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Tobacco Addiction: Effect on Human Health

Chapter 1

Smoking and Primary Cancer among Chinese Urban Men: A Novel Study Design in Practice

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Abstract

In the 1980s, the hazard of smoking has been widely recognized by general public in western countries. While, during the same period, China had developed rapidly as the world's leading tobacco production and consumption country. However, due to the delay of smoking consumption peak comparing with developed countries, the smoking related research in China had lagged behind western developed countries for nearly 50 years and basic epidemiological information regarding to the health problems associated with smoking was lacking. Therefore, it was imperative to undertake nationwide research to depict the hazards related with smoking comprehensively and systematically, which was crucial for forming preventive public health policy as well as raising awareness of health risk caused by cigarette smoking among general population in China. However, it is almost impossible to achieve this goal in a short period by directly applying traditional epidemiological research designs, such as cohort design and case-control design, both of which are most frequently used in epidemiological etiological studies. In cohort studies, subjects need to be followed up for a period of time to observe the occurrence of the events of interest, which usually takes a long time for a cohort study to mature. While for case-control studies require control group to be an approximate random sample of base population, which is hardly to meet in large-scale population studies. Therefore, there was a call for methodological innovation, through which the impact of smoking on Chinese could be evaluated within relative short period with less resource consumption.

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In 1989-1991, a nationwide retrospective mortality survey was conducted in China, which involved 24 major cities and 79 rural counties that covered 67 million of a population, and about 1.2 million adult (age 30 years or above) death during 1986 ~ 1988 were collected. As the first and largest mortality survey in China, the survey was characterized by simultaneously collecting the smoking information of the deceased individuals as well as their surviving spouses, by interviewing the living spouses of deceased people or other informants. This research had received great attentions and aroused considerable repercussions both home and abroad, and it played an important role in convincing opinion leaders, politicians and the general public of the importance both of the problem and of the urgent necessity to address it. The project's primary report, which was published on BMJ in 1998, implemented proportional mortality ratio analysis in evaluating the risks of smoking on different causes of death systematically and comprehensively and presented a whole picture of smoking hazards in Chinese adults. In proportional mortality ratio analysis, the case group consists of deceased people who died from a disease that assumed be related with smoking, and the control group includes deceased subjects with death causes assumed to be unrelated with smoking. Then, differences between the proportions of smokers in case and control groups can be used to calculate the risk ratios. The application of proportional mortality ratio analysis has the strength that any bias affecting assessment of the habits of those in the case group should similarly affect assessment of the habits in the control group. However, the control group has a poor representativeness for base population because the base population consist of living people, which might lead to underestimating the risk of smoking. In addition, we cannot investigate the association between smoking and those death causes involved by control group in proportional mortality ratio analysis.

To address these problems and make a progress from methodological perspective, sex-matched case-spouse control design had been proposed by Chinese epidemiologist Boqi Liu and his colleagues. By utilizing this novel control selection strategy in the data from 1989 nationwide survey, an approximate random sample can be drawn from the base population and the problems mentioned above could be successfully resolved. Using this design, series of studies had been conducted to investigate the hazards of smoking among Chinese adults from both etiology and public health awareness perspectives. In this chapter, we will mainly introduce the theoretical framework of this design, as well as the applications of this design based on the national mortality survey data by investigating the relationship between smoking and primary cancer death among Chinese urban men. In addition, the methodological evaluation of this novel design will also be discussed.

Key words: smoking; chinese; cancer death; case-control study; control selection

1. Introduction

In the 1980's, owing to findings from a large number of smoking-related researches and the tobacco control measures, the hazard of smoking had been gradually recognized by general population in western developed countries, the perception of cigarette smoking had undergone a complete change and the smoking had been viewed as a lethal addiction [1-7]. Meanwhile, China, as the largest developing country with 20% of the world's population, had experienced a dramatic soar in cigarettes consumption and accounted for 30% of the world's cigarette consumption at that time, owing in large part to the earlier and more intensive consumption of cigarette. It was estimated that nearly 67% of males and 4% of females become smokers before the age of 25 and the average cigarette also increased at the same period [8-10].

The epidemic of cigarette smoking in China in 1980s had posed a tremendous threat on health for Chinese population. Moreover, the dramatic growth of tobacco consumption, unless prevented, will result in not only human health but also an economic burden of medical and health costs. In contrast with this severe situation, China was at a different stage of smoking epidemic comparing with western developed country and the smoking-related research in China had lagged behind for nearly 50 years, with few reliable or country-wide research had been done. So the information on hazard caused by smoking is scanty and the awareness of the health risk of smoking was low among general population, which made it urgent to carry out nationwide study to investigate how large the effect of smoking on health and where in China the hazard is the greatest [11]. While, it is almost impossible to achieve this purpose within a relative short period by employing traditional epidemiological study designs, such as cohort and case-control study design, both of which are frequently used in epidemiological etiological research. Because in cohort studies, a number of subjects should be followed up for a period of time to observe the occurrence of the events of interest, which thereby usually takes a long time for a cohort study to mature [12]. While for case-control studies, despite being quick, inexpensive and easily to be carried out, has a requirement that the control group should be an approximate random sample of the base population, which is hardly to meet in large-scale population studies [13]. Therefore, there was an urgent need to found a time-saving and cost-effective method to assess the hazard associated with smoking in consideration of the severe smoking epidemic in China at that time.

In 1989-1991, a nationwide retrospective mortality survey was conducted in China, with 24 major cities being chosen non-randomly to represent a wide range of area, which included Beijing, Tianjin, Shanghai, Harbin, Hangzhou, Changsha, Xi 'an, Lanzhou, Chengdu, Chongqing, Zigong, Guiyang, Guangzhou, Nanjing, Fuzhou, Kunming, Huangshi, Yangquan, Changchun, Jilin, Shenyang, Dalian, Zibo, Luoyang. A total of 79 rural counties were also selected through stratified random sampling from the 2000 counties whose cancer rate in 1973–1975 were recorded in the Chinese cancer atlas (as shown in Figure 1). As the largest mortality

survey in China, the survey covered 67 million of a population and about 1.2 million adult (age 30 years or above) deaths during 1986~1988 were collected. Besides, by interviewing the surviving spouses or other informants, smoking information of deceased person as well as their surviving spouses (other family members or informants if there was no surviving spouses) were collected during the survey.

Figure 1. Location of study areas: 24 major cities (large circles with names) and 79 rural counties (unnamed circles).

As the first and largest nationwide smoking-related study, the 1989 nationwide mortality successfully combined descriptive and analytic epidemiology studies, which belong to different epidemiologic study types. Therefore, the hazard associated with smoking in China could be assessed within only three years, for which the western countries has spent also 50 years. And this research is considered to be a milestone in terms of describing the mortality pattern and smoking pattern, and estimating the harm caused by smoking in China, which had a profound effect on raising the concerns as well as consciousness of the smoking hazard both for the government and the general population. The project's primary report, which was published on BMJ in 1998, estimated the tobacco attributable mortality in middle or old age from neoplastic, respiratory, or vascular disease by employing proportional mortality ratio analysis (PMR) [14]. In PMR analysis, all deceased people are divided into two parts. Those deaths are assumed to be related with smoking were enrolled as cases, whereas for others whose death causes are assumed not related with smoking were enrolled as controls, including infective or parasitic, diabetes, parkinsonism, other nervous or mental disease, renal disease, hepatic disease (chiefly due to chronic hepatitis B infection), peptic ulcer, other digestive disorders, other medical disorders, road traffic accidents, suicide or homicide, and other non-medical reasons. The strength of proportional mortality analyses is that any bias affecting assessment

of the habits of those in the case group should similarly affect assessment of the habits in the control group. Hence, comparison of proportion of smokers can be made between case group and control group to calculate the risk ratios (smoker versus nonsmoker) for mortality from those causes [14]. Moreover, another prominent advantage for PMR is that the criteria for eligible controls can be established conveniently. However, a primary limitation of proportional mortality analysis is that the controls cannot represent the base population due to the reason that the base population consists of living subjects, Which is an obvious violation of study base principle for control selection in case-control study. In addition, it is inevitable that smoking is associated with some certain kinds of deaths in the reference group (for example, some of those from gastric ulcer), which means that proportional mortality analyses might underestimate the risk of smoking.

In light of the these problems, Chinese epidemiologist Boqi Liu, who led the 1989 mortality survey, proposed sex-matched case-spouse control design along with his colleagues, which made it possible to draw an approximate random sample as a control group in large population. More importantly, the implementation of this design could provide an alternative method to give accurate estimate of early smoking-attributable mortality in a nationwide level, which investigate the relationships between smoking and broader range of disease, including those diseases being involved by control group in PMR methods. Since the development of this novel design, it has gained a wide spread attention and recognition both in home and abroad.

By using this innovative design method, we have carried out series of studies (Appendix 1), through which the tobacco hazards were investigated from the following aspects: (1) smoking and deaths from various cancers. Particularly, we proved the positive association between smoking and glioma, which is a rare cancer and its association with smoking remains controversial; (2) smoking and deaths from respiratory disease, including chronic obstructive pulmonary disease and tuberculosis; (3) smoking attributable deaths and life expectancy reduction, which bear significant implications in raising the awareness of hazards associated with smoking in the public for the sake of being easily comprehended and accepted by general individuals. In addition, the hazard of passive smoking, which has been attracted more and more attention, has also been investigated based on the nationwide population-based study. In the following sections, we will introduce the sex-matched case-spouse control design and illustrate the application of this novel design in practice by investigating the association between cigarette smoking and death from primary cancers among Chinese urban men based on data from the 1989-1991 nationwide survey.

2. Methods

2.1 Ascertainment of death cause

In 1989 mortality survey, causes of death were sought chiefly from official death cer-

tificates and supplemented, if necessary, by reviewing medical records or by discussing (a few years after the death) with local health workers, family members or both. The findings were recorded as part I and part II of a standard death certificate, Which were available from local government offices. The underlying cause of each death was coded according to the World Health Organization's International Classification of Diseases, 9th revision (ICD-9). Underlying causes were coded by 100 clerks in five teams, each under a trained oncologist from the Ministry of Health with experience of coding standard death certificates using ICD-9 (international classification of diseases, ninth revision). Some batches of data sheets were coded by two teams and the differences discussed to develop consistent coding conventions. At ages 35-69 only 0.4% of causes were ill defined (codes 780-99), but at older ages 4% were ill defined (3% urban, 6% rural).

2.2 Ascertainment of smoking exposure

All surviving spouses (28.1% of total interviewees in urban areas; 20.7% of total interviewees in rural areas), other family members (35.6% in urban areas; 27.1% in rural areas), or other informants including family relatives and local informants (36.3% in urban areas; 52.2% in rural areas) were interviewed. Interviews were conducted with either the living spouse or another member of the identified household when the spouse was deceased. A short structured questionnaire that had two sections were employed for asking the same questions, one for the deceased person and another for a living person (deceased person's spouse or the other informants). The information concerning smoking history was provided by living informants who described their own smoking habits as well as those of their deceased partners, including types of tobacco (cigarette, hand-made cigarettes or other forms of tobacco), duration of smoking, starting age and average number of cigarettes consumed per day. These data were used to determine whether people had ever smoked by 1980, a period of time prior to the onset of their disease. This approach was used to help guarantee that there was a reduced chance that the interviewee's smoking habits being changed by the disease eventually caused death during 1986–1988. A nonsmoker was defined as a person who had never smoked during his life or had only smoked infrequently during young age.

2.3 Sex-matched case-spouse control design

The scheme of sex-matched case-spouse control design is illustrated in Figure 2. In this design, people aged 30 years old or above who died from any cause–associated smoking during the years 1986-1988 may be defined as cases. Meanwhile, controls were the surviving spouses of those who died of any condition during the same period, whose age range was the same for the cases when his or her spouse had died. This control selection procedure is based on the assumption that in 1980s individuals in the control group had smoking habits similar to those from the base population, and there is no significant relationship between couples in

terms of tobacco use.

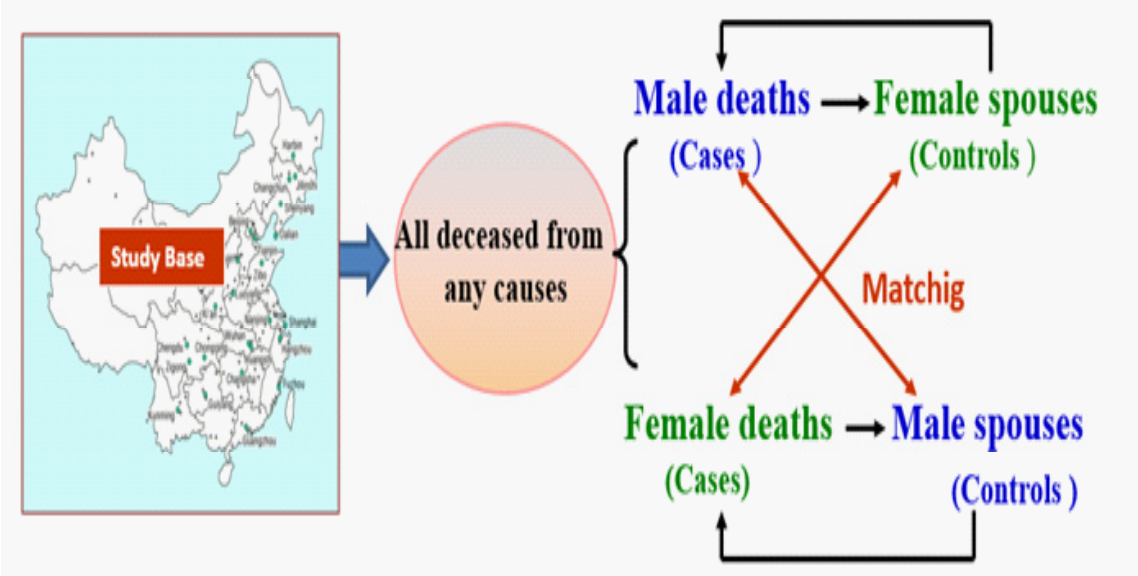

Figure 2: Sex-matched case-spouse control design

In our current study, by implementing living spouse control design, a total of 77,883 urban men aged 35 years or older who died from primary cancers during 1986-1988 were selected as cases, the primary cancer was defined as (n; %) lung cancer (24,794;31.8%), stomach cancer (19,044;24.5%), liver cancer (17,086;21.9%), esophagus cancer (9285;11.9%), pancreaticcancer (2638;3.4%), prostate cancer (1662;0.8%), bladder cancer (584;2.1%), and minor sites cancer which combining lip, oral cavity and laryngeal (2790; 3.6%). The control group was defined as living spouse of those all-caused deceased females during 1986-1988, with the age range was the same for the cases when her spouse had died, and 63,878 subjects were selected as controls.

2.4 Statistical methods

A non-conditional logistic regression model was used to estimate the risk of smoking and conduct a trend test with adjustment for confounders. Odds ratio (OR) with 95% confidence intervals (CIs) were used to estimate the effects of smoking history on the cancer deaths. Attributable fraction (AF), calculated as (OR–1)/OR, was employed to express risk attributing to cancer deaths for the smoking population.

Equivalence evaluation was carried out by testing the equivalence between sex-matched case-spouse control design (denoted by $O\hat{R}_1$) and PMR design (denoted by $O\hat{R}_2$), respectively. Then, the 95% confidence interval (95% CI) for $O\hat{R}_1 / O\hat{R}_2$ were calculated.

We employed the proportion of similar response (PSR) to establish equivalence limit [15], which can be defined as follow

$$PSR = \int \min\{f(x), g(x)\}dx$$

f(x) and *g(x)* are probability density function for cumulative distribution function $f(x)$ and *g(x)*. The more closely the value of PSR approach to 1, the more similar of the two distributions. If the PSR is larger than a predefined value, the two distributions can be regarded as identical and equivalent. In practice, the default value is usually 0.9 or 0.8. In our study, we adopted 0.9 as equivalent standard and thereby the equivalent limit is set to be 0.2513. After conversion, we obtained the equivalent limit of (0.78 1.29), which indicates that equivalence could be concluded if $0.78 < \hat{OR}_1 / OR_2 < 1.29$.

Then, parameter interval method and bootstrap percentile method were employed in estimating the confidence interval. In parameter interval method, we firstly estimate the correlation coefficient *p* between $\hat{OR}_1$ and $\hat{OR}_2$ by using bootstrap technique. Then, confidence interval can be constructed using the following formula:

$$(\ln \hat{OR}_1 - \ln OR_2) \pm u_{0.05/2}\sqrt{se_1^2 + se_2^2 - 2\mathrm{r}\, se_1 se_2}$$

Where se_1 and se_2 denotes the standard error of $\hat{OR}_1$ and $\hat{OR}_2$

In bootstrap method, 100 samples were obtained using re-sampling methods, then 5th percentile and 95th percentile for $\hat{OR}_1 / \hat{OR}_2$ can be obtained and then be compared with the predefined equivalence limit to assess the equivalence.

By re-sampling from the original data set using stratified sampling strategy repeatedly, with the sample size range from 100-25000 (which could satisfy sample size requirement for most case-control study), we evaluate the consistence as well as the stability of the ORs for primary cancers obtained using PMR and the sex-matched living spouse control design respectively under various sample sizes.

All analyses were performed using SAS 9.3 statistical software package (SAS Institute). All *P* values were two-sided except for trend tests, in which one-sided *P* values were used; a *P* value less than 0.05 was considered statistically significant.

3. Results

3.1 Prevalence of smoking among cases and controls

In general, the smoking prevalence for case group and control group were 69.2% and 56.7% respectively. And as shown in Figure 3, the highest smoking prevalence was found in 35-40 age group for control group, while in the case group the peak was found at around 60 years old. In addition, The prevalence of smoking in case group was higher comparing with

control group in almost all age groups, and the gap gradually increased with age at the start and turned stable after 50 years old.

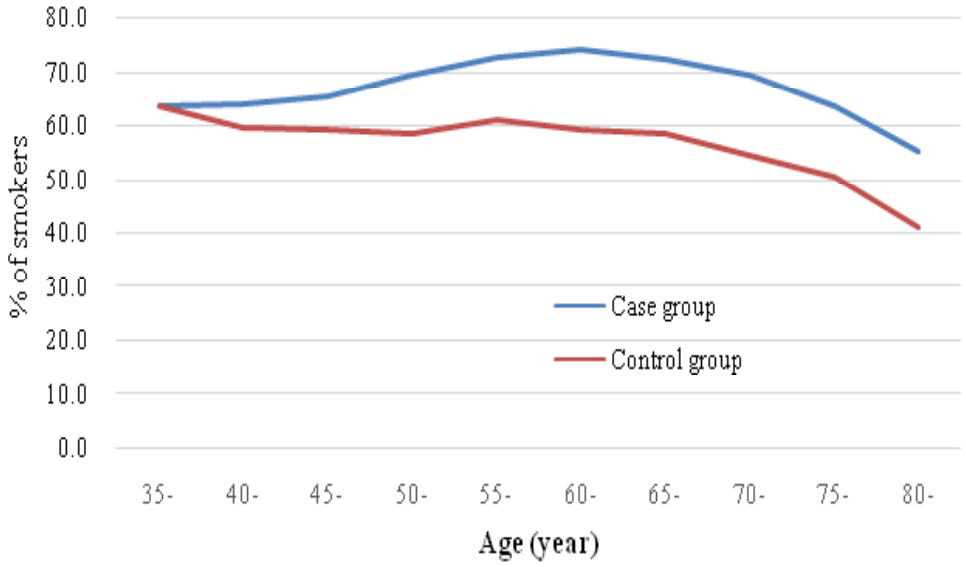

Figure 3: Smoking prevalence by age group in case group and control group, 1990

3.2 Risks for death from primary cancers

The crude and adjusted odds ratios (ORs) of deaths from various primary cancers between smokers and non-smokers are shown in Table 1. There is a relative large variation in risks across the primary cancers, among which lung cancer has the highest association with smoking, and the OR was 2.91 (95% CI: 2.81-3.01). While, the association between smoking and prostate cancer deaths is the lowest, and the OR was 1.04 (95% CI: 0.88, 1.22).

Table 2 to Table 5 present the adjusted ORs for association between smoking and deaths from four major cancers: lung cancer, stomach cancer, liver cancer and esophageal cancer. Although the ORs were influenced by age, the associations were significant for lung cancer across all age groups, and the peak of risk was found in 60-64 age group. However, for other major cancers, significant associations were not observed in lower age groups. For instance, the risk of smoking on stomach cancer death were not observed among individuals under 50 years old.

Table 1. Effect of smoking on various primary cancer deaths

	Crude OR (95% CI)	**Adjusted OR[a] (95% CI)**	**AFs (%)**
Primary cancers combined	1.72 (1.68, 1.76)	1.74 (1.70,1.78)	42.5
Lung cancer	2.91 (2.81,3.01)	2.98 (2.88,3.08)	66.4
Stomach cancer	1.27 (1.23,1.32)	1.32 (1.27,1.36)	24.2
Liver cancer	1.38 (1.33,1.43)	1.33 (1.28,1.38)	24.8
Esophagus cancer	1.72 (1.64,1.80)	1.84 (1.75,1.93)	45.7

Pancreatic cancer	1.33 (1.23,1.45)	1.34 (1.24,1.45)	25.4
Bladder cancer	1.25 (1.13,1.39)	1.45 (1.31,1.60)	31.0
Prostate cancer	1.04 (0.88,1.22)	1.25 (1.06,1.48)	20.0
Minor sitescancer[b]	1.67 (1.54,1.82)	1.66 (1.53,1.81)	39.8

Abbreviations: AFs, attributable fraction; [a]Adjusted for age and area of residence; [b]Combining the lip, oral and throat cancer

Table 2. Age-specific risk of lung cancer deaths associated with smoking

Age group (year)	No of smokers	Total deaths	Adjusted OR[a](95% CI)
35-	189	257	1.59 (1.19,2.13)
40-	278	389	1.70 (1.34,2.15)
45-	643	868	1.98 (1.67,2.34)
50-	1651	2119	2.52 (2.25,2.83)
55-	2934	3598	2.81 (2.55,3.09)
60-	3945	4726	3.47 (3.19,3.79)
65-	3995	4903	3.10 (2.85,3.36)
70-	3319	4172	3.23 (2.97,3.52)
75-	1810	2474	2.70 (2.44,2.99)
80-	864	1288	2.95 (2.59,3.37)
Total	19628	24794	2.98 (2.88,3.08)

[a]Adjusted for age and area of residence.

Table 3. Age-specific risk of stomach cancer deaths associated with smoking

Age group (year)	No of smokers	Total deaths	Adjusted OR[a](95% CI)
35-	165	261	0.99 (0.76,1.29)
40-	211	350	1.03 (0.82,1.30)
45-	382	624	1.09 (0.91,1.30)
50-	933	1464	1.26 (1.12,1.42)
55-	1552	2361	1.22 (1.11,1.34)
60-	2092	3168	1.34 (1.24,1.46)
65-	2437	3730	1.33 (1.23,1.44)
70-	2139	3479	1.33 (1.23,1.44)
75-	1285	2215	1.37 (1.24,1.51)
80-	694	1392	1.43 (1.27,1.62)
Total	11890	19044	1.32 (1.27,1.36)

[a]Adjusted for age and area of residence.

Table 4. Age-specific risk of liver cancer deaths associated with smoking

Age group (year)	No of smokers	Total deaths	Adjusted OR[a](95% CI)
35-	403	667	0.88 (0.73,1.05)
40-	538	866	1.11 (0.95,1.30)
45-	887	1467	1.06 (0.93,1.20)
50-	1604	2468	1.33 (1.21,1.47)
55-	2039	3026	1.32 (1.20,1.44)
60-	1923	2822	1.48 (1.35,1.61)
65-	1582	2383	1.39 (1.27,1.53)
70-	1126	1782	1.43 (1.29,1.59)
75-	604	1041	1.37 (1.20,1.56)
80-	291	564	1.54 (1.29,1.83)
Total	10997	17086	1.33 (1.28,1.38)

[a]Adjusted for age and area of residence.

Table 5. Age-specific risk of esophagus cancer deaths associated with smoking

Age group (year)	No of smokers	Total deaths	Adjusted OR[a](95% CI)
35-	39	57	1.25 (0.71,2.20)
40-	80	108	1.94 (1.25,3.00)
45-	194	253	2.27 (1.68,3.07)
50-	400	537	2.09 (1.71,2.56)
55-	862	1146	1.93 (1.68,2.22)
60-	1131	1534	1.93 (1.72,2.18)
65-	1264	1783	1.72 (1.54,1.92)
70-	1222	1764	1.88 (1.69,2.10)
75-	803	1283	1.66 (1.47,1.87)
80-	428	820	1.58 (1.36,1.84)
Total	6423	9285	1.84 (1.75,1.93)

[a]Adjusted for age and area of residence.

A dose-response relationship between smoking variables, such as years of smoking and number of cigarettes smoked daily with the deaths from four common cancer, were found (Table 6). For lung cancer death, the ORs were 1.21 (95% CI: 1.10–1.32), 2.06 (95% CI: 1.94–2.20), and 3.30 (95% CI: 3.18–3.42) for smokers with <20, 20–29, and ≥30 years of smoking(trend test P<0.001) and 1.70 (95% CI:1.60–1.81), 2.23 (95% CI: 2.13–2.33), and 4.57 (95% CI: 4.39–4.76) for smokers with 10, 10–19, and ≥20 cigarettes smoked daily (trend test P<0.001) after adjustment for age and area of residence compared with non-smokers. This trend was similar for other major cancers.

