of HNSCC but are aware about the carcinogenic role of tobacco.

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