Table 6. Dose-response relationships between smoking and deaths from four major cancer (Odds ratio (95% CI))

	Lung cancer	Liver cancer	Stomach cancer	Esophagus cancer
Non-smoker				
Years of smoking				
<20	1.21 (1.10,1.32)	0.87 (0.80,0.94)	1.03 (0.94,1.12)	1.12 (0.98,1.29)
20–29	2.06 (1.94,2.20)	1.38 (1.30,1.46)	1.23 (1.15,1.32)	1.65 (1.50,1.81)
≥30	3.30 (3.18,3.42)	1.39 (1.34,1.45)	1.35 (1.31,1.40)	1.91 (1.82,2.00)
P for trend	<.0001	<.0001	<.0001	<.0001
Cigarettes smoked daily				
<10	1.70(1.60,1.81)	1.32(1.24,1.40)	1.23(1.16,1.30)	1.48(1.36,1.61)
10–19	2.23(2.13,2.33)	1.20(1.15,1.26)	1.20(1.15,1.26)	1.53(1.44,1.63)
≥20	4.57(4.39,4.76)	1.52(1.46,1.59)	1.49(1.42,1.55)	2.13(2.01,2.26)
P for trend	<.0001	<.0001	<.0001	<.0001

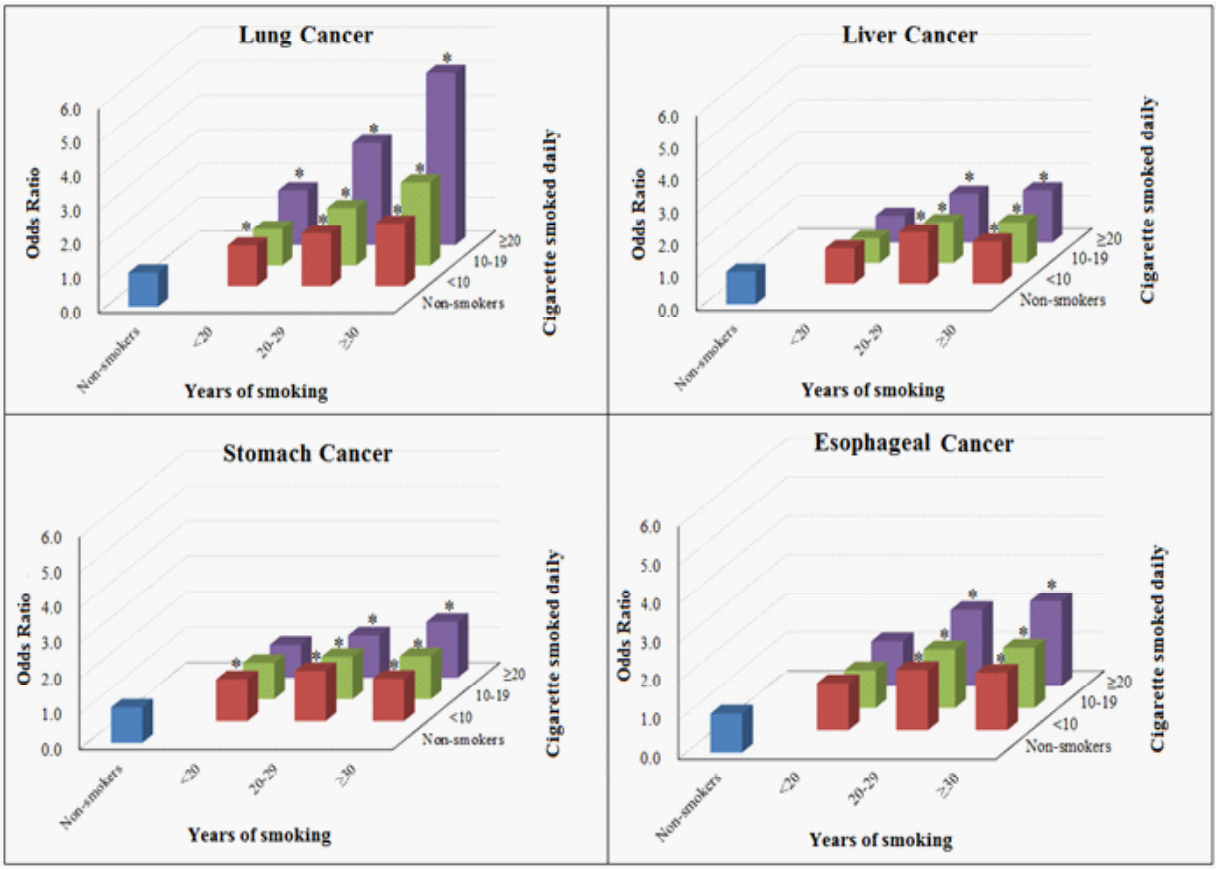

Figure 4. Effects of combining years of smoking with cigarettes smoked per day on death from lung, stomach, liver and esophagus cancer, respectively (adjusted for age and area of residence, **P*<0.05).

To express the synergistic effects of years of smoking and number of cigarettes smoked daily, by using non-smokers as the common reference group,and found that the risk dramatically increased with years of smoking and the number of cigarettes smoked daily (Figure 4).

In particular, in long term heavy smokers (defined as individuals with ≥30 smoking years) and ≥20 cigarettes (equivalent to one pack) smoked daily, the risk increased by 407% (OR=5.07; 95% CI: 4.87 –5.28) for cancer after adjustment for age and area of residence. While the corresponding increased risk were 62% (OR=1.62; 95% CI: 1.55 –1.70), 57% (OR=1.57; 95% CI: 1.51 –1.65) and 120% (OR=2.20; 95% CI: 2.07 –2.34) for liver cancer, stomach cancer and esophagus cancer, respectively.

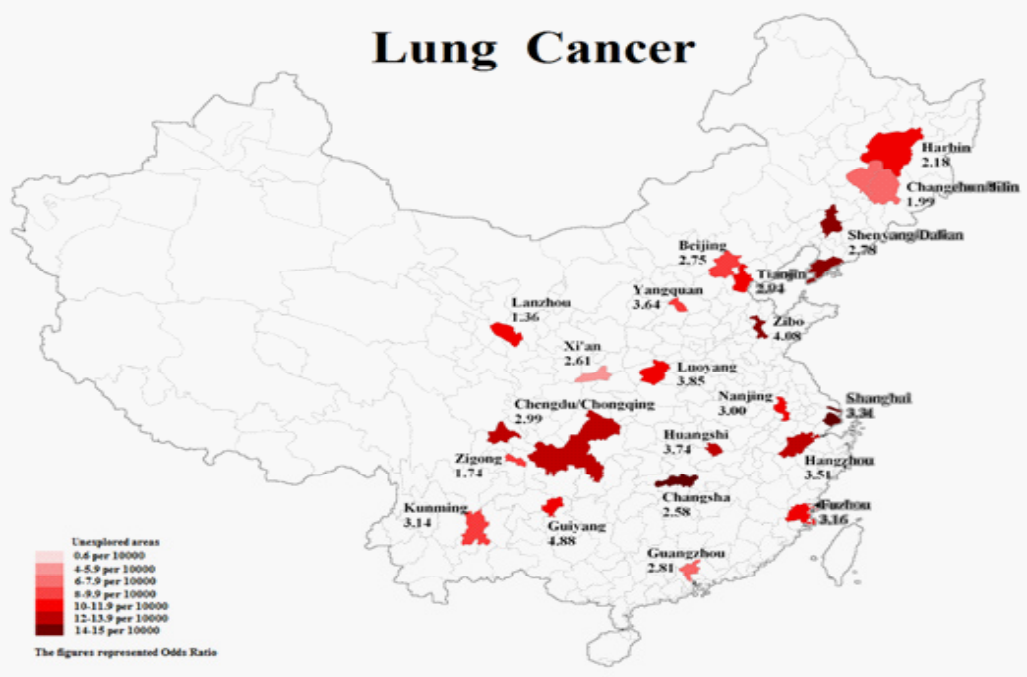

Figure 5. The distribution of mortality rate of lung cancer and its association with smoking among men in 24 cities in China

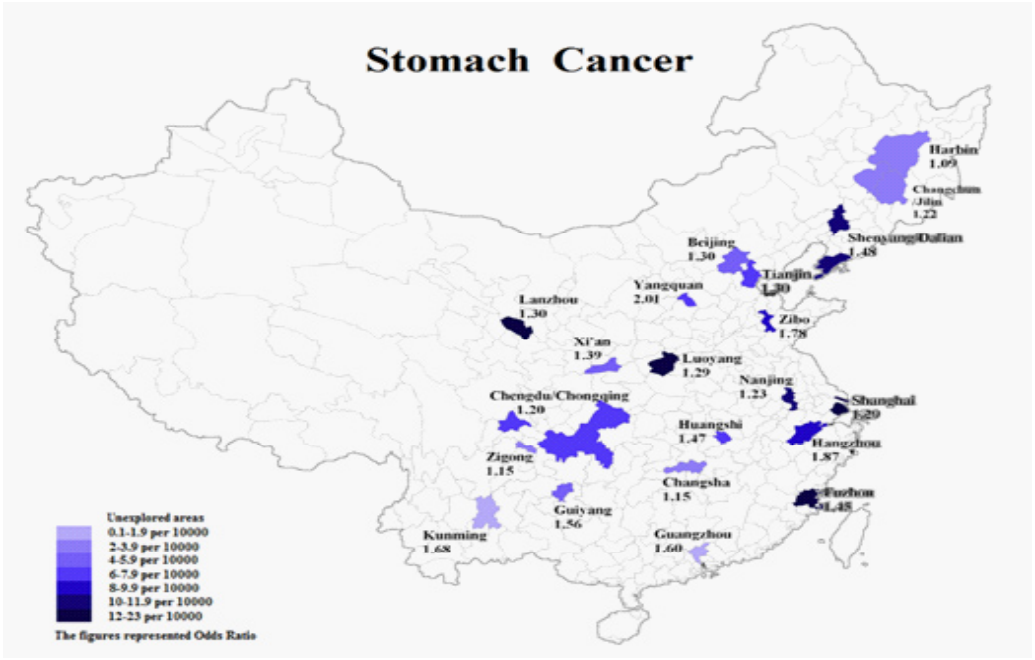

Figure 6. The distribution of mortality rate of stomach cancer and its association with smoking among men in 24 cities in China

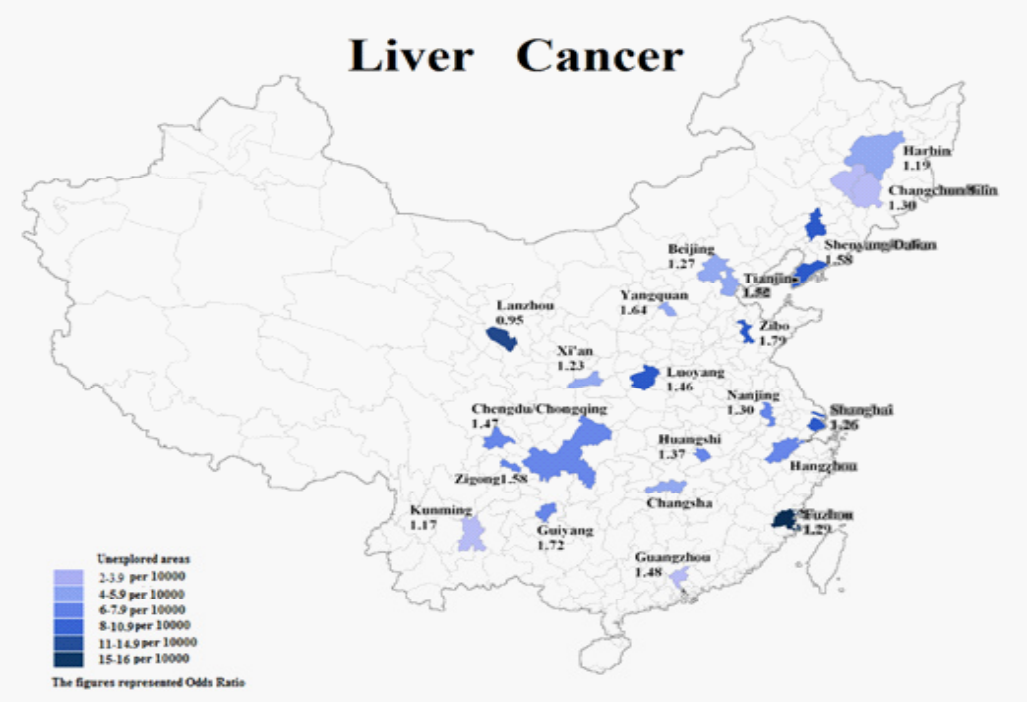

Figure 7. The distribution of mortality rate of liver cancer and its association with smoking among men in 24 cities in China

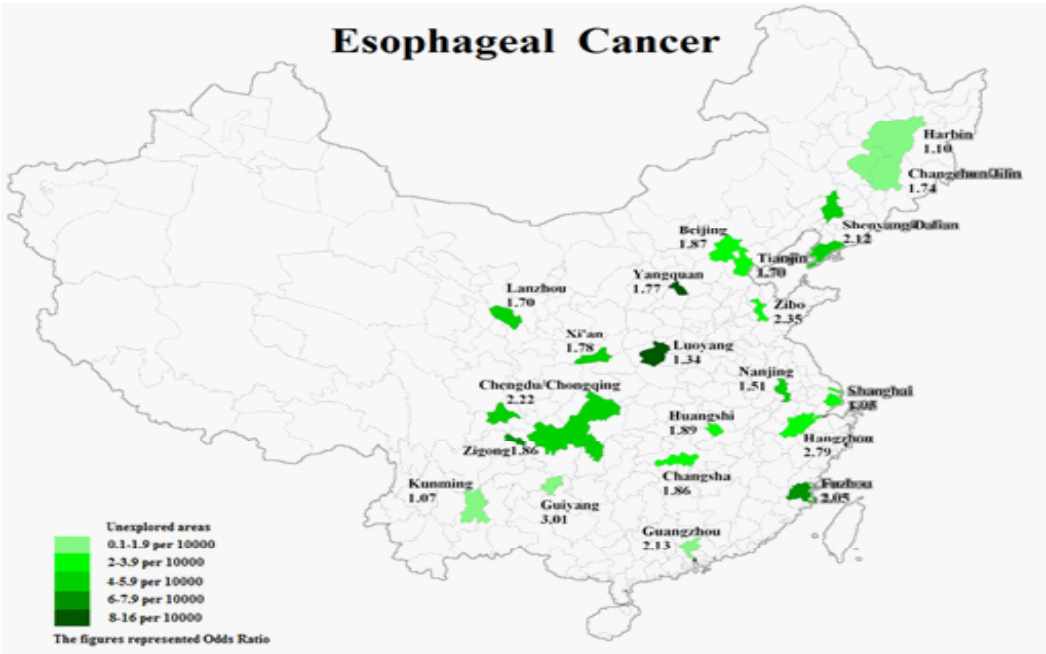

Figure 8. The distribution of mortality rate of esophagus cancer and its association with smoking among men in 24 cities in China

Figure 5 to Figure 8 present the regional features of mortality rates of four major cancers and their association with smoking. The background of the map represents the level of cancer mortality in the city and the darker color means a higher mortality rate. The figures next to the city represent the ORs. The results reveal that, although there is a considerable regional difference in cancer mortality, the associations between smoking and major cancer deaths are significant across all 24 cities. However, the distribution of the risk of smoking and major cancer death is not necessarily congruent with the distribution of cancer deaths.

3.3 Equivalence test and performance in the context of various sample size

Table 7 and 8 show the equivalence evaluation results using bootstrap percentile method and parametric interval method to calculate the 95% confidence interval of OR_1/OR_2, respectively. And the results suggest that whether for a primary cancer alone or in combination of them, there is no 95% confidence interval of OR_1/OR_2 exceeding the pre-defined equivalence limit (0.78-1.29), suggesting a high consistence between PMR method and sex-matched case-spouse control design method in estimating the risk of death from primary cancers.

Tables 7. Equivalence evaluation results using bootstrap percentile method

Death cause	Lower limit	Upper limit	Equivalence[a]
Combining primary cancer	0.973	1.015	√
Lung cancer	0.973	1.009	√
Stomach cancer	0.961	1.003	√
Liver cancer	1.001	1.047	√
Esophagus cancer	0.943	0.989	√
Pancreatic cancer	0.979	1.026	√
Bladder cancer	0.921	0.964	√
Prostate cancer	0.894	0.945	√
Minor sites cancer[b]	0.980	1.017	√

[a] Equivalence limit is 0.78-1.29; [b] Combining the lip, oral and throat cancer

Table 8. Equivalence evaluation results using parametric interval method

Death cause	Lower limit	Upper limit	Equivalence[a]
Combining primary cancer	0.974	1.020	√
Lung cancer	0.971	1.012	√
Stomach cancer	0.957	1.010	√
Liver cancer	0.995	1.051	√
Esophagus cancer	0.936	0.993	√
Pancreatic cancer	0.974	1.027	√
Bladder cancer	0.910	0.969	√
Prostate cancer	0.889	0.946	√
Minor sites cancer[b]	0.975	1.023	√

[a]Equivalence limit is (0.78-1.29); [b]Combining the lip, oral and throat cancer

By employing re-sampling techniques, we obtained ORs for lung cancer using PMR and the sex-matched case-spouse control design under various sample size settings, and the results are shown in Figure 9.

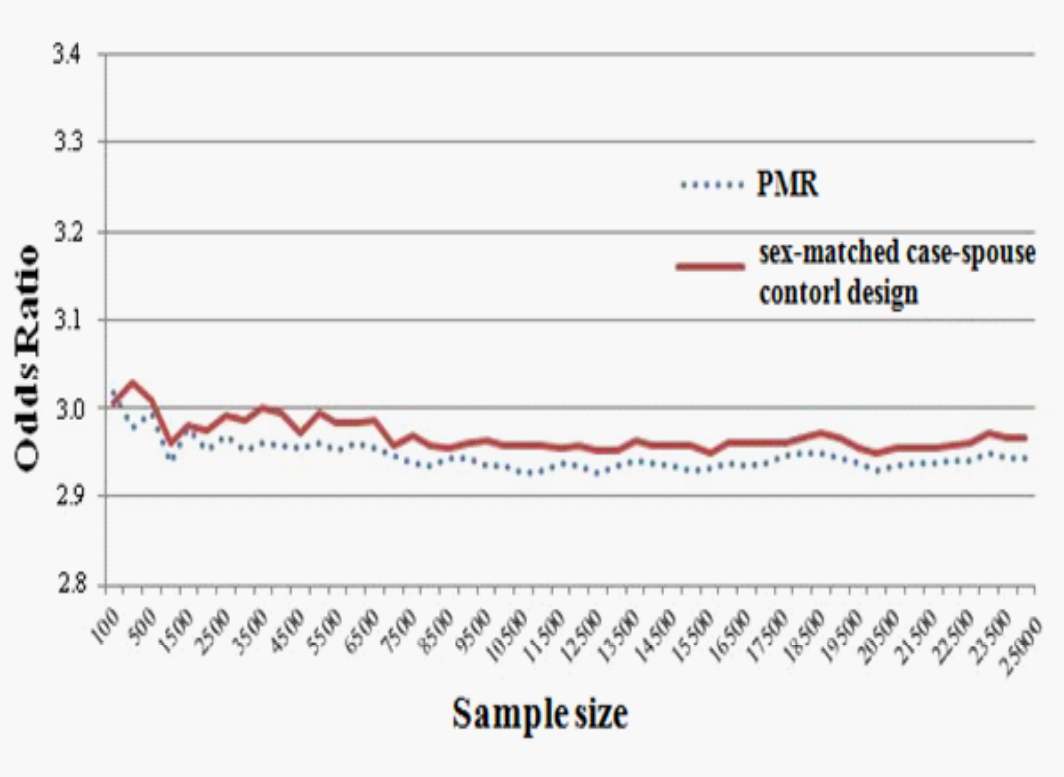

Figure 9. ORs for lung cancers obtained using PMR and the sex-matched case-spouse control design under various sample sizes scenarios

Highly consistent results were observed in the comparison between ORs obtained using two control selection strategies in the context of various sample size, except for the scenario with small to medium sample size (100 to 1500) for which small fluctuations (two contrasting trends) were observed, and with the increase of sample size, the ORs tend to be stable. Moreover, we found that the estimates of ORs for living spouse design methods were always higher than for PMR method.

4. Discussion

4.1. Theoretical exploration for sex-matched case-spouse control design

As a study design with considerable long history, case–control study has been firmly ensconced and being in wide spread use in etiological epidemiology studies, and it is a very efficient way of identifying an association between an exposure and an outcome [16]. Selection of an appropriate control group is critical in case-control study because study conclusions are based on a comparison of the exposure histories provided by cases and controls, therefore, the validity of the results in case-control study heavily relies on the appropriate selection of controls [17]. Overall, the key issue in control selection is that control group should be an unbiased sample of the base population that generated the cases, and theoretically speaking, the “simplest” way to do this is to randomly sample controls from the base population. However, in practice, it might be difficult to define or characterize the base population, especially for large-scale study with nearly a million subjects being investigated, it might be too time-

consuming or otherwise infeasible to obtaining a probability sample of controls from the base population, which makes the selection of controls from the base population the primary challenges.

By enrolling the living spouses of deceased as control group in a nationwide mortality survey, sex-matched case-spouse control design successfully address the problem of selecting controls in a population-based case-control study in large retrospective mortality survey. The development of this design was totally complying with the basic principles for control selecting in case-control study, and there are several crucial assumptions in applying this method, among which the most important one is that the distribution of all causes of deaths in the base population is approximately random, so is their living spouses. Therefore, an approximate random sample of the base population can be obtained by selecting the living spouses as controls. What is noteworthy is that all causes deaths in the base population are assumed to be approximately random, rather than the death from a particular cause, because, as a matter of fact, the distribution of some kinds of deaths have regional characteristics, such as esophagus cancer. Therefore, although such death might happen non-randomly within base population, the all-cause death can still be regarded to be random because any systematical variation can be offset within such vast base population covering 67 million population.

The randomness mentioned above is vital in ensuring the representativeness of the living spouse to the base population. Therefore, it is reasonable to conclude that the living spouses had smoking habits similar to those of the base population, which is reinforced by a study finding that the spouse based prevalence were highly consistent with those in the 1984 and 1996 nationwide surveys of smoking prevalence [18,19].The representativeness of the base population or even general population is crucial in estimating the prevalence of disease and the attributable risk.

Another important assumption for this novel design is that there is no significant relationship in tobacco use between couples, in other words, the smoking status of either party of a couple does not influence the smoking status of his or her spouse. Therefore, the smoking status remains mutually independent between each other for any couples. This assumption is crucial for effect estimation in case-control studies because a correlated smoking habits within couples could undermine the representativeness of living spouse in terms of smoking. Besides, a strong association of smoking habits between couples could also attenuate the estimated risk. To validate this assumption, the Kappa coefficient of agreement test on smoking habits of couples were calculated, which are 0.076 in urban areas, and 0.163 in rural areas, indicating a very weak association between couple's smoking habit. Nevertheless, couples share some common environment (such as living habit, dietary habit, et.al), which seems to be reasonable in China. These shared habits could essentially be removed by cross-matching, through which the distribution of some unknown or unmeasured confounders tend to be same [20].

In the face of a severe and still growing epidemic of smoking in Chinese population during 1980s, the development of sex-matched case-spouse control design method provided a time-saving and resource-economic way in evaluating the hazards of smoking on Chinese population health. In living spouse control design, the exposure information of cases and controls can be collected simultaneously. The convenience of the information collection makes it relatively practical and feasible to be conducted in large-scale study (e.g. a nationwide population based case-control study). Besides, this new design has the advantage in estimating smoking-related deaths for all causes, That is, one exposure to risk of all causes of deaths can be investigated by conducting one study, and risk of smoking on a variety causes of death can be evaluated using one control group. Moreover, from methodological perspective, there is no "gold standard" in epidemiological surveys, therefore,the inclusion of multiple control groups selected by different criteria is preferable to only one control group [21-25]. The development of sex-matched case-spouse control design provide an alternative control selection strategy, affording opportunity in preventing disastrous consequence caused by potential bias and demonstrating consistence in the finding, which thereby enhances the validity of the study.

4.2 Methodological evaluation of the sex-matched case-spouse control design

Although the underlying rationale of the design has gradually gained recognition both home and abroad, there was still a need of methodological evaluation to further validate the methodological rationality and reinforce the validity of this novel design. In view of this point, we used the PMR method to create an "active control" group, which has been treated as a routine control selection design. Then, the comparison could be made between the ORs obtained from the sex-matched case-spouse control design and PMR, and the equivalence of the results could add weight to the rationality and validity of the new design.

Equivalence study has been widely employed in clinical medicine development and clinical trials, with most clinical study activities are aimed at showing that equivalence can also be claimed for generic versions of innovator drugs and for such diverse entities as medical protocols, surgical techniques and medical devices [26-30]. However, there is no such standard criteria for how to evaluating and supporting such equivalence claim in epidemiological survey data [31-33]. Therefore, in our current study, for the first time we applied the equivalence test method in evaluating the consistence of estimated risks obtained through different control selection strategies in case-control study.

The results of equivalence evaluation suggest that the 95% confidence intervals were entirely covered by equivalent limit, indicating a high consistence between the two control groups when estimating the association between smoking and primary cancer death.Our study bears the advantage that a large adequate sample size in each compared group can insure consistency between the initial design and final analysis based on confidence intervals. Moreover,

a large adequate sample size in each compared group will make a high probability (1- β, β is type II error) to insure that the upper/low limit of CIs will not excess the selected criterion. However, it should be noted that an absolute equivalence between the two methods could never be confirmed, and it can only be concluded that the actual difference is unlikely to exceed a particular range, which is the equivalent limit determined by the size of the study and the specified probability of error.

In addition, it is worth noting that the sex-matched case-spouse control design method was initially developed and applied in the context of nationwide mortality survey with extreme large sample size. To investigate the stability of this novel design under various sample size, which is crucial for its further extensive application, we took lung cancer as an example and employed re-sampling techniques to evaluate the stableness of the estimation of risk. The result shows that, although both PMR and living spouses control method showed a certain degree of fluctuation for sample sizes less than 2000, the absolute volatility is negligible (with minimum of 2.97 and maximum of 3.02), which provides a guarantee for its application in case-control studies with relative small sample size. Moreover, the estimate of OR for living spouses design is higher than PMR under various sample size, which indicates that the novel design is more sensitive and may provide more accurate estimate of the hazard of tobacco smoking in general population.

4.3 The hazard of smoking on primary cancer among Chinese urban adult men

To illustrate the application of sex-matched case-spouse control design, we investigated the relationship between smoking and death from various primary cancers among urban adult men in China, these cancer deaths accounted for a considerable fraction of the overall cancer deaths.The results indicated that the effect of the smoking is highest for lung cancer, which is the fourth leading cause of cancer death for Chinese men [34], and is the primary cause of cancer mortality in some cities. Our findings clearly illustrate that lung cancer is about three times as common among urban male smokers as among otherwise similar non-smokers. Moreover, despite the effect is relative trivial in magnitude, we demonstrated a significant association between cigarette smoking and prostate cancer, which remains a matter of debate and previous published data suggested no clear evidence of a causal relationship [35].

After stratified by age groups, we observed increased risk of death from the four major cancers (lung cancer, stomach cancer, liver cancer, esophagus cancer). However, the risk peaked at around 60-70 years old and then declined, which might be explained by potential competitive risk from other diseases, such as the respiratory disease and cardiovascular disease, which also cause considerable burden in China, especially for older people.

Estimating the dose-response relationships between smoking and cancer deaths can serve as important tools in assessing the long-term harm of smoking on health. Although most indi-

viduals realize that smoking is dangerous to their health, many smokers do not comprehend the actual magnitude of long-term exposure. Our findings clearly illustrate that the risk in various kinds of cancer deaths increased consistently with years of smoking and the number of cigarettes smoked daily regardless of age and area. Especially for lung cancer, the risk observed in long term heavy smokers (≥30 years and ≥20 cigarettes/day) was higher than other duration and dose groups. Those findings illustrate the accumulative effects of smoking is potential and harmful, and for current smokers, immediate cessation should be highly encouraged because it offers the only realistic way to avoid a substantial increase in lung cancer mortality caused by further continuation of smoking.

It is a remarkable fact that the risks of smoking in this study for some cancer death are not as strong as those observed in western developed countries (e.g. esophagus cancer and lung cancer) [36], which might be due to:(1) the absolute level of death is higher in China; (2) although China has a long history of smoking, the peak of consumption of cigarette lagged behind the developed countries for nearly 50 years, and the harm caused by smoking was still in an early stage.

In addition, although the smoking prevalence and cancer mortality varied widely among different regions, the association between smoking and primary cancer deaths were significant statistically in all the cities. However, the regional distributions of the risk of smoking on death from main cancers (lung cancer, stomach cancer, liver cancer and esophagus cancer) were not congruent with the regional distribution of the mortalities for primary cancer, that is, the risk is not necessarily high in cities with a relative high cancer mortality, while for some cities with lower mortality rates, higher relevance between smoking and cancer deaths be observed. For example, the strength of the association between smoking and lung cancer deaths in Luoyang is the third highest among the 24 cities, with OR up to 3.85, While lung cancer mortality was 8.50/ million, which was below the average level. This could be explained by the relative high absolute mortality rate for those cities with high cancer death rates, which might attenuate the relative risk for smokers.

Some limitations of this study must be considered when interpreting the results. First, the mortality survey included 90% of all deaths, and 10% of the identified death certificates failed to list an informant (spouse and others). These non-ascertained cases and controls might differ with regard to their smoking status when compared to the ascertained cases and controls, which may lead to selection bias. However, given the overall high ascertainment rate and the large sample size, the results are not likely to be materially affected. Second, not all of the deaths were pathologic diagnosed. For instance, 14.4% of urban esophagus deaths were diagnosed only by clinical symptoms or by other methods, which might lead to some misclassification. Selection bias could occur if these misclassified causes of death were associated with a lower prevalence of smoking and the relative risk of smoking would tend to be underestimated

in this study. Third, recall bias, a measurement error from surviving spouses that might attenuate the association between smoking and deaths from primary cancers. Finally, we made no direct adjustment of those variables that also influence the occurrence of cancer, such as alcohol consumption, environmental pollution and socioeconomic status.

5. Conclusion

As an alternative control selection method in case-control study design, sex-matched case-spouse control design can be considered to be a valid and efficient control selection method for population based large-scale case-control study (e.g. nationwide case-control study). The most prominent advantage of this design is that an approximate random sample can be drawn through this method in a time-saving and resource-economical manner.

6. Acknowledgments

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Tobacco Addiction: Effect on Human Health

Chapter 2

Premalignant and Malignant Lesions of the Oral Cavity: Tobacco as an Etiological Factor

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Abbreviations

WHO: World Health Organization; OSMF: Oral Submucous Fibrosis; HPV: Human Papilloma Virus; OSCC: Oral Squamous Cell Carcinoma; NFHS: National Family Health Survey; GATS: Global Adult Tobacco Survey; GYTS: Global Youth Tobacco Survey; PVL: Proliferative Verrucous Leukoplakia; TSG: Tumour Suppressor Gene; UV: Ultra Violet; DNA: Deoxyribonucleic Acid; HTLV: Human T-Cell Lymphoma Virus; HIV: Human Immunodeficiency Virus; CD: Cluster Of Differentiation; EBV: Epstein-Barr Virus; HHV8: Human Herpes Virus 8; HBV: Hepatitis B Virus; P53: Tumor Protein 53; Rb: Retinoblastoma Protein; ICAM: Intercellular Adhesion Molecule; TGFR: Transforming Growth Factor Beta Receptor; MMP: Matrix Metalloproteinase; TIMP: Tissue Inhibitor Of Metalloproteinase

1. Introduction

The precancerous lesion was defined by World Health Organization (WHO), in 1978 as a morphologically altered tissue associated with a significantly increased risk of cancer. Precancerous lesions of oral cavity include oral submucous fibrosis (OSMF), Plummer Vinson syndrome, erosive lichen planus, dyskeratosis congenita, chronic hyperplastic candidiasis, Cowden's syndrome, discoid lupus erythromatosus, dystrophic epidermolysis bullosa, and xeroderma pigmentosa [1]. In 2005, WHO decided to use the term 'Potentially Malignant Disorders (PMD)' as it describes that the pathological condition may transform into cancer.

Precancerous lesions that are identified PMD are leukoplakia, erythroplakia, palatal changes associated with reverse smoking, oral lichen planus, OSMF, and discoid lupus erythromatosus [2]. Causative factors for PMDs include human papilloma virus (HPV), candida, tobacco, 'gutkha', areca nut, vitamins (such as, A, B, C, D, and E) deficiency and minerals (such as, iron, calcium, copper, zinc and magnesium etc) deficiency [3].

The majority of oral cancers are squamous cell carcinomas. The tongue, buccal mucosa, oropharyngeal region and floor of the mouth are the commonest sites for occurrence of the disease. Lesser affected regions of the oral cavity are the lips, gingiva, dorsal tongue and palate sites. Approximately ninety-five percent of oral squamous cell carcinoma (OSCC) occurs in people older than 40 years, with an average age at diagnosis of approximately 60 years [1]. Lip carcinoma accounts for approximately 12% of all non-cutaneous head and neck cancer. While most of the lip cancer involves lower lip, upper lip cancer involves only 2-7% of lip cancers, followed by commissural areas (<1%) which are less susceptible to OSCC [4]. Most common lip carcinomas are basal cell carcinoma and squamous cell carcinoma. Less common carcinomas are keratoacanthoma, minor salivary gland tumours, melanoma and mesenchymal tumours. Other head and neck cancers include tumours of the salivary glands, thyroid glands, lymph nodes, bone and soft tissue. The incidence of oral cancer is age-related, which may reflect time for the accumulation of genetic changes and duration of exposure to endogenous and exogenous factors (including chemical and physical irritants, viruses, hormonal effects, cellular aging and decreased immunological responses etc). Evidence from long term follow-up from immuno-suppressed patients after solid organ and hematopoietic stem cell transplantation elucidates that immunosuppression increases the risk of development of OSCC [5].

Tobacco consumption in smokeless and smoking form is considered as the major risk factors for premalignant and malignant lesions in oral cavity. Dried tobacco leaves are mainly smoked in cigarettes, cigars, pipe tobacco and flavored shisha tobacco. They are also consumed as snuff, chewing tobacco and dipping tobacco. Tobacco contains the alkaloid nicotine, a potent para-sympathomimetic stimulant. It also contains several potent carcinogens that include nitrosamines, polycyclic aromatic hydrocarbons, nitrosodicthanolamine, nitrosoproline, polonium etc [6]. Prevention and control of tobacco-induced oral mucosal lesions is the prime requisite currently and mainly involves measures undertaken at primary, secondary and tertiary levels. Primary prevention plays a pivotal role in controlling tobacco induced lesions and measures can be taken at policy level, community as well as individual level.

2. Global scenario for Oral Cancers and Tobacco Addiction

Worldwide, oral carcinoma is one of the most prevalent cancers and is one of the 10 most common causes of death. In 2012, approximately 145,400 deaths and 300,400 new cases were reported worldwide from oral cavity cancer (including lip cancer). The highest rates are

found in Melanesia, South-Central Asia, and Central and Eastern Europe (Figure 1), while the lowest is in Western Africa and Eastern Asia [7]. In USA alone, a total of 48,330 incidences of new oral cancers were reported in 2016, among which 34,780 were male and 13,550 were female. The estimated deaths in USA due to oral cancers were 9,750, among which 6,910 were male and 2,660 were female in 2016 [8]. In high-income countries, smoking causes approximately 71% of deaths due to oral cavity cancer (including pharynx) while the burden is 37% of deaths for low-to-middle-income countries. Alcohol is accountable for about 33% and 14% of deaths in high and low income countries, respectively [9]. However, for last few decades oral cavity cancer incidence rates have decreased significantly in Asia, Northern America, and Australia for both males and females, and in Southern and Western Europe for males only. Although, due to tobacco epidemic, the rates of oral cavity cancer incidences have increased in several countries of Eastern and Northern Europe for both sexes and in Southern and Western Europe for females only [10].

Oral use of smokeless tobacco is widely prevalent in the South-East Asia Region; the different forms include chewing, sucking and applying tobacco preparations to the teeth and gums. Smokeless tobacco products and betel quid with or without tobacco are the major risk factors for oral cavity cancer in Taiwan, India, and other South Asian countries [11]. Greater than 250 million people use smokeless tobacco products in the South-East Asian subcontinent; approximately 17% of total population in South-East Asia uses oral tobacco out of which 95% belong to India and Bangladesh [12]. Increasing use has been reported not only among men, but also among such vulnerable groups as children, teenagers, women of reproductive age and by immigrants of South Asian origin wherever they have settled. A global epidemiological study held among the cohort of young individual revealed high (10-20%) prevalence of use of smokeless form of tobacco in adolescents (13-15 year) in South-East Asia. Among the downtrodden youths (45%-71%) prevalence of tobacco use has been studied in South-East Asia [13]. High incidence rates have been reported in developing nations like India, Pakistan, Bangladesh, Taiwan and Sri Lanka. While an increasing trend has been observed in Pakistan, Taiwan and Thailand, a decreasing trend is seen in Philippines and Sri Lanka. The mean age of occurrence of cancer in different parts of oral cavity is usually between 51-55 years in most countries [14-16].

Considering all age groups men are more affected than women. It is true when we observe the male versus female incidences for oral cavity cancers in different parts of Asia. The age-standardized rate for 2012 study for incidences per 100,000 people showed highest rates for male and females in Melanesia, which are 22.9 and 16 respectively. The incidences oral cancer for male and females are 9.9 and 4.7 in South Central Asia, 4 and 2.5 for South-Eastern Asia; 2.7 and 1.6 for Western Asia and 2.4 and 2.2 for Eastern Asia respectively [7]. Among African continents the oral cancer incidences for male and females are 6.3 and 2.3 for Southern

Africa, 4.5 and 2.8 for Eastern Africa; and 1.7 and 1.4 for Western Africa respectively. Among European continents, the oral cancer incidences for male and females are 9.1 and 2 for Central & East Europe, 7.9 and 3.2 for Western Europe, 5.9 and 3.2 for Northern Europe and 5.8 and 2.1 for Southern Europe respectively. Among Americans, Northern America has highest incidences of oral cavity cancers, 7.2 and 3.2 for male and female respectively. South America has 5.3 and 2.4, and Central America has 2.6 and 1.7 incidences of oral cavity cancers for males and females respectively per 100,000 people [7]. Australia has incidences of 8.3 and 3.7 for males and females respectively out of 100,000 people. Aetiological factors for oral carcinoma in these countries include high rate of smoking tobacco, increased alcohol consumption, diet low in fruits and vegetables. Overall incidence of oral cancer in Australia is decreasing. There is a high prevalence of oropharyngeal carcinoma due to HPV infection in these countries, which has a better prognosis than that induced by smoking [17,18].

3. Tobacco consumption in India

Tobacco addictions, in the forms of both smoking and smokeless, are the main reasons for the increasing incidence of oral cancers in India. The social awareness for the hazards of tobacco use is very minimal in India. Moreover, the low socio-economic status and low nutritional value-diet, lacking vegetables and fruits, contribute towards higher risks for cancer development. In addition, viral infections, such as HPV and poor oral hygiene, are other important risk factors. Poor oral hygiene has been advocated as a risk factor for oral cancer. This has been assessed by measuring tooth loss or status of the dentition and periodontal disease. Poor general oral condition associated with increased risk of development of OSCC in both genders [19,20]. However, from current trend of tobacco smoking among young people aged 15 years and over in India, WHO predicts that the tobacco usage will be reduced by 30% in 2025 with respect to the statistics for 2010 (Table 1) [16].

In some parts of India, such as the states of Bihar and Maharashtra, smokeless tobacco use is more common than smoking. Apart from regional preferences due to different socio-cultural norms, the preference for smokeless tobacco is inversely related to education and income. In countries of South Asia, particularly India, traditional values do not favour smoking by the young or by women, but there is no such taboo against using smokeless tobacco. Most women, who use tobacco in India, use it in smokeless forms. In India it has been estimated that roughly one-third of women and two-thirds of men use tobacco in one form or another. In an epidemiological study conducted in eight rural areas of India, smokeless tobacco use was 3–53% among men and 3–49% among women. Moreover, 2-26% of men and 0–4% of women of these areas were indulged in consuming both smoking and smokeless forms of tobacco [21]. A study conducted by National Family Health Survey (NFHS) during 2005–06, found that tobacco use is more prevalent among men, illiterates, poor, and vulnerable section of the society [22]. Another study conducted among individuals of 15 years of age or older in 2009–10

by Global Adult Tobacco Survey (GATS) indicates that 47.9% adult males and 20.3% adult females are tobacco users. About 24.3% males and 2.9% females of the adults use smoke, while 32.9% males and 18.4% females use smokeless tobacco. Global Youth Tobacco Survey (GYTS) conducted a study among 24,000 students (aged 13-15 years) in 2009, and found that 14.6% students were tobacco users in India [23].

Smokeless tobacco use showed a variation of usage from 7.2% to 59.4% in different states of India. In a survey from Mumbai, the smokeless tobacco use was 57.1% among women and 45.7% among men [12]. In Trivandrum and Kerala chewing habits were observed by 26.8% men and 26.4% women of the studied population [24]. In Jammu & Kashmir, Goa, Himachal Pradesh, Haryana, Punjab, Kerala, Andhra Pradesh, Tamil Nadu, Delhi, Karnataka, Meghalaya, Rajasthan and West Bengal individuals mainly consumed smoking forms of tobacco whereas in Maharashtra, Uttar Pradesh, Sikkim, Madhya Pradesh, Assam, Orissa, Bihar, and Arunachal Pradesh smokeless tobacco use predominated. In Gujarat, Manipur and Mizoram areas, equal frequency of usage of smokeless and smoking tobacco was observed among men while among female the ratio was 5:1 respectively. In North Eastern states of India females are reported to be the extensive user tobacco Gul than men and a significant population of Assam, Meghalaya, Nagaland, Sikkim, and Mizoram are affected with the tobacco epidemic. Tobacco water is used extensively in North East Indian states, especially Mizoram and Manipur. Frequency of use of tobacco water use was almost similar among male and females [12,25].

Who surveyed a study on tobacco addiction for more than 10 years of span with 35,288 respondents of Karnataka and 29,931 respondents of Utter Pradesh. According to the study, tobacco use in smokeless form was predominant among women and among men less than 30 years in both urban and rural areas; however, smoking was the predominant form of tobacco use among men more than 30 years age. The overall prevalence for use of smokeless tobacco was observed to be 13.9% in Karnataka (13.4% among men and 14.4% among women) and 17.5% in Uttar Pradesh (24.3% among men and 6.6% among women). Use of smokeless tobacco was higher among females as compared to males in the age-groups above 40 years in Karnataka [25]. In Uttar Pradesh, the proportion of men using smokeless tobacco was higher than women, in all age-groups and prevalence of smokeless tobacco use increased with age in both sexes. Betel-tobacco quid was found to be extensively consumed in Karnataka, but had limited practice in Uttar Pradesh. The prevalence rate of use of this tobacco modality was 14.2% (26.9% among males and 0.6% among females) in Karnataka and 2.0% (2.3% among males and 1.4% among females) in Uttar Pradesh. Overall, a higher prevalence among rural areas was observed in all age-groups of Karnataka as compared to urban areas, but the trends were variable in different age-groups in Uttar Pradesh. An inverse correlation of decrease in prevalence rates of betel-tobacco quid/smokeless tobacco use with increasing educational

levels was observed in different age-groups in Karnataka, and similar pattern was noticed only among females in Uttar Pradesh. An inverse association of betel-tobacco quid use with increasing family income levels was observed in Karnataka but not in Uttar Pradesh[12,25].

4. Premalignant disorders of the Oral Cavity

The mechanisms for transformation of the oral premalignant disorders into malignancies are not well understood; among many hypotheses the 'field cancerization theory' is the most accepted. Field cancerization involves the formation of multiple areas of premalignant disease with a higher-than-expected rate of multiple local second primary tumors. Many theories have been proposed to explain the occurrence of carcinomas in specific sites. One theory states that multiple squamous cell lesions occur irrespective of each other. This is due to the exposure of the oral cavity to carcinogens at the same time leading to multiple genetic aberrations in the entire area. Another theory states that multiple lesions arise due to the migration of dysplastic and altered cells with two different patterns, (a) migration of malignant cells through the saliva (micro metastasis); (b) intra-epithelial migration of the progenitor cells of initially transformed malignant cells. The mechanism is different from the metastasis, since metastatic cells are usually transported through lymphatic and vascular system from primary cancer sites [26,27].

Potentially malignant oral lesions reflect underlying cellular changes, which are either red or white or mixed red and white appearance. Along with the clinical manifestations, the associated cellular changes are termed as dysplasia, which is defined as loss of uniformity of individual cells and their architectural orientation. The WHO 2005 classification recognizes five histopathological stages in the epithelial precursor lesions: (i) Squamous hyperplasia: It can be acanthosis and basilar hyperplasia. The histopathology shows regular stratification without cellular atypia. (ii) Mild dysplasia: The architectural disturbance is restricted to the lower third of the epithelium accompanied by mild cytological atypia. (iii) Moderate dysplasia: The dysplastic change extends to the middle third of the epithelium with moderate cytological atypia. (iv) Severe dysplasia: The dysplastic changes involve more than two-thirds of the epithelium with severe cytological atypia. (v) Carcinoma-in-situ: Full thickness architectural disturbance in the viable cell layers accompanied by pronounced cytological atypia [2,28].

Oral leukoplakia is defined as a predominantly white lesion of the oral mucosa. The disorder can further be divided into a homogenous and a non-homogenous type. The homogenous form is clinically characterized as a white, well-demarcated plaque with an identical clinical appearance all throughout the entire lesion (Figure 2). The surface texture may vary from a smooth thin surface to a leathery appearance referred to as "cracked mud". Another characteristic feature is the lack of a peripheral erythematous zone in homogenous leukoplakia. If the surface texture is homogenous but contains papillary (nodular) or exophytic components, the leukoplakia is also regarded as nonhomogenous. The nonhomogenous type of oral leukopla-

kia may show white patches or plaque amidst red tissue. Due to the concomitant occurrence of white and red areas, the nonhomogenous leukoplakia is also called erythroleukoplakia or speckled leukoplakia. The clinical manifestation of the white areas may vary from large white verrucous areas to small nodular structures. Both homogenous and nonhomogenous leukoplakia may be observed in all sites of oral mucosa [28]. Oral leukoplakia includes white component dominated by papillary or finger-like projections, which are referred to as verrucous or verruciform leukoplakia. Oral leukoplakia with this clinical manifestation but with a more angry-looking growth pattern and recurrence rate is designated as proliferative verrucous leukoplakia (PVL) having high malignant potential. As the common surface pattern is similar to oral papillomas, the PVL is suspected to have a viral etiology. Oral erythroplakia has not been so well studied as leukoplakia. It is defined as a red, velvety, plaque-like lesion of the oral mucosa that cannot be characterized as any other definable lesion (Figure 3) [28,29].

5. Signs & symptoms of oral malignant lesions

The classical complaint of patients suffering from OSCC is discomfort which compels them to immediate treatment. Dysphagia, odynophagia, otalgia, limited movement, oral bleeding, neck masses and weight loss may occur with advancement of the disease. The high risk sites for oral carcinoma include the lower lip, the anterior floor of the mouth and the lateral borders of the tongue. The patient may develop tissue changes, which include a red, white or mixed red and white lesion and a change in the surface texture producing a smooth, granular, rough or crusted lesion or ulceration (Figure 4). The lesion may be flat or elevated and ulcerated or nonulcerated and may be minimally palpable or indurated. Loss of function of tongue can affect speech, swallowing and food intake [28,29].

Lymphatic spread of oral carcinoma usually involves the submandibular and digastric nodes, the upper cervical nodes, and finally the remaining nodes of the cervical chain. The nodes most commonly involved are those that are on the same side as the primary node, although the closer the tumour is to the midline and the more posterior in the oral cavity or oropharynx, the more common are the involvement of the bilateral or contralateral nodes. The nodes are not tender until and unless they are associated with secondary infection or an inflammatory response is present, which may occur after biopsy [28]. Oral malignant melanomas are relatively rare cancers and occur commonly in the maxillary gingival, more frequently on the palate with fewer incidences in the mandibular gingival [30]. Though, these lesions are biologically aggressive, they are clinically asymptomatic in the early stages and usually present merely as a hyperpigmented patch on the gingival surface (Figure 5).

Verrucous carcinoma is described clinically as papillary, verrucoid, fungating or cauliflower-like and may develop from progression of proliferative verrucous leukoplakia that progress to carcinoma (Figure 4). Verrucous carcinoma rarely spreads through lymphatic route

and generally remains locally destructive. There are a few other variants of SCC other than verrucous carcinoma. Basaloid carcinoma is composed of solid growth of basaloid cells with small cystic spaces. It has been suggested that HPV-associated cancers of oral cavity are more likely to have basaloid features. Spindle cell carcinomas are rare variants of SCC, where epithelial changes ranging from prominent dysplasia to frank SCC in conjunction with a dysplastic spindle cell element [28].

6. Etiopathogenesis for Oral Cancer

Carcinogenesis is a genetic process that leads to a change in tissue morphology and cellular behavior. The development of oral malignancy is attributed to a powerful alliance of two factors: nicotine and carcinogens. Nicotine is addictive and toxic, but it is not a carcinogen. This addiction, however, causes people to use tobacco products continually, and these products contain many carcinogens. Carcinogens alter the gene responses for proto-oncogenes and tumour suppressor genes (TSGs) that ignite the pathogenesis of oral carcinoma. Other genetic factors which play a major role in oral cancer include chromosomal aberrations in the telomere region of chromosome, genetic mutations of proto-oncogenes and TSGs, or epigenetic changes like DNA methylation or histone modification [6]. In addition, angiogenesis, immune function and hemostatic regulation of surrounding normal cells also play important roles in disease pathogenesis (Figure 6).

Tobacco and alcohol are great risk factors for oral and oropharyngeal cancers. Nitrosamines, polycyclic aromatic hydrocarbons, nitrosodicthanolamine, nitrosoproline, and polonium are the potent carcinogens in tobacco. Carbon monoxide, thiocyanate, hydrogen cyanide, nicotine and its metabolites are major constituents in tobacco smoke. Epidemiological studies have found that up to eighty percent of oral cancer patients are associated with smoking. In addition to the risk of primary cancers, the risk of secondary and recurrent primary oral cancers is related to continuation of smoking after cancer treatment. A follow up study for 1 year of primary oral cancer patients (after surgery) found that 18% of these patients develop a recurrent oral cancer, and those who continued to smoke had a 30% risk of secondary oral cancer development [31]. It has been suggested that the deleterious effect of smoking on the development of cancer decreases 5 to 10 years post stoppage. The incidence of OSCC varies worldwide and may be explained partly by differences in the use of tobacco products. Benign hyperkeratosis and epithelial dysplasia have been reported following short-term use of smokeless tobacco and chronic use is associated with an increasing incidence of malignant lesions [32].

Genetic damage is the step towards the process of carcinogenesis. Three classes of carcinogenic agents have been identified: 1) chemical, 2) radiant energy and 3) microbial agents. Another cause of oral carcinogenesis is mechanical trauma; for example, sharp cuspal edges of teeth causing trauma to the buccal mucosa or lateral border of the tongue. Chemicals and radi-

ant energy are the primary causes of cancer in humans whereas oncogenic viruses are involved in the pathogenesis of cancer in mainly animals and some human tumours.

Chemical carcinogens have highly reactive electrophile groups that directly damage the DNA. There are two types of chemical carcinogens: direct and indirect acting. Direct acting agents (for e.g. alkylating agents used for chemotherapy) are carcinogenic from the initial states, whereas indirect acting agents (for eg. benzopyrene, azo dyes, aflatoxins) are not carcinogen during initial states and are converted into carcinogens by endogenous metabolic pathways. Hence, polymorphisms of endogenous enzymes that are critical for metabolic conversion of chemicals compounds (such as, cytochrome P-450), may promote carcinogenesis.

Radiations, such as UV rays of sun, X-rays, radio-nucleotides etc, can also induce DNA breakage and considered as carcinogen. Ionizing radiations cause chromosomal aberrations, translocations and less frequently point mutations, leading to genetic damage and carcinogenesis. UV induces the formation of pyrimidine dimers within DNA, and may lead to carcinoma and melanoma of the skin [28].

The study of oncogenic retroviruses in animals has provided spectacular insights into the genetic basis of cancer. The human T-cell lymphoma virus-1 (HTLV-1) has been demonstrated to cause cancer in humans. HTLV-1 has been reported to be associated with a variant of T cell leukemia/lymphoma in humans. Similar to the HIV, HTLV-1 has affinity for CD4+T cells, and these T cells are the major victims of neoplastic transformations. The role of viruses, such as HTLV-1 and HPV are a new parameter for the pathogenesis of human cancers. The HTLV-1 genome, in addition to the usual retroviral genome, contains a unique region called the pX which encodes a major TAX protein, which turns on genes for cytokines and their receptors in infected T cells. Although this proliferation is initially polyclonal, the proliferating T cells are at increased risk for secondary mutations that lead to the outgrowth of a monoclonal leukemia. By interfering with several transcription factors, such as NF-κB, the TAX protein can transactivate the expression of genes that encode cytokines, cytokine receptors and co-stimulatory molecules. This inappropriate gene expression leads to autocrine signaling loops and increased activation of promitogenic signaling cascades. Furthermore, TAX can drive progression through the cell cycle by directly binding to and activating cyclins. In addition, TAX can repress the function of several tumour suppressor genes that control the cell cycle, including P16 and P53 [33,34].

Recently, several DNA and RNA viruses, have been identified and correlated to the developement of OSCC. Four DNA viruses HPV, Epstein-Barr virus (EBV), human herpes virus 8 (HHV8), and Hepatitis B virus (HBV) are gaining immense attention in cancer biology because they are strongly associated with human cancer. Very recently, the association of HPV, specifically HPV-16 and -18, with oral squamous cell carcinoma is also gaining importance.

Certain EBV gene products contribute to oncogenesis by stimulating a normal B cell proliferation pathway [34,35].

7. Conclusion

Oral mucosa, salivary gland and jaws are the sites of oral cavity that are affected during pathogenesis. Neoplastic lesions of oral cavity include fibromas, leukoplakia, erythroplakia and OSCC. The risk for erythroplakia to undergo malignant transformation is higher than leukoplakia. OSCC covers 90% of cancer of oral cavity and is highly associated to tobacco and alcohol consumption. In recent times a trend in oral cancer is observed in young adults and the risk of OSCC is being increased. Among young adults with any habit of tobacco consuming, tongue and buccal mucosa are the sites for occurrence of oral cancer. Immune factors, dietary factors, genetic factors and oral sex are the major etiology of OSCC in young patients. Furthermore, according to the Oral Cancer Foundation, oral cancer now affects one woman for every two men, as compared to the earlier trend of six men for every woman. The incidence of HPV associated oral lesions is also increasing in recent years. HPV oncoproteins, mainly E6 and E7 bind to Rb (retinoblastoma) and p53 thus regulate their functions. More than 30 different types of tumors may arise in salivary glands, among which mucoepidermoid carcinoma, composed of mixtures of sqamous and mucous cells, is the most common malignant tumor. Mucoepidermoid carcinoma is reported to disregulate the Notch signaling pathway. Both pre-malignant and malignant lesions of oral cavity are mainly caused by tobacco, a great risk factor for different diseases. Tobacco specific nitrosamines have been detected in saliva of tobacco chewers. These nitrosamines are carcinogen that modify DNA and cause mutagenesis. The underlying mechanisms for OSCC still remain unknown. Several reports documented the involvement of several proteins, including P53, Rb, ICAM-5, TGFR, MMP-8, and TIMP-1 in development of OSCC. Moreover, OSCC develops frequently in immune-suppressed individuals, because the disease aggressiveness is directly associated with altered immune responses. Several approved drugs, targeting diverse factors of cancer are clearly not enough for the present times (Figure 7). New drugs are being developed targeting each of the enabling signaling molecules that are contributing in cancer development, thus hold promise as cancer therapeutics. Moreover, social awareness against tobacco and self-awareness for early diagnosis are required worldwide for oral cancer prevention.

8. Table

Table 1: Current trend of tobacco smoking among young people aged 15 years and over in India [16].

CURRENT TOBACCO SMOKING (%)										
Year	Men			Women			Both sexes			
	Lower 95% CI	Point estimate	Upper 95% CI	Lower 95% CI	Point estimate	Upper 95% CI	Lower 95% CI	Point estimate	Upper 95% CI	Estimated no. of current smokers
2000	23.7	33.8	46.9	3.7	5.7	7.8	14.0	20.2	28.0	138,505,200
2005	22.1	28.0	34.3	2.9	3.8	4.7	12.8	16.3	19.9	124,176,100
2010	19.1	23.5	28.1	1.9	2.5	3.0	10.7	13.3	15.9	111,856,400
2015	13.7	19.9	26.3	1.2	1.7	2.2	7.6	11.0	14.6	101,399,700
2020	9.5	17.0	25.3	0.7	1.1	1.7	5.6	9.3	13.8	91,913,300
2025	6.4	14.6	25.1	0.4	0.8	1.3	3.5	7.9	13.5	83,514,000
Voluntary target (30% relative reduction from 2010 to 2025		16.5			1.8			9.3		

Men - Fitted current tobacco smoking (%) Women - Fitted current tobacco smoking (%)

9.Figures

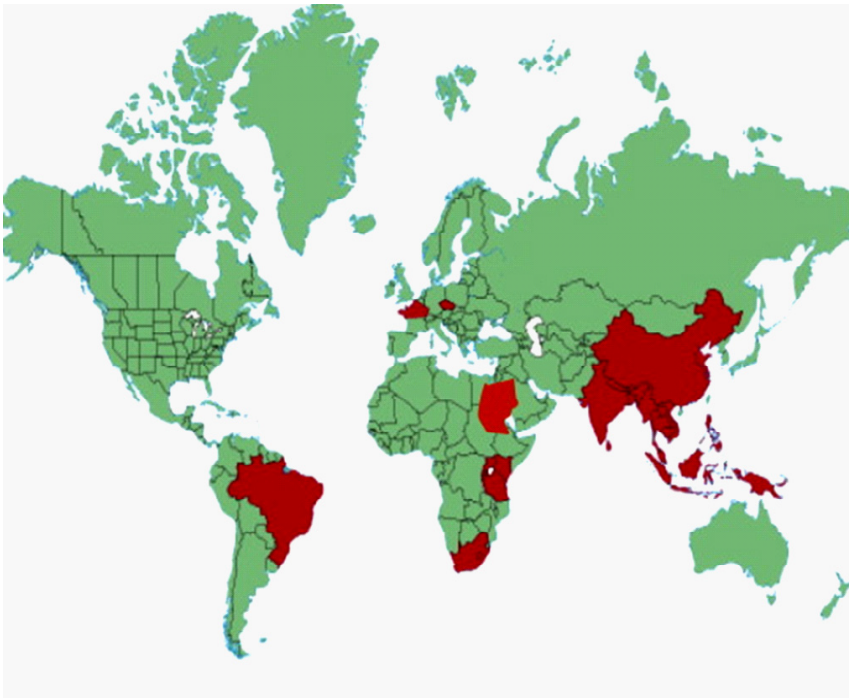

Figure 1: Countries with high incidence and mortality from oral cancer (in red). The areas characterized by high incidence rates for oral cancer (excluding lip) are found in the South and Southeast Asia (e.g. Sri Lanka, India, Pakistan and Taiwan), parts of Western (e.g. France) and Eastern Europe (e.g. Hungary, Slovakia and Slovenia), parts of Latin America and the Caribbean (e.g. Brazil, Uruguay and Puerto Rico) and in Pacific regions (e.g. Papua New Guinea and Melanesia) [15].

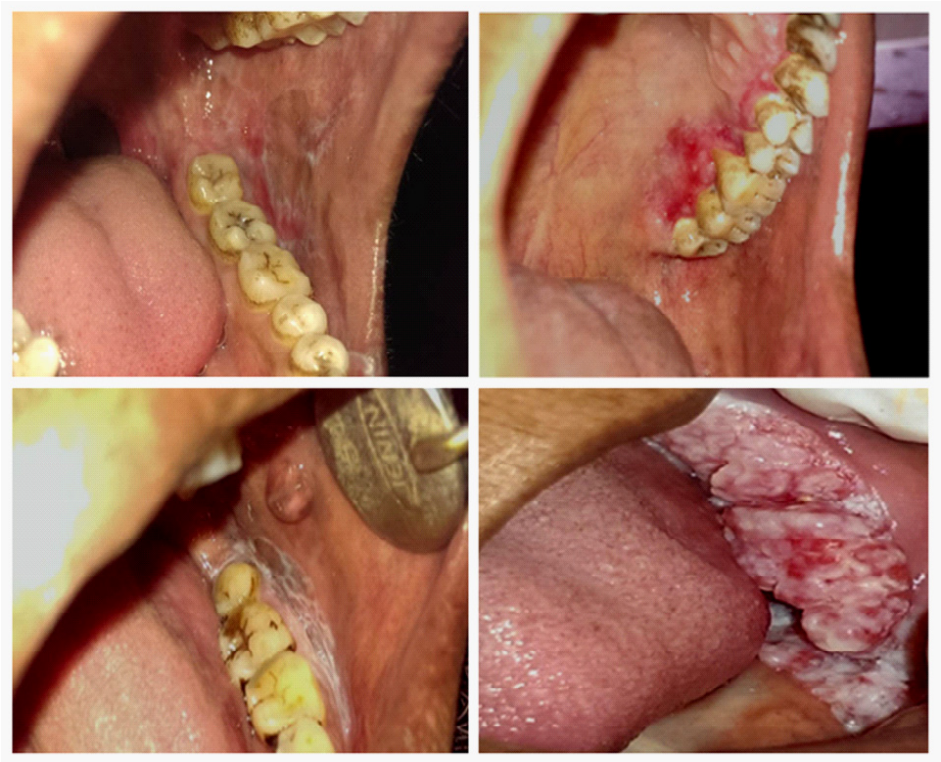

Figure 2: (in clockwise manner) Reticular lichen planus in the left buccal mucosa of a patient; erosive lichen planus in the upper left palatal aspect of an immunocompromised patient; proliferative verrucous leukoplakia in a patient having habit of retaining 'gutkha' in the buccal vestibule; speckled leukoplakia in the left buccal vestibule (all pictures were taken in the Dept of OP&OD of Burdwan Dental College & Hospital).

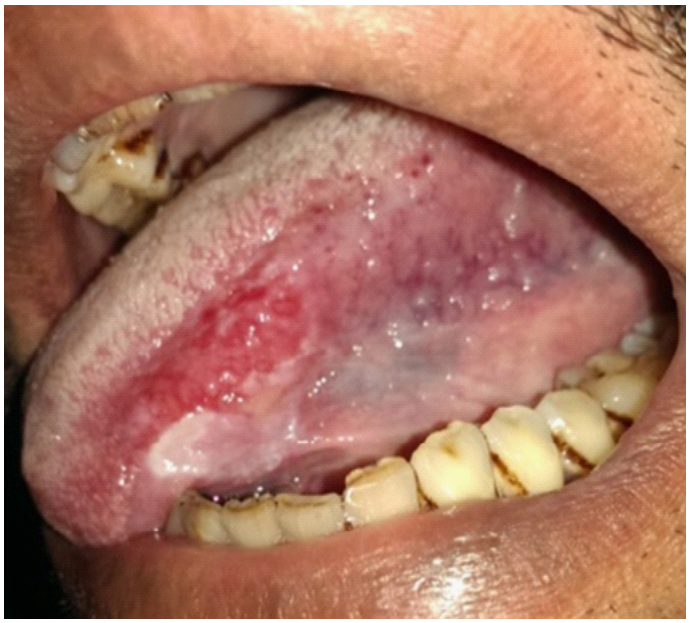

Figure 3: An ulcerated area on the left lateral border of the tongue of a patient suggestive of erythroplakia with areas of leukoplakic growth situated posterior to the reddened area (pictures taken in the Dept. of OP&OD of BDC&H)

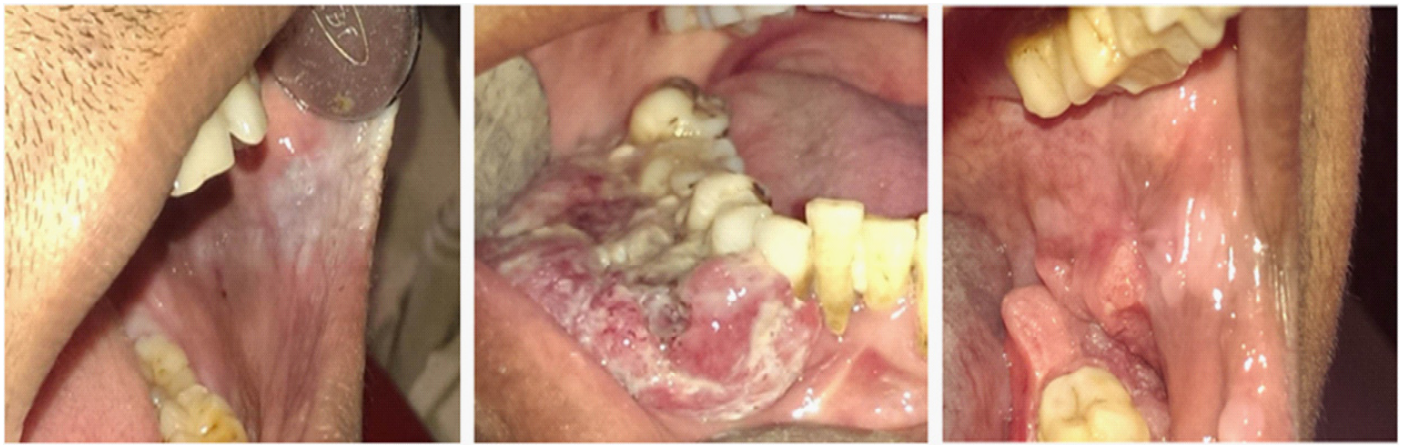

Figure 4: (from left to right) Hyperkeratotic area significant of epithelial dysplasia seen in the left buccal mucosa; Carcinoma in situ of the left buccal mucosa; Squamous Cell Carcinoma of the right buccal mucosa and associated alveolus. The lesion was associated with extensive bone loss, (pictures taken in the Dept. of OP&OD of BDC&H)

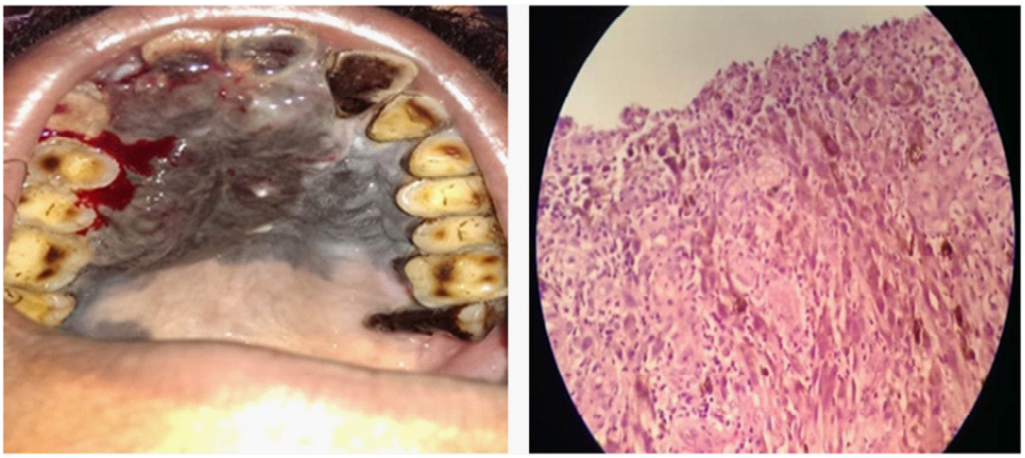

Figure 5: Malignant melanoma of the hard palate(left); Histological section stained with H&E showing malignant melanoma from sample collected from the same patient (right) (slide picture taken in the Dept. of OP of BCD&H).

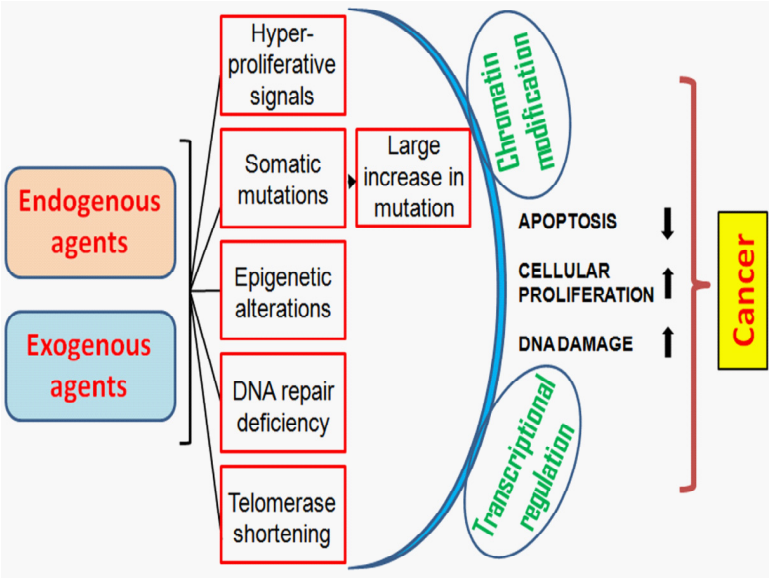

Figure 6: Endogenous and exogenous agents affecting somatic mutations, epigenetic alterations, telomerase shortening and DNA damage that leads to cancer progression through chromatin modifications and transcriptional regulation of oncogenes.

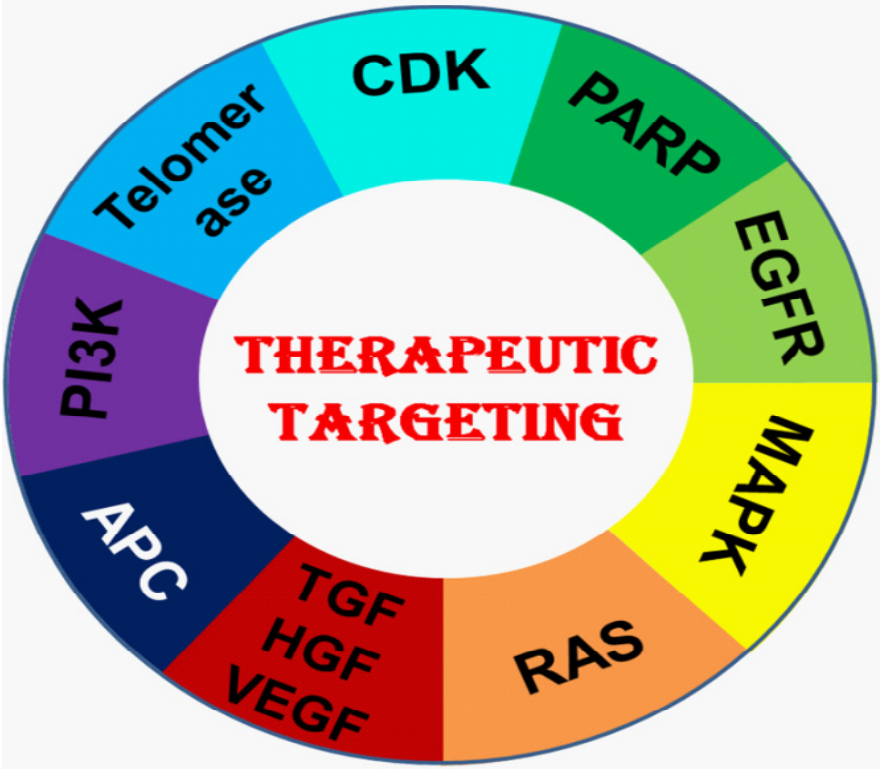

Figure 7: Different signaling molecules associated to cancers that are targeted for therapeutic purpose. CDK, Cyclin-Dependent Kinase; PARP, Poly ADP-Ribose Polymerase; EGFR, Epidermal Growth Factor Receptor; MAPK, Mitogen-Activated Protein Kinase; TGF, Transforming Growth Factor ; HGF, Hepatocyte Growth Factor; VEGF , Vascular Endothelial Growth Factor; APC, Anaphase-Promoting Complex, PI3K, Phosphatidylinositide 3 Kinase.

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Tobacco Addiction: Effect on Human Health

Chapter 3

Tobacco and its Ill-Effects

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1. What is Tobacco?

Tobacco is a plant called 'nicotiana tabacum', whose dried leaves are used to make different forms of tobacco [1]. All forms of tobacco contain nicotine - the chemical responsible for addiction [2,3]. Although extremely lethal, tobacco is cultivated in many regions of the world and is legally available [1].

Tobacco products can be broadly classified into smoked and smokeless products. Smoked forms of tobacco are those substances which are burnt and the resultant smoke is inhaled or held in the mouth [4]. While on the other hand, smokeless tobacco products are used either orally or nasally without burning the product [5]. The different types of tobacco products have been listed below [4,5,6].

Smoked forms of tobacco
Cigarettes
Cigar
Bidis
Kreteks
Pipes
Hookah
Cheroots
Chuttas
Dhumtis
Hookli
Chillum

Smokeless forms of tobacco	
Gutkha	Snuff
Zarda	Snus
Khaini	Toombak
Khiwam	Chimo
Mawa	Iq'mik
Gul	Maras
Gudhaku	Shammah
Mishri	Tobacco chewing gum
Naswar	Tobacco tablets
Betel quid	Red tooth powder

2. Health Effects of Tobacco Use

Tobacco, both smoked as well as the smokeless variety has been known to cause a number of health hazards. It is the leading preventable risk factor responsible for global burden of deaths [7,8], estimated to kill half of its users [9,10]. Six of the eight leading causes of death in the world are attributed to tobacco use [11] (**Figure 1**).

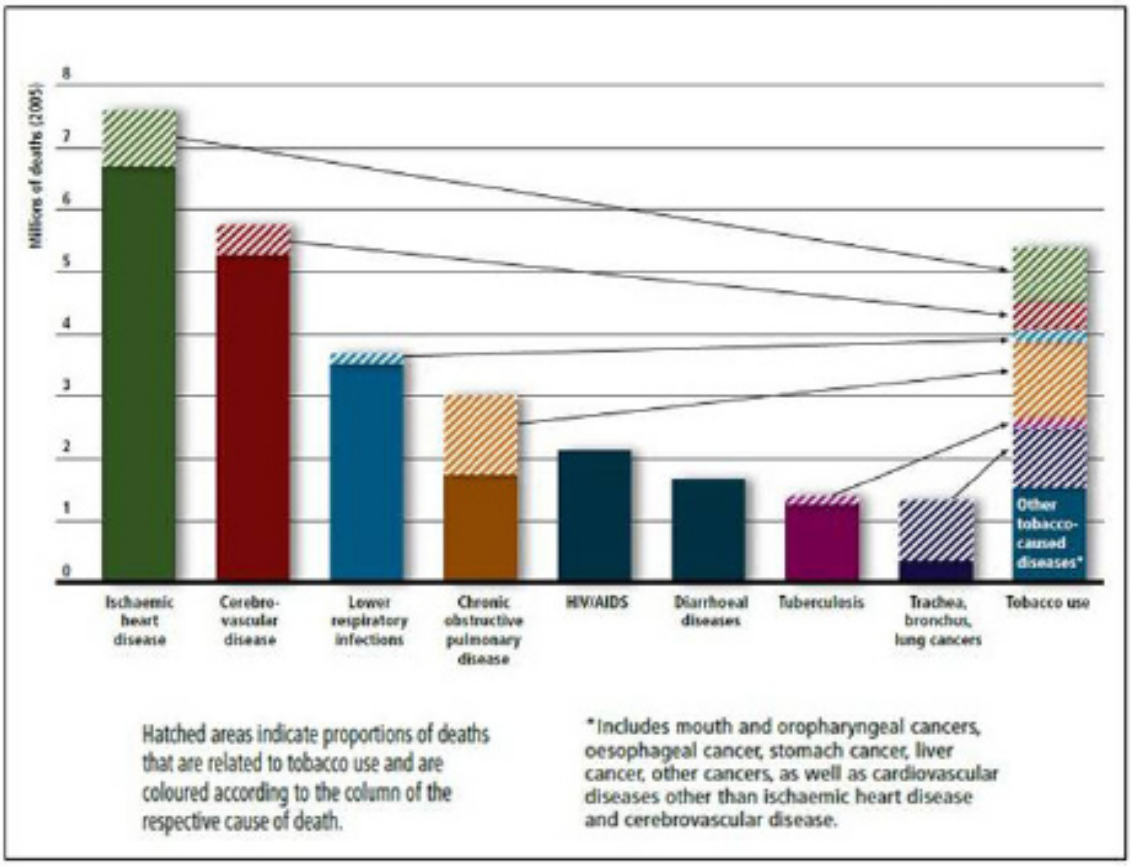

Figure 1: Proportion of deaths related to tobacco use [11]

Tobacco caused an estimated 5.1 million deaths globally in 2004, or almost one in every eight deaths among adults aged 30 years and over [8].

Tobacco in any form is damaging to the human body and almost every organ is affected by its use [12,13] (**Figure 2**). No tobacco product is less harmful than the other – they all have the same consequences, health-related as well as socio-economic. The most vulnerable population groups affected by tobacco are the youth, middle-aged men and even new born infants.

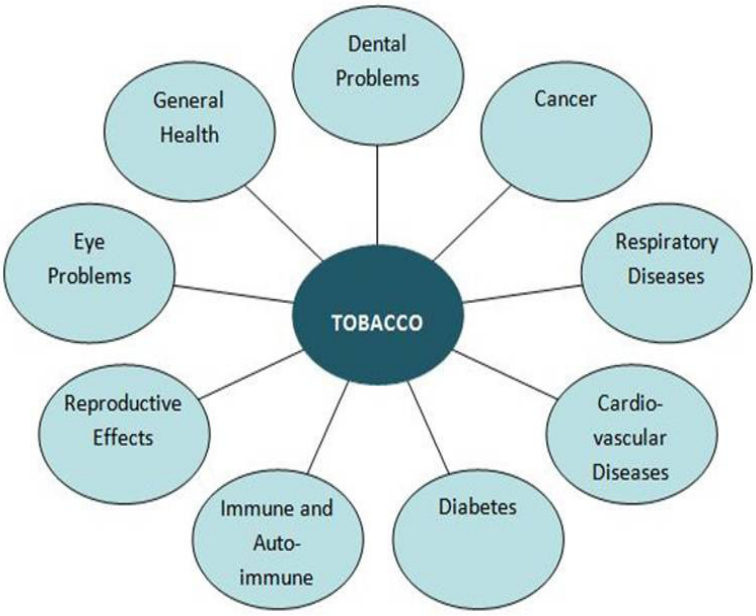

Figure 2: Health Effects of Tobacco Use

2.1. Health effects of smoked tobacco products

Cigarettes are the most commonly used smoked tobacco products across the world [6,10]. Some other forms that are popularly smoked in specific countries, particularly those in the South East Asia region, are pipes, cigars, bidis, kreteks, hookah, cheroots, chuttas, dhumtis, hookli, chillum, etc. [6].

Globally, smoking causes about 71% of lung cancer, 42% of chronic respiratory disease and nearly 10% of cardiovascular disease [8].

2.1.1. Cancer

Population studies have shown a causal link between smoking and lung cancer [8] (**Figure 3**). Among industrialized countries, where smoking has been common, smoking is estimated to cause over 90% of lung cancer in men and about 70% of lung cancer among women [1].

The risk of developing cancer of the lung for the combined group of pipe smokers, cigar smokers, and pipe and cigar smokers is greater than for non-smokers, but much less than for cigarette smokers [14].

Smokers of all ages have death rates two to three times higher compared to non-smokers. Those who smoke cigarettes, lose on average, about 10 years of life compared to non-smokers [15]. The average male cigarette smoker has approximately a 9 to l0 fold risk of developing lung cancer and a heavy smoker has at least a 20 fold risk, in comparison with non-smokers [14].

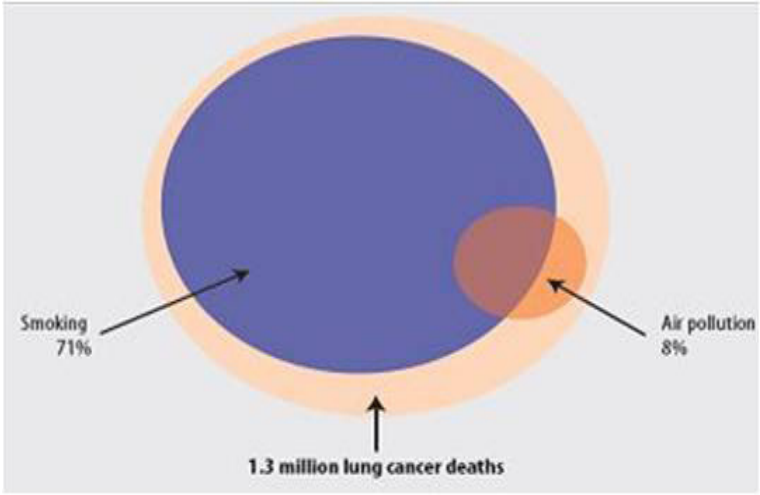

Figure 3: Lung cancer deaths in 2004: proportion attributed to smoking and air pollution [8]

Apart from lung cancer, smokers tend to develop cancer at other sites such as the oral cavity, larynx, oesophagus and urinary bladder [11] (**Figure 4**). Pipe smoking in particular leads to lip cancer [10,12,16].

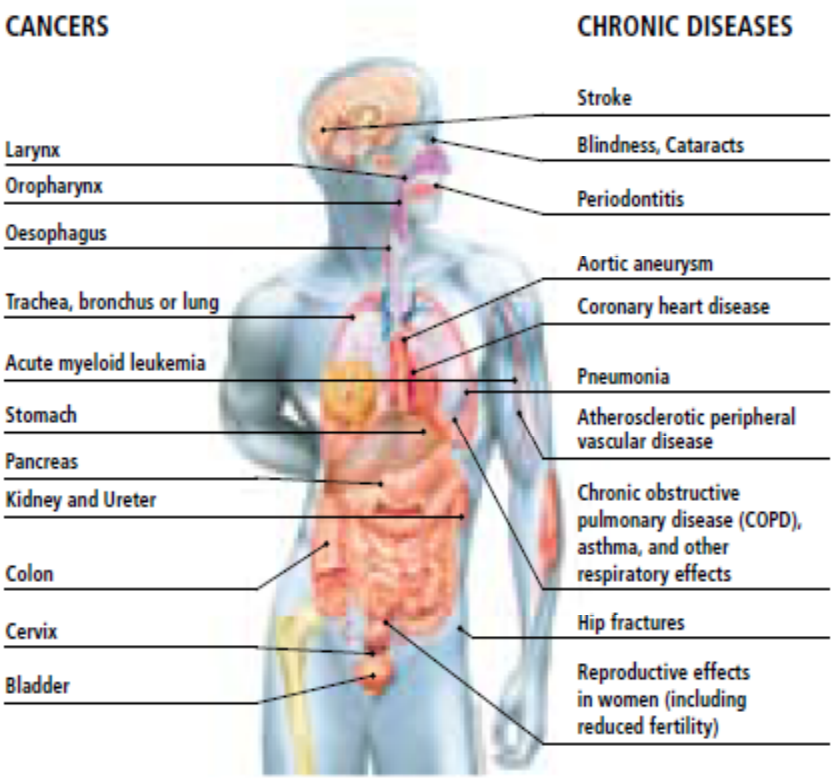

Figure 4: Health Effects of Using Smoked Tobacco Products [11]

2.1.2. Respiratory problems

Inhaled smoke and its components get deposited and absorbed into the lungs, affecting the respiratory system, exacerbating chronic lung diseases, and increasing the risk for respiratory infections [14].

Smokers also particularly suffer from pneumonia and reduced lung function [16].

2.1.3. Cardiovascular disease: [12]

Over the last two decades a considerable number of epidemiologic studies on different populations, employing different techniques, have shown with remarkable consistency a significant relationship between cigarette smoking and an increased death rate from coronary heart disease. Smoking leads to thickening of arteries, there by increasing the cardiovascular disease risk [10].

2.2. Other Health Effects of Smoking [10,12,16]:

1. Affected Vision: Smoking affects the vision leading to blindness and cataract.

2. Reproductive System: Smoking also affects the reproductive system of both males and females. In females, it has been known to cause ectopic pregnancy and reduced fertility, while in males it is linked with erectile dysfunction. Smoking during pregnancy can lead to still births and congenital abnormalities in the unborn, growing foetus.

3. Effects on the Oral Cavity: Inhaled smoke leads to bad breath, dark lips, loose teeth and gum problems.

4. Immune and auto-immune disorders: It compromises the equilibrium of the immune system, leading to general adverse effects on the body, altered immune function, and systemic inflammation. It increases the chances of arthritis and hip fractures.

5. Diabetes and Stroke: Smoking reduces the oxygen and blood circulation to vital body parts. Reduced oxygen to the brain leads to stroke. Diabetics who are smokers have trouble regulating their blood sugar levels leading to further complications [17].

The discussion on health effects of smoked tobacco products would be incomplete without a mention of hookah smoking. Hookah or water-pipe smoking has become popular, especially among the youth (**Figures 5 and 6**). It is a relatively new and disturbing trend that has the same dangers as any other smoked tobacco.

Figure 5: Youth Smoking Hookah (Source: AFP)

Hookah or water-pipe smoking is a social activity mostly done in groups, where the pipes are often shared among users [18]. Sharing of hookah carries the risk of transmission of infectious diseases such as tuberculosis. But, the commonly held belief is that, hookah smoking is a safe alternative to cigarette smoking. However, water-pipe and cigarette smoke contain similar toxic agents such as carbon monoxide, tar and nicotine as well as carcinogens such as arsenic, beryllium, chromium, cobalt and lead [18,19].

Most hookah smoking sessions typically last 60 minutes, where in a smoker may take around 200 puffs and inhales approximately a litre of smoke [20]. In contrast, cigarette smoking which lasts 5 to 7 minutes, where the smoker takes 8 to 12 puffs inhaling about 0.5 to 0.6 litres of smoke [21].

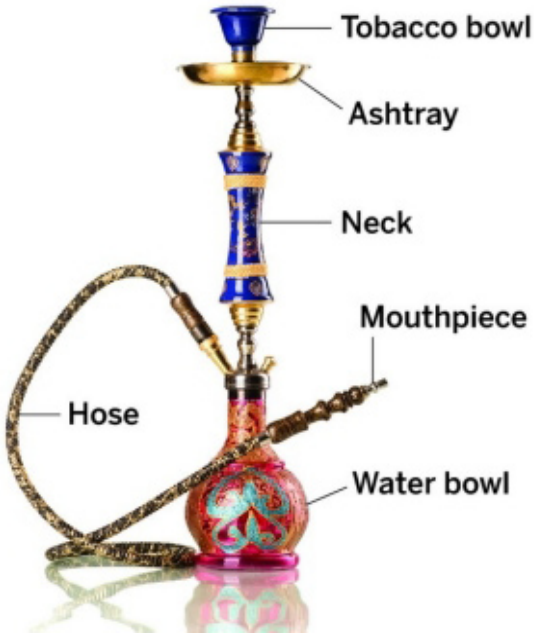

Figure 6: Hookah Components (Source: Shutterstock)

An hour long hookah smoking session involves inhaling 100 to 200 times the volume of smoke inhaled with a single cigarette [22]. Thus the amount of smoke inhaled in one hookah

session is equivalent to smoking around 100 cigarettes [20]. This form of smoking is made appealing to the youth by adding additives and flavours to make it more culturally and socially acceptable [20,23].

Hookah smoking also known as water-pipe tobacco smoking has been declared a public health problem by the World Health Organization and other authorities [23].

2.3. Health effects of smokeless tobacco products

Over 300 million people around the world, most of whom are south asians, use smokeless tobacco products [10]. Smokeless tobacco is consumed without burning the product, and can be used orally or nasally. Tobacco pastes or powders are used in a similar manner and applied to the gums or teeth. Fine tobacco mixtures are usually inhaled and absorbed in the nasal passages [5].

Smokeless tobacco contains carcinogens such as tobacco specific nitrosamines, cadmium, polonium, formaldehyde, lead and formaldehyde [29].

Oral smokeless tobacco products are placed in the mouth, cheek or lip and sucked or chewed [5]. These sites are thus common sites for development of oral cancers [29]. (**Figure 7**).

Red and white coloured lesions (called precancerous lesions) occur in the mouth of smokeless tobacco users. Failure to stop using tobacco can lead to progression of these lesions to cancer [5]. The presence of these lesions usually occurs in the location of the mouth where the smokeless tobacco product is kept. Oral cancer risk and severity increases with duration and intensity of tobacco use [5].

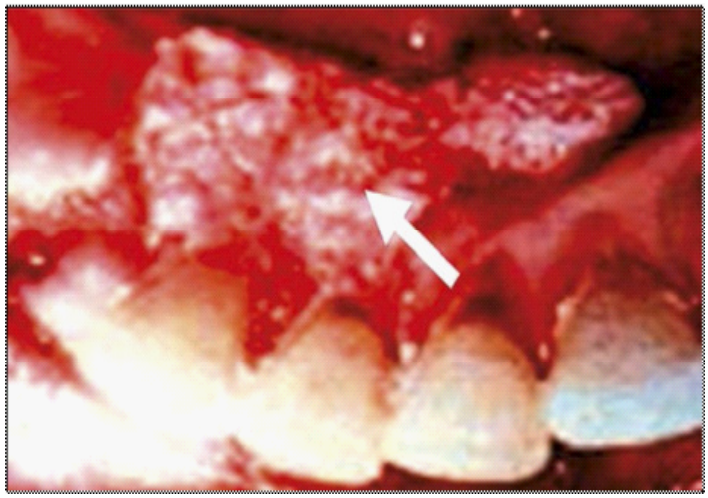

Figure 7: Oral Cancer caused by Smokeless Tobacco [29]

Apart from oral and oro-pharyngeal cancers, smokeless tobacco use increases the risk of cancer of the oesophagus and cardiovascular disease. It can also lead to gum problems [13].

Aesthetically, smokeless tobacco stains teeth and causes bad breath. It can also cause

cancer of the head and neck [30].

2.4. Health effects of passive smoking

Environmental Tobacco Smoke (ETS) or second hand smoke is the combination of smoke emitted from the burning end of a cigarette or other tobacco products and smoke exhaled by the smoker [24]. ETS causes disease not only in smokers but also in those who do not use any tobacco product. ETS contains the same toxic components that are released during active smoking [25]. It affects both adults and children [11] (**Figure 8**).

Exposure to tobacco smoke causes lung cancer in healthy non-smoking adults [12,24,25]. Breathing other people's smoke is also a cause of ischaemic heart disease, increasing a person's risk by a quarter [26].

Children who involuntary and unknowingly get exposed to tobacco smoke emitted in the environment face an increased risk for developing pneumonia, bronchitis, respiratory irritation and ear problems. In children who suffer from asthma, passive smoking exacerbates the disease [24,25].

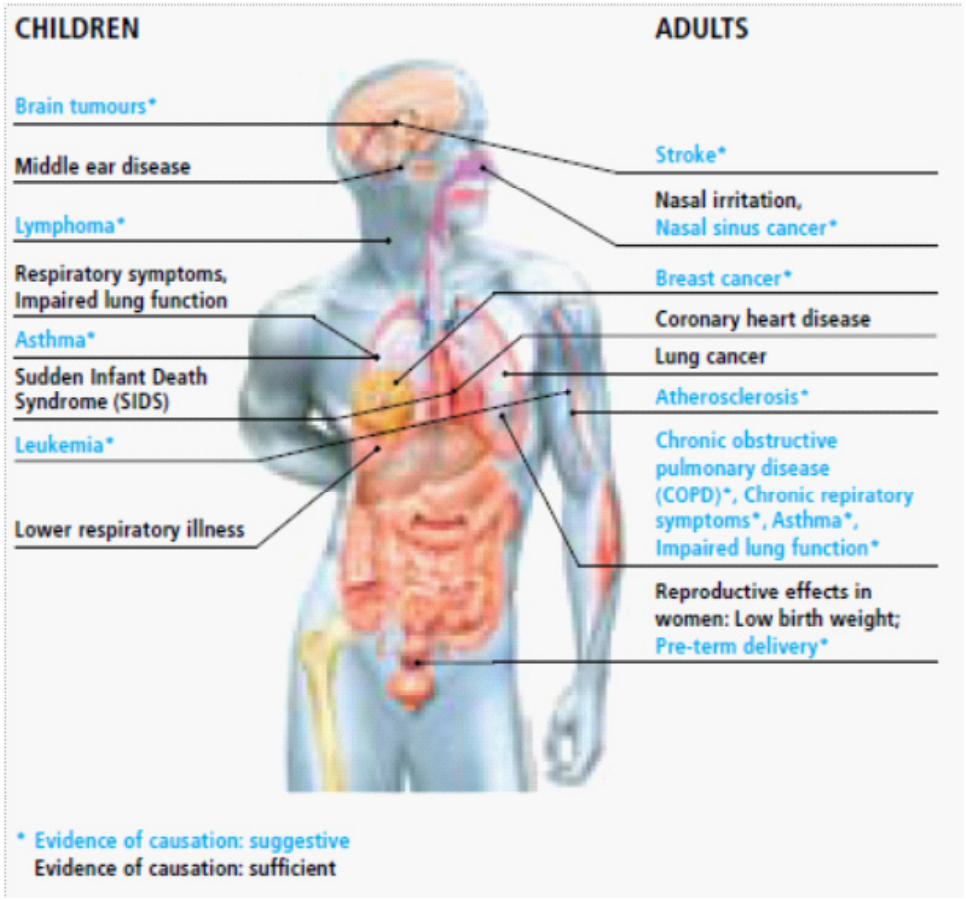

Figure 8: Health Consequences of Second-hand Smoke Exposure [11]

Unborn foetuses are also susceptible to the dangers of tobacco smoke. Smoking during pregnancy is not only dangerous for the mother but also for the growing unborn foetus [10] (**Figure 9**).

Maternal smoking during pregnancy results in passive smoke exposure for the foetus (referred to as tertiary smoke). This exposure results in an increased risk of low birth weight and foetal and infant deaths [24,25].

Tobacco use in pregnancy affects the intrauterine foetal development. The risk of Sudden Infant Death Syndrome (SIDS - death of an infant less than one year of age) is three times more when mothers smoke [27]. Nicotine exposure changes the intensity and timing of brain cell development of the growing foetus [28].

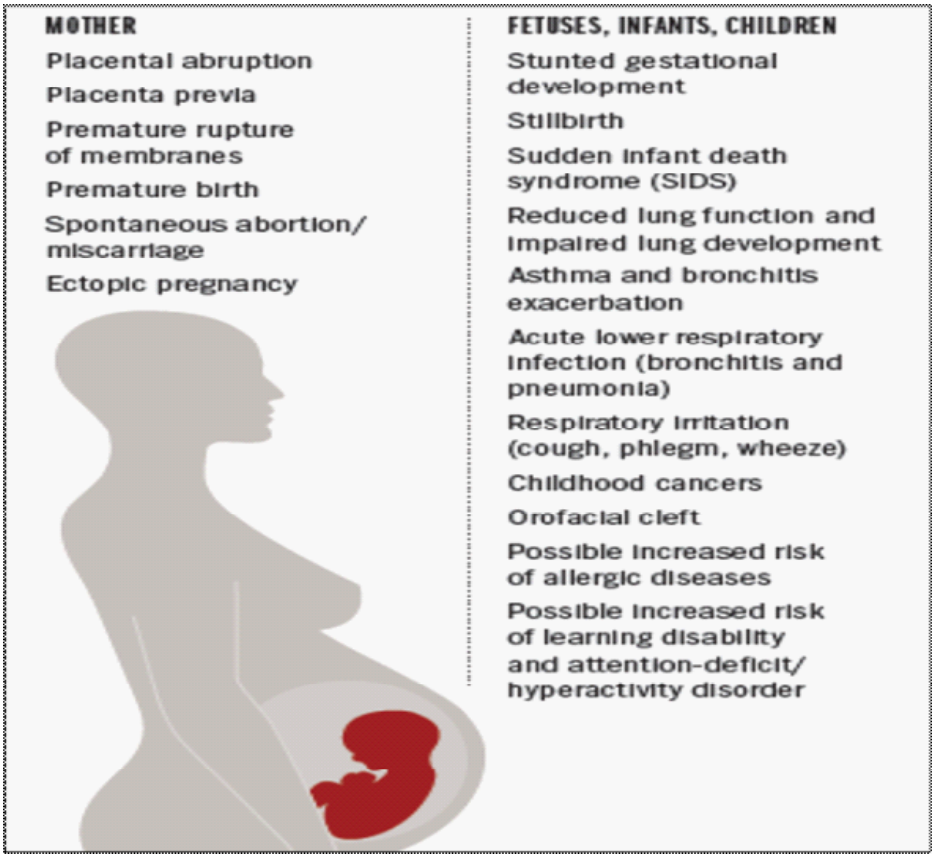

Figure 9: Effects of smoking mothers on their children [10]

3. Social Consequences of Tobacco Use

Apart from affecting the body, tobacco has an impact on human development and the environment. Addicted users spend more money on tobacco products than on household essentials. Discarded cigarette butts and tobacco wrappers are a source of waste. It is estimated that 1.69 billion pounds of cigarette butts wind up as toxic thrash, which is roughly equivalent to the weight of 177,895 endangered African elephants [10].

Tobacco related illnesses cost billions of dollars each year, imposing a heavy economic toll on countries, both in terms of direct medical care for adults and lost productivity. Tobacco use also affects the poorest people. More than 80% of the world's smokers live in low- and middle income countries, harming health, incomes, earning potential, labour productivity, and undermining human capital accumulation – a critical factor for sustainable economic growth

and social development [31,32] (**Figure 10**).

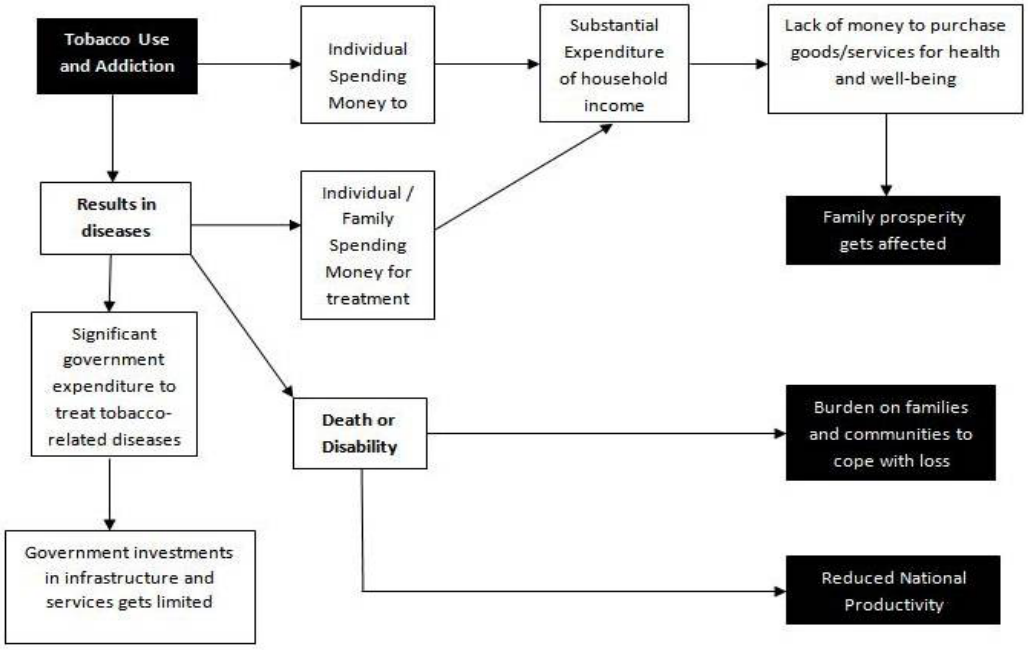

Figure 10: Social Consequences of Tobacco Use

4. What Makes Tobacco Products So Harmful?

According to the U.S. Food and Drug Administration, tobacco contains harmful and potentially harmful substances including more than 50 cancer causing substances [33]. In fact, the cigarette is actually an elaborately designed miniature chemical factory [34]. Some of the substances present in tobacco are nicotine, acids, pesticides, coal tar, carbon monoxide, carbon dioxide, hydrogen cyanide, phenols, benzopyrene, nitrogen dioxide, hydrogen cyanide, chromium, arsenic, acids, etc. [5,14,25,29].

These harmful substances which when inhaled, ingested or absorbed in the body cause direct or indirect harm to tobacco users or non-users [35]. All tobacco products contain a complex mixture of toxic, carcinogenic and addictive substances that are responsible for majority of the harmful effects. Additionally, substances which facilitate product initiation, make cessation difficult and increase the urge to use are also present.

5. Why is it Difficult to Curb this Menace?

In spite of the fact that tobacco products in any form are harmful, millions of people the world over continue to use these deadly products. Apart from the presence of nicotine that makes tobacco consumption an addictive habit which is difficult to get rid of, the tobacco industry has a strong market presence to counter the anti-tobacco activities of governments and non-government organizations.

As part of their marketing, the tobacco industry indulges in social responsibility initia-

tives and public relations campaigns to gain a respectable position in society [36]. The industry also finances research to create an evidence base against the proven harmful effects of their products. All of this creates a favourable image of the tobacco industry in the minds of the people, thereby leading them to believe that the products manufactured are supposedly free of harm.

The industry is constantly manoeuvring and interfering in the political and legislative processes, by getting access to government officials, funding political campaigns, and negotiating seats at policy-making forums [37]. There have been numerous examples from across the world of how the industry weakens and delays anti-tobacco legislations [38].

In the guise of protecting the interests of tobacco cultivators and consumers, the tobacco industry introduces special interest groups to governments to show that they are fighting for the economic rights of farmers and human rights of consumers to smoke. Moreover, the farmers and consumers are manoeuvred into believing that the industry supports their welfare [37, 38].

Another tactic that the industry utilizes is creating new customers by targeting children, youth and women through attractive packaging, misleading advertisements and nomenclatures.

A classic example of misleading promotions is from India, where smokeless tobacco products such as gutkha and a non-tobacco product called paan masala are sold under the same brand name with identical packaging [39] (**Figure 11**). Since advertising of tobacco products is banned in India [40], promotion of paan masala creates a recall value for the said brand and for the tobacco based product that it is barred from being directly promoted.

Figure 11: Similar looking tobacco and non-tobacco products

Newer and 'safer' alternatives are introduced by the tobacco companies such as e-cigarettes and products such as paan masala which although do not contain tobacco, are equally harmful due to the presence of betelnut / areca nut (supari). The International Agency for Research on Cancer (IARC) has reported that areca nut chewed without tobacco is carcinogenic [41,42]. The industry cleverly packages this product to appeal to children through attractive pictures of cartoon characters and movie personalities (**Figure 12**).

Figure 12: Non-tobacco products marketed at children [43]

Tobacco companies constantly engage in product innovation and trap customers who are trying to quit smoking. As a result, users switch to supposedly 'less harmful' substances based on claims that these products reduce the risks caused by cigarette smoking.

Products such as smokeless tobacco and nicotine delivery devices (electronic cigarettes or personal vaporizers, etc.) have long been marketed as a 'safe alternative to cigarettes'. Electronic cigarettes (e-cigarettes) are hand held battery-operated items which when heated create emissions that are inhaled by the user and later exhaled into the environment [44,45]. Nonusers can be exposed to these emissions, which contain toxic substances and carcinogens [46,47].

The newest entrant in this list of products are the 'heat-not-burn' tobacco products (smokeless or non-burning cigarettes), where in the tobacco is heated to release nicotine, but combustion and smoke is prevented [45,48].

The US Family Smoking Prevention and Tobacco Control Act of 2009 describe such products as 'modified risk tobacco product' (MRTP). They are sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products [45,49].

Tobacco companies claim that these products reduce the number and the levels of harmful substances generated by tobacco products. However, their potential benefits as a smoking cessation aid or as safe alternatives to cigarettes have not been substantiated by research. In fact the little data that exists, points towards greater harm caused by such products than the intended benefits.

Moreover, these products can lead to nicotine addiction and can serve as a gateway to experimenting with other deadly forms of tobacco.

Tobacco companies claim to manufacture newer and safe products. However, there is no epidemiological evidence to prove so. Tobacco in any form is unsafe (34).

Regulating tobacco products, offering cessation help to tobacco users and strong anti-

tobacco government policies and stringent laws are the only treatments to control this rapidly spreading and deadly epidemic.

6. Conclusion

Tobacco use is projected to kill 1 billion people during the 21st Century [4]. If current trends continue, by 2030 tobacco will kill more than 8 million people worldwide each year, with 80% of these premature deaths among people living in low- and middle-income countries [9]. The tobacco industry finds new ways to deceive people, serving as a disease vector and spreading the tobacco epidemic [5,35].

Tobacco companies claim to manufacture newer and safe products. However, there is no epidemiological evidence to prove so. Tobacco in any form is unsafe [34].

Regulating tobacco products, offering cessation help to tobacco users and strong anti-tobacco government policies and stringent laws are the only treatments to control this rapidly spreading and deadly epidemic.

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Tobacco Addiction: Effect on Human Health

Chapter 4

Maternal Cigarette Smoking and Fetal Programming of Cardiovascular Dysfunction Late in Life

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Abstract

Maternal tobacco smoking during pregnancy and lactation remains a major public health concern and is associated with a higher risk of poor pregnancy outcomes. It is well known that the adverse environmental exposure within the critical window of gestation period can initiate aberrant fetal development that leads to cardiovascular diseases in adulthood, a phenomenon called programming. Here, we summarize several epidemiological and experimental studies that demonstrate the association between maternal nicotine or tobacco exposure during pregnancy and the development of cardiovascular dysfunction. This chapter also presents some novel epigenetic molecular mechanisms underlying the maternal smoking/nicotine-induced fetal programming of the adult cardiovascular disease. Taken together, a smoke-free environment during pregnancy is essential to improving health outcomes and reducing the risk for future cardiovascular diseases. A better understanding of the epigenetic molecular mechanism underlying the effects of perinatal smoking exposure on programming could provide novel insights into the therapeutic strategies for cardiovascular diseases.

Key words: smoking; nicotine; offspring; cardiovascular dysfunction

1. Introduction

In the last half-century, great attention has been focused on the adverse effects of tobacco smoking on human health, especially on fetal development [1]. Although an increasing number of women realize the critical health issues of smoking during pregnancy and make decisions to cease smoking, the prevalence is still unsatisfactory [2]. There are about 6.8% to 12.3% of women who smoke during pregnancy in the United States and more than 10% of women in Europe [2,3]. In Asian countries, such as China, 2.4% of the women also smoke [4].

Tobacco smoke contains numerous chemicals which are not only harmful to smok-ers, but also to secondhand non-smokers and the fetus while in utero [5]. This toxic smoke consists of tar, heavy metals (such as lead, cadmium, and chromium), hydrogen cyanide and gaseous phases such as carbon monoxide (CO), carbon dioxide, nitric oxide, and the notori-ous nicotine [6-8]. Nicotine, one of the most hazardous substances and carcinogens in tobacco smoke, can easily cross the placenta entering the circulation of the fetus, and penetrate into the mother's milk [9]. In fact, the fetus tends to be more prone to injury because nicotine is concentrated in the fetus at levels at 15% higher than maternal levels [10]. To investigate the adverse effects of tobacco smoke in the fetal cardiovascular system, the relationship between maternal smoking during pregnancy and the increased risk of cardiovascular disease has been extensively studied [11,12]. Furthermore, previous studies have demonstrated that nicotine exposure in the fetal period could lead to fetal programming of cardiovascular dysfunction later in life [13-16].

2.Maternal Cigarette Smoking and Development of Congenital Heart Defect (CHD)

2.1. Maternal cigarette smoking increases the risk of CHD in offspring

CHD is one of the leading causes of perinatal and infant morbidity and mortality which involves the structural abnormalities of the heart and large arteries [17,18]. CHD has a high incidence of 6 to 12 per 1000 live births around the world [18]. There have been substantial studies about the genetic and chromosomal risk factors for CHDs; however, the etiology of CHDs is still an enigmatic [19,20]. Growing evidence suggest that maternal smoking is one of the important risk factors for the development of CHDs [12]. In the 1970s, investigators from London and UCLA reported that, across all races, offspring of tobacco smoking mothers had a higher incidence of CHDs from 7.3% to 8.1%, respectively [21,22]. In addition, epidemiological studies have further confirmed that maternal tobacco smoking acts as a significant risk factor for CHD [23,24]. For example, meta-analysis evidence shows that smoking exposure (odds ratio (OR) =2.766, 95% confidence interval (CI): 1.982-3.859) during maternal pregnancy is the main risk factor of neonatal CHDs [25]. Furthermore, there is approximately a 10% relative

increase in the incidence of CHDs which appears in infants whose mothers were addicted to tobacco smoking during pregnancy [12].

2.2. The effect of maternal cigarette smoking on CHD is dependent on the pregnancy period and exposed dosage

The first trimester of pregnancy, especially at 11-13 weeksgestation, is one of the most important periods for changes in the maternal serum levels of the placental growth factor and other factors. These factors could induce CHDs in the fetus [26]. There are 3 subtypes of CHDs associated with maternal tobacco smoking during this period: pulmonary valve (PV) anomalies, pulmonary artery anomalies and the isolated atrial septal defect (ASD) (the secundum type) [11]. Coincidentally, in the Baltimore-Washington study, first-trimester maternal cigarette smoking contributed to the right ventricular outflow tract (RVOT) defect (OR=1.32, 95% CI: 1.06-1.65), PV stenosis (OR=1.35, 95% CI:1.05-1.74), secundum ASD (OR=1.36, 95% CI: 1.04-1.78), L-transposition of the great arteries (L-TGA) (OR=1.79, 95% CI: 1.04-3.10) and truncus arteriosus (TA) (OR=1.90, 95% CI: 1.04-3.45) [27]. In a population based case control study in the USA, mothers who were addicted to tobacco smoke before pregnancy and/or during the first trimester of pregnancy had a higher risk of having the congenital septal defect in their infants [28].

The risk of increased CHD is not only dependent on the maternal smoking exposure time but is also dependent on the smoking dosage. Mothers who were heavier smokers were significantly increased by the incidence of congenital septal defect in their infants [28]. In another population-based study among 14,128 non-patent ductus arteriosus cases with CHDs, the maternal first trimester of tobacco smoking shows a dose dependent increase in the risk of CHDs in their offspring [29]. In this study, the adjusted odds ratio (aOR) for the anomalies of pulmonary vein and pulmonary artery are increased as the dose of maternal tobacco smoking increased. The dose response was especially strong among offspring with a septal defect and left ventricular outflow tract obstruction with a four-fold and six-fold rise from medium maternal smoking to heavy maternal smoking, respectively [12,30,31].

2.3. The effect of maternal cigarette smoking on CHD may be dependent on the genetic and epigenetic background

In mothers with a functional gene defect, maternal smoking exposure could put offspring closer to the threshold of CHDs as compared with normal genetic mothers [30]. It was reported that mothers with the GSTM1 and GST1 deletion tend to have increased chances for development of CHDs in their children, if they have a higher hair nicotine concentration [30]. Previous studies have shown that Gata4 and Tbx5 are two cardiac transcription factors which play an important role in the development of CHDs [32, 33]. Maternal nicotine exposure could promote DNA hypermethylation, resulting in an inhibition of the Gata4 and Tbx5 gene

expression in both differentiating embryonic bodies and their offspring hearts [34]. This suggests that epigenetics may play a key role in the maternal cigarette smoking-mediated development of CHDs.

3.Maternal Cigarette Smoking and Fetal Programming of Adult Cardiovascular Dysfunction

Fetal hemodynamic changes in response to maternal tobacco smoking during pregnancy, and such changes may be associated with either cardiovascular adaptation or maladaptation in their offspring [35]. Previous studies have suggested that cardiovascular dysfunction in adulthood may be programmed from its onset early in the prenatal period [36,37]. Therefore, we provide some key evidence to show that maternal smoking may predispose fetal programming to adult cardiovascular dysfunction.

3.1. Fetal programming of atherosclerosis of aorta late in life

Atherosclerosis of the aorta is one of the inducing factors of heart disease causing cardiovascular dysfunction [38]. One of the earliest signs of atherosclerosis of the aorta is increased aortic intima–media thickness (aIMT) [39]. In animal models, nicotine administration (3 and 6 mg/kg/day) in female rats during gestation had significantly increased aIMT in their offspring [40]. Another similar study showed that rat pups had twice the thickness of the intima in the maternal nicotine exposure period during pregnancy as well as in the lactation period as compared with the saline control group [38]. In a human study located in a Turkey hospital, it was reported that increased mean and weight-adjusted aIMT was detected among neonates whose mother had smoked [41].

3.2. Fetal programming of adult hypertension

Hypertension is a worldwide cardiovascular disease with a prevalence among 30% of the world's population that damages multiple organs such as the heart, lung, brain, and kidney [42]. Extensive studies demonstrate that hypertension is considerable when associated with maternal tobacco smoking exposure in utero which is characterized by a higher systolic and/or diastolic blood pressure in childhood [2,43]. Epidemiologic studies have shown that maternal tobacco smoking increases blood pressure not only in newborns [44,45], and children [46,47], but also in adults [48]. Maternal smoking-induced hypertension is most likely associated with the action of nicotine. Nicotine is a ganglionic agonist which could stimulate neurotransmitter (such as norepinephrine) release. Indeed, direct treatment with nicotine during pregnancy has been shown to increase risk of hypertension in adulthood among different animal models [14, 15,49,50].

The effect of maternal smoking/nicotine exposure on the development of hypertension

in offspring is complex, with many underlying mechanisms. Previous studies have demon strated that the functional changes of endotheliocytes, perivascular adipose tissue and kidneys may contribute to the maternal smoking/nicotine exposure-induced hypertension [43]. It's well known that the renin-angiotensin system plays a key role in the regulation of blood pressure. Recent studies suggest that alterations of the renin-angiotensin system may be one of the important mechanisms contributing to the fetal programming of hypertension [51,52]. In the kidneys, the angiotensin receptor type 1 (AT1R) gene is downregulated in offspring rats of maternal rats exposed to cigarette smoking [53]. On the other hand, AT1R expression and the ratio of AT1R/ AT2R in vasculatures were increased in adult offspring whose mothers had been treated with nicotine [54]. The overexpression of AT1R results in the enhanced capability of Angiotensin II-induced vasoconstriction, and the consequent development of hypertension in adult offspring. In addition, it has been shown that prenatal nicotine exposure can inhibit baroreflex sensitivity which maintains blood pressure steadily by a rapid negative feedback loop [55]. Further more, nicotine exposure during pregnancy could increase arterial reactive oxygen species (ROS) production, which enhances vascular reactivity, resulting in the development of hypertension in offspring. Inhibition of ROS could block maternal nicotine-induced hypertension [56], suggesting that heightened ROS production may be one of the molecular mechanic linkers between maternal nicotine exposure and the fetal programming of adult hypertension.

3.3. Fetal programming of arrhythmia

In addition to the development of hypertension in offspring, previous studies have also found an irregular fetal pulse and permanent development of arrhythmia in adulthood following exposure to nicotine during pregnancy [57]. It has been reported that maternal cigarette smoking acutely increases the fetal heart rate, which may be due to an increase in sympathetic activity [58]. However, the chronic long-term effects of fetal nicotine exposure on the heart are some what different and may result from alterations in heart development. Fetal nicotine exposure during pregnancy has been shown to alter the types of nicotinic receptors that facilitate excitatory inputs to cardiac vagal neurons, which may be responsible for the bradycardia observed in offspring [59]. Moreover, cardiac cycle irregularity and single/multiple dropped cardiac cycles have been detected in fetal sheep prenatally exposed to nicotine [57]. This cardiac conduction dysfunction and malignant arrhythmia may be one of the major causes for sudden infant death and cardiac dysfunction-induced by maternal cigarette smoking [57,58]. Studies in rats and sheep models have suggested that uterine hypoxia may be considered as one of the potential mechanisms underlying prenatal nicotine-induced arrhythmia in offspring [57]. In adult offspring rats, maternal nicotine exposure caused myocardial fibrosis and cardiac remodeling. Given the fact that myocardial fibrosis and cardiac remodeling are the major factors for the development and progression of atrial fibrillation, these factors may be other

potential mechanisms underlying nicotine-mediated arrhythmia in offspring [50,55,60]. Previous studies have demonstrated that in the three month-old offspring rats prenatally exposed to nicotine, heart rates are significantly increased, with loose, confused myofibril arrangement and excessive ECM accumulation. Mean while, the cardiac eject function was impaired and diastolic LV posterior wall thickness had thickened [55]. The TGF-β1 geneplays a key role in the development of myocardial hypertrophy and fibrosis [61]. Previous findings have shown that the expression of TGF-β1 was increased with the β-myosin heavy chain in offspring born to prenatal and postnatal nicotine-treated dams.This suggests that the TGF-β1 genes may be one of the important mechanisms in perinatal nicotine-induced cardiac hypertrophy and arrhythmia [62].

3.4. Fetal programming ofpulmonary arterial hypertension (PAH)

Pulmonary arterial hypertension is another cause of cardiac dysfunction [62]. Pulmonary fibrosis is one of the key factors in the pathology of PAH with an excessive accumulation of the extracellular matrix protein (ECM) which contributes to vascular stiffness through a decrease in tissue and vessel compliance [63]. It has been reported that maternal nicotine exposure adversely affects lung development and function in fetuses and neonates [64,65]. In pregnant rats treated with nicotine, male adult offspring showed higher collagen content and expressions of collagen 1 and 3 in the lungs, associated with an increase in the expressions of AT1R and the ratio of AT1R/AT2R in the lung tissues [66]. In addition, the expressions of TGF-β1, CTGF and Smad3 were also increased in the lung tissues [66]. This evidence suggest that maternal cigarette smoking could induce the occurrence and development of pulmonary fibrosis in adult offspring and increase susceptibility to PAH [66].

Epithelial-mesenchymal transition (EMT) is one of the key factors in the pathogenesis of pulmonary fibrosis. Recent studies have shown that prenatal nicotine exposure can directly regulate EMT-related protein expression [67,68]. EMT related protein expressions were significantly higher as early as postnatal day 7 in the maternal nicotine exposed group [68]. The maternal smoking/nicotine-mediated enhanced the EMT-related protein expression which may contribute to the development of pulmonary fibrosis and pulmonary arterial hypertension in offspring [68,69].

3.5. Fetal programming of heart ischemia-sensitive phenotype

Human epidemiological studies suggest a link between adverse intrauterine environments and an increased risk of ischemic heart disease in adulthood [70]. Our previous studies in a pregnant animal model have demonstrated that fetal nicotine exposure reprogrammed cardiovascular function and induced the development of a heart ischemia-sensitive phenotype in adult offspring [16,71]. Nicotine exposure in pregnancy significantly enhanced Ischemia/Reperfusion (I/R) injury in the left ventricle (LV) and led to poor outcomes of LV function

with a lower coronary flow rate in the adult offspring [16,71]. Furthermore, the development of heart ischemia-sensitive phenotype is associated with a significant decrease in protein kinas Cε(PKCε) in the hearts [16]. PKCε plays a pivotal role in cardioprotection from heart ischemia and reperfusion [72]. This evidence suggest that prenatal nicotine exposure-induced fetal programming of the PKCε gene expression pattern in the developing heart. This may be one of the key molecular mechanisms underlying why the nicotine-mediated increased its'heart susceptibility to ischemia/reperfusion injury in adult offspring.

Another vital mechanism that contributes to myocardial ischemia/reperfusion injury and heart dysfunction is oxidative stress. Maternal smoking is associated with the increased levels of reactive oxygen species (ROS) in offspring [73]. Furthermore, fetal nicotine exposure leads to increasing levels of ROS in fetal, neonatal and adult tissues [74,75]. Our recent studies in the pregnant rat model have demonstrated that fetal nicotine exposure increases cardiac ROS production, which leads to an epigenetic downregulation of the cardioprotective protein, PKCε gene expression and an upregulation of cardiac GSK3 βphosphorylation. This results in an increase in the heart I/R injury and dysfunction [76].

Protein disulfide isomerase (PDI) acts as a cardioprotector and a survival factor during ischemia [77]. In the perinatal nicotine exposed rat adult offspring, PDI levels were significantly decreased in the heart tissues, associated with decreased levels of superoxide dismutase enzymes, mitochondrial complex proteins, and the tissue inhibitor of metalloproteinase-4 [78]. These findings suggest that fetal nicotine exposure could program cardiac PDI expression, leading to promote oxidative stress and mitochondrial damage, consequently increasing heart ischemic injury in adulthood.

4.Conclusion

Maternal cigarette smoking is one of the most perinatal insults. Maternal smoking during pregnancy not only induces fetus growth restriction, but also affects fetal organ development. Epidemiological human and animal studies have shown that fetal cigarette smoking exposure is a major risk factor for the development of CHD and other cardiovascular diseases later in life. As the prevalence and incident use of tobacco cigarette or e-cigarettes continues to increase worldwide, an understanding of their risks during pregnancy becomes a pressing need in areas of public health. Although we begin to understand that fetal smoking exposure could affect fetal cardiovascular development and consequently lead to cardiovascular disease in adulthood, the epigenetic molecular mechanisms are still not fully understood. A better understanding of those mechanisms is critical and could help professionals to identify early biomarkers and provide new leads in the development of the preventive diagnosis and therapeutic strategies of maternal smoking-mediated fetal programming of cardiovascular disease.

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Tobacco Addiction: Effect on Human Health

Chapter 5

Tobacco Use and Its Genotoxic Effects in Pregnancy

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Abstract

The current chapter discusses recent findings in humans on genetoxic effects on the fetus of prenatal exposure to smoke tobacco. Tobacco Smoking is the most widespread substance dependence in the World, and is a rapidly increased including in pregnant women and serious public health problem worldwide. Nicotine readily crosses the placenta and the fetuses of mothers. Recent studies have suggested a direct contribution of nicotine the addictive component of tobacco and tobacco smoke to human carcinogenesis, and it remains the most common harmful substance to which pregnant women are exposed. Smoking during pregnancy increases maternal health, and also it has deleterious effects on the fetus; premature birth, intrauterine fetal death, intrauterine growth retardation and congenital anomalies. It is known that cigarette smoking has genotoxic effects and causes mutations. The newborns of smoking mothers have elevated frequencies of chromosome damages and DNA strand breaks. Our results also strongly suggest that nicotine is hazardous to the human fetal cells and adult cells. The toxic substances from cigarette smoke induce chromosomal aberrations (CAs) in vitro and could potentially increase levels of aneuploidy in the fetus. This possibility is consistent with the genotoxic effects in fetal cells from smoking during pregnancy are most likely caused by cigarette constituents, providing a potential mechanism for polyploidies and aneuploidies in fetal cells or embryo. Moreover, increased levels of aneusomy in fetus are correlated with low implantation rates, spontaneous abortions and fetal losses. Due to the harmful effects of cigarette, pregnancy is one of the ideal times to quit smoking. Because, mothers should

repeatedly be awared about the harmful effects of cigarette on their baby health. They must be achieved for quitting smoking before pregnancy, and stop smoking especially if you can get pregnant or no pregnancy before you quit smoking.Likewise, healthcare units and maternity wards should hold lectures and explain the harm of tobacco to health and the environment.

1. Cigarette Smoking Habits and Turkey

The use of narcotic stimulants and the habit of these substances are very important for human and community health. It is known that cigarette and tobacco derivatives are addictive, and is one of the most important threats to human health. Smoking is the most widespread substance dependence in the world. Smoking habit is a complex illness that is not clear the genetic inheritance model and causes many permanent illnesses without recycling. Addiction to tobacco has significant psychosocial aspects, but the basis of physical addiction is nicotine. Tobacco use kills more than 7 million people each year in the world, and was the second leading cause of deaths in 2017 [1]. If the consumption of cigarettes continues this way it is envisaged that around 10 million people will die in 2025 due to cigarette smoking worldwide, 70% of which will be seen in developing countries [2,3].

Smoking is one of the major health problems in Turkey as well as the problem of many developed and developing countries in the World [4]. The acute toxicity of nicotine and the longer-term exposure has adverse effects on reproductive health, lung growth and development, neurocognitive function and cognitive decline, psychiatric morbidity, immune function, cancer risk, and cardiovascular disease. In recent years, however, some important steps have been taken in the social field to combat smoking. Nevertheless, Turkey still ranks 11th in the ranking of most cigarette consuming countries. In Turkey, close to 15 million people (27.1%) (10.6 million men and 3.9 million women) currently smoking every day, and cigarette consumption has increased by 52% over the last 10 years. At the same time, 5 million people are also exposed to cigarette smoke. The frequency of tobacco use is higher in males (41.5%) than in females (13.1%). Among tobacco users, 23.8% use daily tobacco (37.3% of men, 10.7% of women). The majority of users of tobacco products (94.8%) are cigarette smokers and only 0.8% use narghile. About half of the smokers (42.1%) also drink the first cigarette in the first 30 minutes after awakening. The average age at everyday cigarette smokers is 17.1 years and most of the smokers (58.7%) have started smoking before the 18th year of legal age[5].

According to the results of the WHO in 2014, passive smoking appeared in 39% of the working population in Turkey. Unfortunately, 55% of our children are exposed to passive smoking by their families. Over1985-2000 period, cigarette consumption in Turkey has increased by 89% [6]. This shows that despite the smoking ban, the rate of passive smoking is increasedto 58% in closed areas. There are 1 million women, 4 million men and about 5 million passive smokers who are exposed to cigarette smoke while they are not smoking at

work in Turkey. Passive smoking is particularly threatening the babies and born babies in the womb. This reduces the chances of a healthy individual in the future. This shows that passive smoking is effective and bad. In Turkey, 120 thousand each year and 300 people every day die due to smoking reasons.

If the current smoking situation continues this way, in 2020, about 10 million people die each year in the world because of smoking and 7 million of them will be fromin developing countries. For this reason, the WHO considers reducing tobacco use and tobacco-related deaths as a top priority [7]. According to the survey results, when compared with 2008 and 2012, smoking rates in Turkey has regressed from 31.2% to 27.1%. This rate decreased from 47.9% to 41.5% for males and from 15.2% to 13.1% for females. The rate of those who started smoking before the age of 15 declined from 19.6% to 16.1%. The most significant decline in the period of 2008 - 2012 has been seen with regard to passive smoking, and it is noteworthy that the rate of smoking in restaurants has decreased from 55.9% to 12.9%. In Turkey, the National Tobacco Control Program by applying determination, MPOWER became the first and only country to fulfill all of the strategies in the policy package [3].

2. How Many Women Smoke During Pregnancy?

Smoking habits among women starting in adolescence can turn into physiological and psychological dependence when it comes to women's marriage. Smoking is the greatest danger in pregnant women; a major health problem because of the damage the fetus gives to pregnant women. Nevertheless, these increases in cigarettes are more common among young people and women, which cigarette companies regard as the target audience. At the same time, especially these companies are trying to increase smoking rates among women by giving messages that smokers look more attractive or more modern, and also encourage cigarette smoking in adolescence, a period when women may feel the most need for themes such as freedom and power. In this period, women's smoking addiction can continue during pregnancy.

Today, it is understood that smoking is an illness and that these people should be treated. Although, cigarette addicts think so because they feel relieved by cigarettes, feel relieved of their stress and are relieved only by taking nicotine. Smoking addiction that develops in women can often continue during pregnancy, and 50-70% of women with cigarette addiction continue to smoke during pregnancy. In a large-scale study conducted, it was determined that approximately one in five women smoked while pregnant. In Turkey, one of the four or five females generally smokes during pregnancy. Smoking in pregnancy is extremely harmful to both mother and baby health. We know that maternal smoking and smoke exposure during pregnancy are detrimental to fetal growth and development. In 2002, about 11.4% of all pregnant women in the USA were using cigarettes[8], and maternal cigarette tobacco smoking is the leading cause of premature morbidity and mortality in the United States [9]. The prevalence

of tobacco use during pregnancy was found to be in 2012, 20.5% in United Kingdom, in 2012, 11.2% in the 2001-2006 in Canada, 69% in pregnant women-Australiannatives (aborigines) and 17.2% in Nepal [10-13]. The prevalence of waterpipe smoking among pregnant women is around 6% in Lebanon [14] and 9% in Jordan [15]. According to WHO date (2005), 22% of women in developed countries are reported to be smoking [16]. Similarly, in our country which is in the status of a developing country, it is known that the number of women smoking increased over the years and that about one in four women smokes.

Smoking or being exposed to smoke, the most extreme example of a systemic human mutagen, is the most important preventable cause of diseases or deaths. Despite these damages of nikotin it is estimated that about 20–27% of women still smoke during pregnancy [17]. Increased cigarette consumption in a society can also increase the risks associated with smoking-related pregnancy. The adverse effect of smoking during pregnancy can be also caused by cigarette smoke in the environment as well as from smoking in pregnant women. Environmental cigarette smoke is known to be a common negative factor for pregnant women. There is not enough number of studies in pregnant women who report smoking status in Turkey. Only, there are few studies on the harms of cigarettes in pregnancy. In a study carried out in Turkey, the percentage of women who had smoked at any time during pregnancy was 17%. The percentage of women who smoked throughout pregnancy period was 9%. The prevalence of current smoking among them on the first day after birth was also 9%. The percentage of current smokers among the husbands of the respondents found as 68% [18]. According to a report prepared by Turkey Statistical Institute in 2012, smoking rate was 41.4% for males and 13.1% for females. At the same time, 11% of pregnant women and 17% of breastfeeding mothers are smoking cigarette [5]. In Turkey, the studies on smoking before pregnancy in women was observed in the range of 35.3%-13,7% rate of smoking1-6, and the rate of smoking in pregnancy was found to be 11.9% [3].

3. Smoking During Pregnancy Harms the Genetic Structure of Mother and Baby

Tobacco smoking and smoke exposure during pregnancy seriously damages both mother and baby health and cause considerable childhood morbidity and mortality. Tobacco smoke is the most extreme example of a systemic human mutagen. Because, normally nicotine passes to fetus, placenta, amniotic fluid and milk of smoking mothers [19]. Thus, maternal smoking continues to be a leading preventable cause of pregnancy complications in otherwise low-risk women. Smoking harms the heart, veins and all other organs of the body, especially the respiratory system. These harmful effects of cigarette are seen in two ways, short and long term. Nicotine and carbon monoxide in the cigarette are extremely harmful to the baby. The baby is fed through placenta and cord. In smokers' mothers, babies can not feed enough and can not develop because they can not carry enough oxygen to the baby. Oxygen is the most important means for the growth and development of infants. When oxygen is reduced in the

mother's blood, the amount of oxygen the baby receives and the amount of nutrients are also decreasing. Therefore, the development of growth and development in the infants of smokers' mothers can occur. When the cigarette is left in the first trimester (the period up to the 12th week of pregnancy), the fetus in the mother's womb is less damaged than in the other periods [20,21]. Approximately one third of infants are exposed to environmental tobacco smoke. Smoking is among the most important preventable causes of intrauterine, infant and childhood diseases and deaths; including low birth weight, premature delivery, spontaneous abortion, placental abruption, perinatal mortality and ectopic pregnancy [22-24].For this reason, smoking cessation in pregnancy is extremely important for mother and baby health.Although knowledge about the negative effects on the fetus and the newborn of smoking during pregnancy is getting increasingly widespread, this habit still remains a great problem worldwide. The International Child Care Practices Study concluded in a survey of 21 centers in 17 countries that an average of 22% of mothers and 45% of fathers were smoking at the time of their child's birth [25].

Recent studies have suggested a direct contribution of nicotine the addictive component of tobacco to human carcinogenesis, and it remains the most common harmful substance to which pregnant women are exposed. Most people know that smoking causes cancer, heart disease, and other major health problems. The effects of smoking for fetus during pregnancy depend on chemical materials of contents. Nicotine, carbon monoxide and cadmium are among the most important ones. The main harmful substance for fetus is acetaldehyde. At the same time, tobacco smoking during pregnancy has been reported as one of the source of oxidant status [26]. Cigarette smoking can lead to oxidative stress for smokers and those exposed to smoking, as well as reduce the level of certain antioxidants. It has been clearly demonstrated that these harmful substances are a potent inducer of DNA strand breaks in human and rodent cells [27-30]. Mutants and free radicals in cigarette are known to cause DNA strand breaks during DNA synthesis and thus block DNA synthesis. DNA damage has even been observed in groups that consist of young populations (19 to 23 years old) with a brief history of smoking [31]. Studies have found that there is a relationship between prenatal environmental smoke exposure and neonatal DNA damage [32]. However, knowledge about the possible genetic effects of prenatal nicotine exposure in humans is to date limited. It has been shown that the exposure to tobacco increases the potential for chromosome breakage at some cancer sites in the genome. In a study, we showed that smokers had a higher frequency of total CA expression compared with non-smokers [33]. Various studies have also found that smoking caused a 10–20% increase in CAs frequency [34], and in vitro exposure of peripheral lymphocytes to smoke, results in higher CAs frequencies [35-38]. A similar study also show a significant increase in the CA frequency in smokers when compared to non-smokers [39]; the incidence of CAs was between 8.1 and 54.2% with a mean of 26.5%.

In an our similar study, the smoking group exhibited a higher frequency of total CA

expression compared with that of the non-smoking control group (P<0.001), but except for 1p36, 3q21 and 5p15 regions, the overall difference was not statistically significant (P>0.05) (. 1) [33]. In the smoking group, FS at 1p36, 3q21 and 5p15 regions were significantly increased. It is interesting that the tobacco compounds were particularly interactive with cancer loci but not with the other loci. It has been noted in studies conducted by other researchers that these three regions are potential sites for both the development of some cancers and the development of smoking habits. These regions may be evidence for a common genetic factor that contributes to smoking. This also shows that the exposure to tobacco increases the potential for chromosome breakage at three cancer sites in the genome. At the same time, there is a relationship between the cancering materials and the cancer gene-regions. What are the molecular mechanisms that provide attraction between these dangerous regions and the carcinogenic substances in cigarettes? Its molecular mechanism is unknown. The other studies demonstrated that a significant increase was found in the frequency of CA and FS between smokers and nonsmokers [39-41].

These observations should stimulate more studies on these chromosomal regions at the molecular, cytogenetic, and population genetic level. These three regions have been previously identified as potential susceptibility loci for several cancers and may have susceptibility loci that are specific for the development of habitual smoking. The 1p36 band is a cancer breakpoint [42]. Reciprocal translocations between 3q21 and other chromosomes are well documented in myelodysplastic syndrome and leukemia [43,44]. Previous studies have shown that the 5p15 region exhibits frequent genetic changes in bronchial epithelial cells in long-term smokers, and in invasive cervical carcinoma, and that these changes arise early during carcinogenesis [45,46]. It is interesting that the tobacco compounds were particularly interactive with cancer loci but not with the other loci. Knowing these mechanisms is very important in terms of prevention of cancer. After that, it will need to work on resolving this relationship.

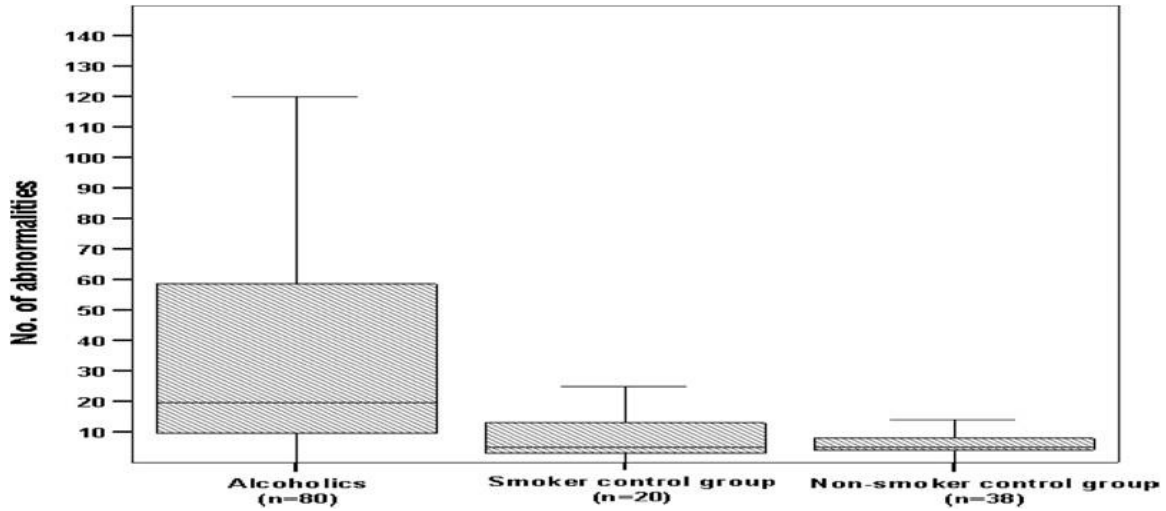

Figure 1. Distribution of chromosomal aberrations in the alcoholic, smoking and non-smoking groups [33].

At the same time, nicotine also affects the cell development of fetus. We personally observed that women who smoked during pregnancy were adversely affected by fetal cell development in routine amniotic cell cultures.The growth of fetal cells in pregnant smokers is later than non-smokers in amniocentesis cultures. According to our findings, there is a significant difference of CAs between nicotine containing medium grown cells and control medium grown cells. Also, this shows that the nicotine passed from mother to fetus it decelerates the development of the baby's cells. Although, information on the in vivo genotoxicity of nicotine is limited, in our study it was confirmed that the nicotine leads to significant direct genotoxic effects on human fetal cells in vitro [47]. The determination of tobacco-specific metabolites in fetal blood and amniotic fluid also supports the possible genotoxic effects of smoking during pregnancy. Meanwhile, some genotoxicity studies [48,49] have demonstrated the existence of an increased incidence of micronuclei and CAs, as well as sister chromatid exchanges in smoking adults; however, only limited data is available on the possible genotoxic risks of smoking on fetuses and newborns.

High DNA damage is also known to influence apoptosis and cell cycle [50]. Most cells have the ability to perform DNA repair. However, if DNA lesions are mis-repaired and the physiological pathway of apoptosis is interrupted, DNA lesions can cause CAs or other changes with the potential of inducing mutagenesis in a multistep mechanism [51]. Certain studies have shown that nicotine induces aneuploidy and polyploidy [52], SCE and CAs in bone-marrow cells of mice [53]. In a similar our study, there are significant differences in the frequency of CAs between medium containing nicotine and control medium ($P<0.001$). This data indicates that nicotine expresses significant direct genotoxic effects in fetal cells *in vitro* [46]. However, the causal relationship between smoking in pregnancy, the induction of genotoxic effects of nicotine, and the frequency of embryonal CAs have not been fully documented. The chromosomal breaks and other damage observed in our study may be related to the proliferation of DNA.

In our related study, approximately 20% of all cells were carriers of numerical CAs (total CAs 22.1%) (ure 2). Tetraploidy and aneuploidies were found to be the most frequent abnormalities. Other studies show a significant increase in the CA frequency in smokers when compared to non-smokers; the incidence of CAs was between 8.1 and 54.2% with a mean of 26.5% [46]. These findings may explain the increased aneuploidy rates in fetal cells, and were in agreement with other cytogenetic studies among smokers [36,37]. The prenatal exposure to nicotine increases the frequencies of premature centromere separation and premature anaphase, in agreement with the results of our study which suggested that nicotine elevates aneuploidy levels in human fetal cells. This data indicates that nicotine expresses significant direct genotoxic effects on human fetal-cells in vitro. This possibility is consistent with the genotoxic effects in fetal cells from smoking during pregnancy are most likely caused providing

a potential mechanism for polyploidies and aneuploidies in fetal cells or embryo.Accordingly, in a few in vivo studies measuring CAs, nicotine has been reported to interfere with oocyte maturation and chromosome disjunction [51], and to induce aneuploidy and polyploidy in mouse bone-marrow cells [54]. The origin of autosomal trisomies has also been investigated, and several studies showed that smoking can be a confounding factor when assessing aneuploidy and DNA damage in epidemiological studies [55]. In utero exposure to tobacco smoke also increases CAs frequencies in the newborns [56]. Various studies have found that smoking caused a 10–20% increase in CAs frequency [57]. All chromosome groups are represented in aneuploidies, but non-disjunction is not a random event in female meiosis. In particular, there is a significant excess of nondisjunction in the acrocentric D (13, 14, 15. chromosomes) and G (21, 22 chromosomes) groups chromosomes [58] or in the G group chromosomes only [59].

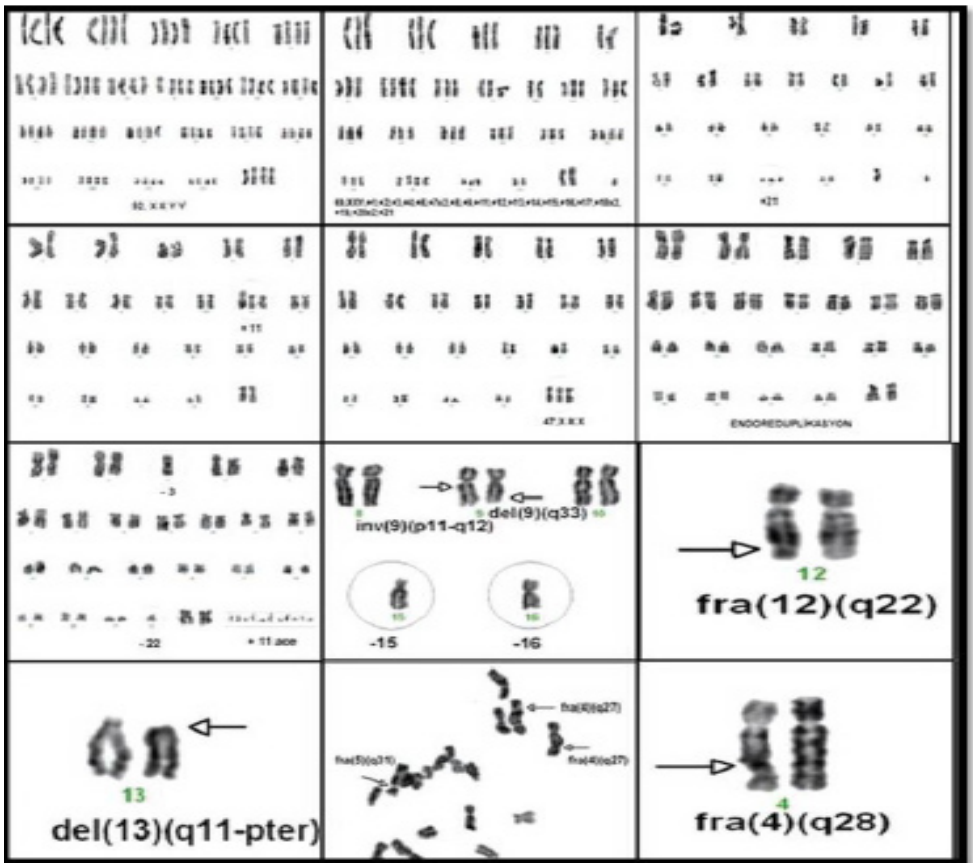

Figure 2: Metaphase and partial metaphase figures showing some chromosomal abnormalities of fetal cells, cultured in medium containing nicotine [46].

This fact has been reported in studies conducted on spontaneously aborted fetuses and live-born infants. In our work on the genotoxic effects of nicotine in smoking mothers; the most common numerical aberrations were chromosome 21 aneuploidies (in 1.7% of all cells and 9% of numerical aberrations), followed by monosomies and trisomies 22, X, 8, 10, 15 and 20, respectively [46]. In particular, there is a significant excess of nondisjunction in the acrocentric G group in our study. This finding shows that G group chromosomes are more sensitive to nicotine in terms of non-disjunction events. Our findings indicate that smoking can be a confounding factor when assessing aneuploidy and tetraploidy in human fetal cells. We speculate that there is an association between prenatal exposure to cigarette smoke and in utero

aneuploidies. Results of this study confirm that the nicotine leads to significant direct genotoxic effects in human fetal cells in vitro, and there is an association between prenatal exposure to cigarette smoke and in utero aneuploidies. Despite the damages explained above it is estimated that about 20–25% of women still smoke during pregnancy [60]. Although knowledge about the negative effects on the fetus and the newborn of smoking during pregnancy is getting increasingly widespread, this habit still remains a great problem worldwide. The widespread use of cigarettes among pregnant women or pre- and post-pregnancy women is a threat to the health of future generations and suggests that these studies should be more active.

We speculate that the toxic substances from cigarette induce structural and numerical CAs in vitro and could potentially increase levels of aneuploidy in the fetus. These findings may explain the increased aneuploidy rates in fetal cells and oocytes of mother, and were in agreement with other cytogenetic studies among smokers [36,37]. Therefore, nicotine could express significant direct genotoxic effects in human cells. This possibility is consistent with the genotoxic effects in fetal cells from maternal smoking during pregnancy, and are providing a potential mechanism for polyploidies and aneuploidies in fetal cells. Just as in a few in vivo studies measuring CAs, nicotine has been reported to interfere with oocyte maturation and chromosome disjunction [53], and to induce aneuploidy and polyploidy in mouse bone-marrow cells [61].

Aneuploidy is one of the most important reason of reproductive biology and reproductive diseases.The origin of autosomal trisomies (13, 18 and 21 chromosomes) has also been investigated, and several studies showed that smoking can be a confounding factor when assessing aneuploidy and DNA damage in epidemiological studies [62]. At the same time, high doses of nicotine increased the frequencies of premature centromere separation and of premature anaphase and reduced the number of oocytes ovulated. Also, it has been suggested that the chromatids arose from premature centromere division at meiosis and it was a major mechanism for the generation of trisomy [63]. Together, these findings indicate that smoking can be a confounding factor when assessing chromosome disjunction (aneuploidy and tetraploidy) in human fetal cells. The human genome is delicately balanced, and for the most part perturbations in the chromosome complement are often incompatible with embryonic development. In particular, there is a significant excess of nondisjunction in the acrocentric G group in our study. This finding shows that G group chromosomes are more sensitive to nicotine in terms of non-disjunction events. For the acrocentric chromosomes 15 and 21, meiosis I errors are the predominant maternal errors, in contrast, for trisomy 18 meiosis II errors predominate. These results strongly indicate that cigarette smoking is hazardous to the viability and function of developing oocytes and their resulting embryos [64].

At the same time, aneuploidy is a well recognised feature of human tumours, and there is a significant correlation between aneuploidy and melanoma thickness. We observed aneuploid

not only in gestation but also in different types of cancer [65,66]. Because, aneuploidy is a well recognised feature of human tumours, and has been proposed to drive tumor development by enhancing genomic instability. The increased incidence of aneuploidy, could contribute to the progression of the disease along with other CAs. Many women are exposed to cigarette smoke. Therefore, the aneuploidy screening is important in pregnancy.

In our study, the numerical changes of sex chromosomes were present in four fetalcells including 47XXX, 45,X[2] and 47,XXY [46]. Smoking appears to induce aneuploidy in sperm for chromosomes 1, 13, and YY disomies, but not for XY, XX, or 7[67-69]. However, sperms of smokers display elevated levels of meiosis II non-disjunction of the sex chromosomes, relative to that of non-smokers [70]. In another our study, the numerical changes of sex chromosomes were present in the maternal cells [64]. The results of our work showing there is a positive correlation between the frequency of aneuploidy and the smoking. Smoking in women may increase sex aneuploidy rates, providing a potential mechanism for aneuploidy in fetal cells or and their resulting embryos. However, sperms of smokers display elevated levels of meiosis II non-disjunction of the sex chromosomes, relative to that of non-smokers [46]. The toxic substances from cigarette exposure induce CAs in vitro and could potentially increase levels of aneuploidy in the fetus.

Some studies found that smoking produced a marginal increase in translocation frequency [71], or caused a significant increase in stable structural aberrations (translocations and insertions) [72]. Also, structural changes were observed in 2.1% of the cells and in 9.7% of the maternal cells with CA in our study. The findings in our last study confirm that the newborns of smoking mothers have elevated frequencies of chromosome translocations and DNA strand breaks. It is known that cigarette smoking has genotoxic effects and causes mutations. There is a positive correlation between the frequency of aneuploidy and the effect of nicotine. Smokingduring pregnancy increases maternal health risks as well as mental and physical problems for the fetus, contributing to multiple adverse outcomes such as preterm delivery and stillbirth. It is well understood that the fetal environment is of tremendous importance during the developmental period in determining health throughout the life of the individual [46].

4. Conclusion

Smoking in women is a rapidly growing and serious public health problem worldwide. Tobacco smoking is a risk factor for numerous disorders, including cancers affecting organs outside the respiratory tract. Nicotine readily crosses the placenta and the fetuses of mothers and are exposed to relatively higher nicotine concentrations than their mothers. Also, it has deleterious effects on the fetus. The results of studies strongly suggest that the newborns of smoking mothers have elevated frequencies of chromosome translocations, DNA strand breaks and numerical chromosomal irregularities. It is known that cigarette smoking has genotoxic

effects and causes mutations. The toxic substances from cigarette smoke induce structural and numerical CAs and could potentially increase levels of aneuploidy in the fetus. Due to the harmful effects of cigarette, pregnancy is one of the ideal times to quit smoking. We can certainly conclude that quitting smoking early on in the pregnancy may avoid genetic effects on the newborn. From a public health perspective, it is essential that pregnant women should be advised to give up smoking from conception and avoid exposure to environmental tobacco smoke during pregnancy. Likewise, healthcare units and maternity wards should hold lectures and explain the harm of tobacco to health and the environment. In pregnancy, by determining the factors effecting smoking status, smoking during pregnancy and passive smoking can be reduced through counselling and education services. Thus health care providers and policy makers need to give special attention in those issues and effective implementation of national guideline for effective curving tobacco consumption epidemic during pregnancy. By this way, the effects of smoking on mother, baby and pregnancy can be reduced.

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Tobacco Addiction: Effect on Human Health

Chapter 6

Tobacco Chewing/Smokeless Tobacco: It's Effect on Human Health

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1. Introduction

Tobacco is a powerful addictive substance which is deliberately consumed all over the world. People enjoy tobacco and its product via various methods in different partsof world. It isconsumed either orally by *smoking* through cigarettes, pipes, cigar, bidi, hukkah, chhutta etc. or *chewed* in the form of raw leaves as khaini, pellets, plugs and snus/dripping or e-cigarettes. It is also taken (sniffed) through nasal route as nasal snuff (luktsnus). Smokeless tobacco is consumed with or without flavouring agents and sweeteners [1,2]. In this method tobacco product is placed between cheek and gums or lower lip and teeth. Thenit is slowly chewed or crushed to release flavour and nicotine and unwanted juicesare expectorated at short intervals till the content finishes. So, it is also known as spitting tobacco.

In South-east Asia region chewing tobacco is very popular habit among all the age groups. It is popularized due to various substitutes of tobacco easily available like pan masala and gutkha which are mixture of tobacco, areca nut slaked lime, catechu, flavouring agents and condiments [3,4]. In thelast few decades small attractive and inexpensive sachets of gutkha and pan masala have been aggressively advertised and marketed by various companies and often claimed to be safer product. These products yield popularity among all ages of male, female and even in school going children particularly in India. Tobacco contains various chemicals among which alkaloid nicotine is a stimulant and highly addictive even in lesser dosage hence, it easily makes a customer a loyal consumer (highly addictive). In addition to nicotine, smokeless tobacco products contain over 30 carcinogens [1]. These products have

been strongly implicated in the recent increase in the incidence of oral cancer,head and neck cancer, oral submucous fibrosis and other oral diseases in very young population in South-East Asia region [1-3]. As gutkha, pan masala and other tobacco products are mixture of several ingredients, where carcinogenic potential is further accentuated. Additionally, these products are potently addictive and enhance early dependence.

2. Types of Smokeless Tobacco and its Products

Smokeless tobacco is not burnt and used by other means than smoking. It is also known as chewing tobacco, oral tobacco, spit or spitting tobacco, dip, chew, and snuff. Now spit less, smokeless tobacco has also been developed in some countries. In South-east Asia region, use of smokeless tobacco is a threat for public health in many countries. These smokeless tobacco products like paan masala, gutkha, mawa, etc are advertised and marketed by various national and international companies which are available world widely. School going children and other people easily get attracted towards these cheaper, scented and small fancy sachets/ pouches of tobacco products. Various types of smokeless tobacco and its products used worldwide are

1. Khaini (India): Chewingraw tobacco leaves is the oldest method of consuming tobacco. It is consumed either as whole sweetened dry leaves or in shredded form with lime. Small amounts are placed at the bottom of lip, between the gums and teeth, where it is gently crushedor rolled to release the content and nicotine. It also stimulates the salivary glands, which leads to the development of the spittoon and isdiscarded periodically.Chewing tobacco is now modernised as several varieties of products like *scrap* (most often as loose leaves), *pellets* (tobacco bites or bits), *plug* (a form of loose leaf tobacco condensed with a binding sweetener) and *twist* (rope-like piece of tobacco twisted together). Nearly all modern chewing tobacco products are produced via a process of leaf curing, cutting, fermentation and processing or sweetening [1-6].

2. Paan/ betel quid: Paan is famous preparation used in South East Asia region. It is prepared by combining betel leaf with areca nut (seed of areca palm), katha, slaked lime, sweetener, mouth freshener and tobacco **[Figure-1]**. The compiled mixture is wrapped / rolled nicely with betel leaf and placed in mouth **[Figure-2]**. Ingredients varies in different regions and the preparation is name daccordingly [7-9]. Paanis chewed for its stimulating and psychoactive effects. After chewing,it produces red colour saliva which is either swallowed or spat out. In India people discard spit leniently here and there which creates unsightly atmosphere.

3. Guṭkha/pan masala/ pan parag/ mawa: It is ready to use preparation of crushed areca nut,tobacco, catechu (extract from the wood of the acacia plant), paraffin wax, slaked lime and sweet or savory flavourings [Figure-3]. Itis largely manufactured in India and exported and soldacross South Asia in small, low cost, individual-sized/sachets/packets. These are widely consumed by all age groups and all strata people in countries like India, Pakistan andBangla-

desh [2,9-10].

4. Gul, mishri, or gudahku: It is a toothpaste/tooth powder like preparation which is applied on teeth and gums. It is used mainly in India and Bangladesh. Men and adults from rural areas use these products at a higher rate than urban residents [2,11].

5. Dissolvable tobacco: It is finely ground tobacco that is pressed into shapes such as orbs, lozenges, tablets, sticks, or strips which slowly dissolve in the mouth. These products appeal to adolescent because they come in attractive packaging, look like candy or small mints, and can easily be hidden from.

6. Toombak and shammah: It is mixture of tobacco, slaked lime, and ash. These products are taken orally, mainly consumed in north and eastern Africa and the Arabian Peninsula [2].

7. Zarda: It contains broken tobacco leaves, boiled with lime and spices. This mixture is dried and colored with vegetable dyes and thenit is mixed with finely chopped areca nuts. It is used alone or with betel leaf in India and Arabiancountries [**Figure-3B** and **D**] [2,7].

8. Naswar: It is a mixture of tobacco, slaked lime, indigo, cardamom, oil and menthol. It is mainly used in Iran, Afghanistan, Pakistan and Central Asia [2,11].

9. Snuff: Itis a dissolvable form of smokeless tobacco which is more prevalent in The United States. It is finely ground form of tobacco that can be dry, moist, or packaged in small packets or pouches [7-8]. It can be consumed through various routes. Dry (fire-cured) powder form is put in the mouth or inhaled through the nose and may require spittingwhereas moist (age cured) is fermented tobacco processed into fine particles is placed between cheek or lip and gums and also requires spitting. Other form is U.S Snus, originated in Norway and Sweden which comes in small tins. It is a non-fermented steam-pasteurized moist powdered tobacco product.Theseare available in ready-to-use pouches that are placed between cheek or teeth and gums and do not require spitting.

3. Incidence of Smokeless Tobacco

The popularity of smokeless tobacco is growing rapidly and its prevalence of use is rising all around the world. People think that smokeless tobacco products are less harmful alternative to smoking, but it hurts and kills the people all the same. Smokeless tobacco and their products are used in a wide variety of forms and available worldwide in many countries of the world [12,13]. It contains many toxic chemicals and carcinogens, which causes negative health effects and deadly cancer. Due to widespread advertisement and exhaustive marketing of smokeless tobacco products, school children and young population are attracted more towards this bad addictive habit.

Smokeless tobacco use is a global problem that is present in low, middle and high-income countries and affects more than 300 million people. The greatest burden of smokeless tobacco use is in the South-East Asia which experiences the highest prevalence including the 89% of the world's users [**Figure- 4**]. It also carries the highest attributable disease burden, and has the different varieties in smokeless tobacco product and forms of its use. According to WHO (2009), tobacco consumption has been increasing at the rate of 2% to 5% per year. It is estimated that number of deaths due to tobacco will increase from 3 million per year world-wide to 70 million per year by 2025 [13-17].

In the 11 countries of the WHO South-East Asia Region, around 250 million adults consume smokeless tobacco which constitutes approximately 90% of global smokeless tobacco users. Its prevalence among youth and adults is higher in males than females **[Figure- 5]**. However, WHO estimates show that smokeless tobacco use among females in south Asia is a major public health threat. Among females, Mauritania (28.3%) had the highest prevalence of smokeless tobacco consumption, followed by Bangladesh (27.9%), Madagascar (19.6%), India (18.4%), and Bhutan (17.3%). Among males, Myanmar (51.4%) had the highest consumption rates of it followed by Nepal (37.9%), India (32.9%), Uzbekistan (31.8%), and Bangladesh (26.4%) [1,2,16-21].

1. India: India has one of the highest tobacco users in the world in number, diversity in product types and forms of smokeless tobacco. It is the only country in the world where overall smokeless tobacco (26%) use is nearly twice as prevalent as smoking (14%) [17-18]. There are more than 30 different types of smokeless tobacco products are available including zarda, gutkha, gul, mishri, betel quid, mawa, pan masala etc. Beside this, people make their own chew by mixing ingredients like zarda, areca nut, lime, spices according to their taste. Due to the extraordinary rate of consumption of smokeless tobacco products in north India, prevalence of oral cancer is high especially in Uttar Pradesh. In India these products are also famous among children and teenagers. It has been reported that 40% of school students and 70% of college students regularly chew gutkha in Mumbai [14,18,22-25].

2. Europe: Smoking is more prevalent than smokeless tobacco in Europe. They consume ST mainly as moist snuff, or snus, or chewing tobacco (twist) and e-cigarettes. Prevalence of ST use among adults varies from 0.1% in Maldova 3% in Denmark, 4% in Switzerland to 24% in Sweden and 31.8% in Uzbekistan. Men have higher rates of ST use than women. In Scandinavia, Swedish snus, a particular type of moist snuff product, is very famous. But migrants from SEAR show higher rate of ST use and specially consume their traditional products like pan masala and gutkha. In Kyrgyzstan and Uzbekistan, naswar is used, which is similar to the product known as nass or naswar in Iran and Pakistan.In North America, moist snuff is the most widely used product [1,2,11].

3. United States: In USA smokeless tobacco use is low (average 3.4%). It is used in two forms: chewing tobacco and snuff or snus. Approximately 6.7% of U.S. male and 0.3% female adults use smokeless tobacco. It is highest in Wyoming (9.1%), West Virginia (8.5%), and Mississippi (7.5%), and lowest in California (1.3%), Columbia (1.5%), Massachusetts (1.5%) [1,26-27].

4. African region: Prevalence of smokeless tobacco use and types of products varies across countries. They are sniffed, chewed, sucked, or applied to teeth and gums. ST prevalence is as high as 28.3% for females vs 5.7 % malesin Mauritania and 22.6% for males vs females 19.6% in Madagascar, 21.0 % for males vs 0.4% females in Algeriaand as low as 0.3% for males vs 1.2% females in Zambia and1.3% males vs 0.2 % females in Ghana [1,2,18].

5. Youth: The findings of the Global Youth Tobacco Survey show that the overall tobacco use especially among students, has not decreased in most of the countries; rather, it has shown an increasing trend. The current use of any form of tobacco ranges from 8.5% (Maldives) to 54.5% (Timor-Leste) among boys and from 3.4% (Maldives) to highest 29.8% (Timor-Leste) among girls.

The current use of smokeless tobacco products among girls aged 13-15 years ranges from 2.3% (Thailand) to 7.9% (India). Prevalence of current smokeless tobacco use among students aged boys 13-15 years in South-East Asia Region is highest in Bhutan 9.4% and 9.0% in India.[1,17-23]

In U.S.A smoking habit has declined among high school children but use of smokeless tobaccohas sharply increased in duration 2011-15 from 1.5 to 15%(highest for e- cigarettes) Recently,in 2015 CDC report it was 10% in high school boys and 1.8% in high school girls [28-29].

4. Side Effects ofSmokeless/Chewing Tobacco on Oral Health

It is a common belief that smokeless tobacco is safer alternative to smoking tobacco or it may be more conveniently used anywhere which also been emphasized by many advertising popular models or companies. Some people start use of smokeless tobacco as toothache remedy or in form of tooth power or paste. In developing countries adolescents start chewing tobacco products specially pan masala/ gutkha/ mawa just curiously or as a time pass or as mouth freshener. As these products are easily available at general store/retail shops or even road side vendors on footpath **[Figure- 6 and 7]**. Sometimes, young girls start use of smokeless tobacco as a means to lose or control weight. Whatever the reason maybe, tobacco is enjoyed by all age groups worldwide. It is a pity that they are unaware of dangerous consequences or toxic properties of tobacco products.

4.1. Toxins in smokeless tobacco

It has been estimated that tobacco, and smokeless tobacco products, contain roughly 4,000 chemical constituents, including nicotine and other toxicants and over 30 carcinogens (substances which have potential to cause or promote cancer) which are believed to play a crucial role in causing the negative health effects besides addiction [30,31,32].

International Agency for Research on Cancer (IARC) documents that traditional smokeless tobacco as well as altered/refined products, such as snuff, chewing tobacco, and betel quid, pan masala, gutkha, mawa, etc. are carcinogenic to humans. It is also found that smokeless tobacco products cause precancerous oral lesions and cancers of the oral cavity, oesophagus, and pancreas as well as reproductive and developmental toxicity [1,6,33]. Smokeless tobacco products differ considerably in their concentrations of nicotine and volatile and non-volatile nitrosamines and other contents because it passes through various steps like tobacco processing, curing, fermentation and storageduring manufacturing. Each step is differed for variety of products or treatment with chemicals which impart formation of toxic substance.

All smokeless tobacco products contain nicotine, and virtually all contain tobacco-specific nitrosamines (TSNAs). Other substances are variable in different tobacco products including N–nitrosamino acids, volatile N–nitrosamines, polycyclic aromatic hydrocarbons (PAHs), volatile aldehydes, inorganic compounds, heavy metals, metalloids and radioactive metals [1,31,34,35]. TSNAs and PAHs are carcinogenic to humans. TSNAs include 5-6 chemicals of which N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)1-butanone (NNK) are most toxic and IARC classify them as Group 1 carcinogens [36-38] [*group-1* carcinogen: means substance is a proved carcinogen in animal models.]. The nitrosamines can be metabolized in human body by target tissues to compounds that can modify cellular genetic material. This alteration can be repaired to some extent but if chronic modification occurs, it leads to mutagenesis/ carcinogenesis (initiation of cancer formation) Toxic metals that have been found in ST products include arsenic, beryllium, chromium, cobalt, cadmium, lead, nickel, mercury, and the radioactive metals polonium-210 and uranium [36,39].

Tobacco products also contain added plant materials such as tonka bean or sweetener, flavouring agents that may further contribute to adverse health consequences. Areca nut containing products like paan (betel quid), gutkha, tombol, pan masala and mawa, are commonly used in South-East Asia, Middle East, South Africa and other parts of the world. [7,17]Areca nut itself is considered by an IARC as group 1 carcinogen and has been associated to oral submucosal fibrosis (OSF) and oral squamous cell carcinomas. Therefore, the health risks associated with smokeless tobacco can vary with use of products, manner and duration of use. When tobacco is mixed with other chemicals or substances with a carcinogenic potential its deleterious effect synergizes [1,9,40-41].

4.2. Mechanism of cancer induction

Mechanism of cancer induction in smokeless tobacco users are shown in **[Figure-8]**. Genotoxic effects occur from smokeless tobacco and its products, paan and gutkha mostly due to the presence of nitrosamine, its compound and heavy metals. The nitrosamine in the chewers' saliva undergoes nitration when it reacts with nitrite in the presence of a catalyst [1,43]. The nitrosamine in tobacco and its products undergoes metabolic activation by cytochrome P450 enzymes. It leads to the formation of N-nitrosonornicotine (NNN), methylnitrosaminobutanone (NNK), methyl-nitrosaminopropionitrile (MNPN) Arecoline and Reactive oxygen species (ROS)are major carcinogen [9], which further leads to DNA damage or methylation. If this damage is not repaired and cause permanent DNA mutations, such as in the RAS oncogene or the TP53 tumor suppressor gene, leading to uncontrolled cell growth and ultimately cancer. Other mechanisms that may contribute to cancer promotion include chronic local inflammation and irritation, oxidative stress, and reactive oxygen species [11,44].

4.3. Adverse effects

Smokeless tobacco users generally keep crushing the contents in oral cavity for a long duration while chronic users may even keep for a full day. The contents of tobacco mixed with saliva; are slowly absorbed through oral mucosa and may also cause local irritation and sensitization. Contents which are absorbed through the oral mucosa and by swallowing saliva, containing nicotine, sweetener, flavour and various toxic substances reach the systemic circulation. In this way these harmful chemicals (carcinogens and other toxicants) circulate throughout the body and may cause cancer and damage multiple organs [42-44]. Little et al in their study compared 245 smokeless tobacco users and 223 non-smokeless tobacco users with same age distribution and found that 78.6% of smokeless tobacco users had observable oral lesions as compare to 6.3% of non-smokeless tobacco users. 85% of the lesions in smokeless tobacco users was located in the primary area of tobacco placement [45].

The adverse effects of using smokeless tobacco or chewing tobacco or their ready-made products can be local or systemic:

1. Local: In the oral cavity its effect is on both soft and hard tissue; including teeth, supporting ligaments and temporo-mandibular joint. Soft tissue includes mucosa and submucosa which is delicate covering of entire oral cavity. Oral mucosal lesions are more severe in people who use smokeless tobacco and its products at an earlier age or for longer duration per day or greater dosages or on more days per month or for years [6]. The benign (precancerous) lesions usually resolve when people stop using tobacco.

2. Systemic: Effects on distant organs of the body.

[A] Local Effects on Soft And Hard Tissues Of Oral Cavity

1. Staining of teeth: Most common effect of chewing tobacco is reddish brown staining/ discolouration of teeth and oral mucosa [46]. Tobacco users lose their sparkling smile **[Figure-9]**. It is caused by various ingredients like betel nut and catechu mixed with lime and other substances. Initially the staining is temporary, but as the user becomes habitual, staining becomes permanent. In India and South-East Asia, the use of pan masala, gutkha and betel nut is so familiar that people do not bother to have coloured tooth and oral mucosa. They do not even hesitate to spit on the public places, official places, streets, lifts and stairs.

2. Betel chewer's mucosa: The oral mucosa at site of placement of betel quid and areca nut becomes loose and irregular with desquamated tags of tissue and underlying areas can also show wrinkled appearance. This condition may be caused due to either direct action of the betel quid or traumatic effect of chewing or both. This leads to tendency for the oral mucosa to desquamate or peel off. The affected areas also show the evidence of incorporation of ingredients of the betel quid in the form of yellowish or reddish-brown encrustations. Betel chewing produces reactive oxygen species (ROS) that is detrimental to oral mucosa and cause damage to lining epithelial cells.

The lesion is often localized and strongly associated with the habit of betel nut/ quid chewing for long durations. The chewer's mucosa usually develops at age of 50 years or more after chronic use [47-48]. Several studies have shown that the prevalence of betel chewer's mucosa may vary between 0.2 to 60.8% in different south East Asian populations. Betel chewer's mucosa is not potentially a precancerous lesion but this condition is often co-exists with other mucosal precancerous lesions such as leukoplakia and oral submucous fibrosis [49-50].

3. Gingival and Periodontal diseases: Due to continuous use of smokeless tobacco for long duration, the hygiene of oral cavity becomes poor which leads to development of many oral problems. Chemicals and toxins that are present in smokeless tobacco and their products make the chewer so addictive and with each chew it irritates the gum tissue and periodontium. It also abrades gingival surface causing it to pull away from teeth (gingival recession) and makes the gingiva more prone to gum diseases like gingivitis, periodontitis etc. Incidence of gingival recession is commonly seen adjacent to the area where the tobacco is held. It is higher among individuals who use snus or snuff than people who do not use smokeless tobacco. Gingival recession can be observed within one year of beginning to use smokeless tobacco [1,6, 50-53].

4. Dental caries and Tooth loss: It is common belief that chewing areca nut is protective for tooth as it contains anti-bacterial properties but in long term it rather harms. Prevalence of dental decay and caries is more associated with the use of chewing tobacco. [54] When the

gingival tissue pulls away (gingival recession) it creates greater risk for tooth decay. The ingredients present in ready to use tobacco products like tobacco, areca nut, catechu and slaked lime (calcium hydroxide) together may cause greater inflammation and injuries than one of these ingredients used alone. [55] Sugars are often added to enhance sweetness and flavour of smokeless tobacco, acts as nutrient medium for growth of microorganisms and hence increases the risk for tooth decay. Grit in betel nut or gutkha causes abrasion of hard coating of teeth (enamel) leading to sensitivity to hot or cold and individual feel pain on taking cold or hot food materials. Gradual abrasion also causes shortening of tooth and eventually tooth loss **[Figure 10]** [47,48,52].

5. Root fracture of tooth: Smokeless tobacco products contains some gritty and hard substances like betel nuts, so in chronic users there is risk of root fracture of tooth, trismus and other pathology of temporomandibular joint [47,56].

6. Bad Breath: Long-term use of smokeless tobacco causes bad breath or halitosis due to production of nicotine and other chemicals in their mouth and thus allows bacterial growth resulting in bad breath. Moreover, due to the habit of continually spitting, saliva gets exhausted leading to dry mouth and person deprived of the protective antibacterial property of saliva. This leads to infection or other harmful consequences [51,57].

7. Alteration of taste and smell: - Chewing tobacco alters person's sense of taste and ability to smell. As a result, tobacco users like to eat saltier and sweet foods which are harmful if consumed excessively [51,54, 57].

8. Nasal Cavity: Some types of ST are inhaled nasally like dry (powdered tobacco) snuff, can cause edema of the mucosa and submucous conjunctive tissue of the turbinates, atrophy of the middle and inferior turbinates, reduction of nasal mucociliary clearance, and chronic rhinitis.

4.3.1. Development of Pre-Malignant Lesions of Oral Cavity

In smokeless tobacco chewers, the hygiene of oral cavity becomes poor day by day with gradual loss of saliva which leads to many oral problems. In the chewer's mouth, the tobacco contents and its toxins mixed with saliva remain in contact with oral mucosa for long duration, causing staining and deleterious effects like desquamation, non-healing ulcer and thickening of mucosa. Gradually these changes progress to precancerous lesions like white patches (leukoplakia), red sores (erythroplakia), tobacco pouch keratosis, quid induced lichenoid reaction, oral submucus fibrosis, tobacco associated melanosis etc. These harmful effects are so gradual in onset that tobacco user can not relate the tobacco as a causative agent and they become so addicted to tobacco that they don't want to give up the habit even on these warning signs and on doctor's advice. Several studies from the United States, Europe, and Asia provide conclusive evidence that smokeless tobacco products, including snus, snuff, shammah, and betel quid

(pan), are strongly associated with the prevalence of oral mucosal lesions such as leukoplakia, erythroplakia, and verrucous hyperplasia and have risk to transform into cancer. [1,9,40-41,43] These mucosal lesions are more severe in those persons who start chewing ST products at an earlier age and use for more hours per day, use greater dosages, or use on more days per month [6].

Pre-malignant lesions of oral cavity developed by chewing tobacco: Chewing tobacco leads to development of various pre-malignant lesions which are

1. Oral sub mucus fibrosis (OSMF): It is a chronic, insidious onset, potential malignant lesion of the oral cavity; characterized by inflammatory and progressive fibrosis of the submucosal tissues, leading to restricted mouth opening or (stiff mucosa). As the disease progresses, the jaws become so rigid that the person is unable to open the mouth or eat food. It may affect the entire oral cavity or may extend to the pharynx. In India, the prevalence OSMF increased over the past four decades from 0.03% to 6.42% [58].

2. Clinical feature: OSMF is characterized by sunken cheeks, thin stiff lips, dryness and burning sensation of the oral mucosa followed by ulceration pain, and change in tone of voice. The oral mucosa also shows a mottled marble like appearance with thick vertical fibrous bands. These bands restrict the mandibular range of motion and causes trismus. So, in these patients maintenance of oral hygiene becomes poor, dentition often is stained as a scarlet colour and develops gingival and periodontal diseases **[Figure-11]**. Condition of patient is extremely debilitating and has a high risk of transformation into the oral cancer. A malignant transformation rate of 7.6% over a period of 10 years was described in an Indian cohort [59].

3. Aetiology: Various factors have been implicated in the development of OSMF; the most common of which is chewing betel nut or tobacco products like pan masala, gutkha, mawa etc. Other causes are autoimmune reaction, vitamin and iron deficiency have been reported [47-49,60-63].

4. Pathogenesis: Recently suggested pathogenesis of OSMF is by action of betel nut and its toxic chemicals due to gradual deposition of collagen in submucosa **[Figure 12]**. The alkaloids present in betel nut, arecadine and arecoline gets converted in to arecadine (active metabolite) and cause stimulation of fibroblasts leading to proliferation and collagen synthesis. It also into excessive amount of collagen deposition. In addition, repeated trauma to mucosa by grit, or chewing areca nut or grit in pan masala, induce inflammation which release various collagen stimulating and stabilizing growth factors which further enhance collagen fibre deposition. All these mechanisms finally result into fibrosis of oral mucosa termed as OSMF [47].

5. Incidence: OSMF is very common in Southeast Asia but has started to spread to Europe and North America due to increasing the widespread habit of pan and gutkha use. The current prev-

alence of smokeless tobacco use is very high in India. Nowadays use of pan masala, gutkha and mawa have popularised among adults and adolescents in India which have been strongly implicated in the recent increase in the incidence of oral submucous fibrosis, especially in the very young / adolescents, even after a short period of use. [11] Kumar BN et al found in his study that OSMF is highly prevalent in smokeless tobacco users as compared to conventional and reverse smokers [47,64-65].

6. Prognosis and treatment: It depends on the degree of clinical involvement. Most of the patients with OSMF presents with moderate-to-severe stage. If OSMF is diagnosed at a very early stage, it is reversible with cessation of tobacco chewing habit alone. Moderate OSMF is reversible with cessation of habit along with some medications and mouth opening exercise whereas severe oral submucous fibrosis is irreversible and potential to develop into malignancy.

7. Leukoplakia: It is a thick and firmly attached white patch or plaque that can be found on oral mucosa, gingiva, tongue, palate, floor of mouth and pharynx. The patch may appear translucent or opaque and raised or ulcerated **[Figure-13]**. Leukoplakia is a clinical term hence other disease must be ruled out by doing tissue biopsy of lesion and its histological examination. Leukoplakia changes with time and progress to more severe lesion. Aetiology: It is caused by chronic irritation due to tobacco chewing, smoking, alcohol and betel nut. Other causes may be sharp cusp of teeth or ill-fitting dentures. It usually occurs at middle age (after 30 years).In India many studies have proven that leukoplakia is strongly associated with smokeless tobacco and areca nut use and seen in young adults and adolescents[51,61,64, 66-68].

8. Type of leukoplakia: There are various types of leukoplakia recognized according to appearance like homogenous, non–homogenous, flat or raised, nodular and exophytic. Verrucous leukoplakia (or verruciform leukoplakia) is type of leukoplakia appear as thick, white, papillary lesions [47-48,69].

9. Prevalence and prognosis: The prevalence and severity of leukoplakia show a dose-response relationship, which is best predicted by the amount, frequency and duration of smokeless tobacco or betel nut use [66,68-6]. Warnakulasuriya reviewed four case control studies that showed relative risk of oral leukoplakia in betel quid chewers **[Figure 14]**. They found that chewing areca (in betel quid without tobacco) raised the odds ratio (OR) to 5 compared with non-chewers (OR=1); adding tobacco to the quid further raised the relative risk by at least three-fold compared with non-tobacco users [67]. Leukoplakia is a pre-step of cancer, that gradually progresses to oral dysplasia (disorganised cells but limited to oral epithelium) to frank cancer (squamous cell carcinoma) [70-71]. Various studies have examined the transformation rates of oral leukoplakia to cancer. Pindborg et al [72] followed 248 patients for a mean period of 3.7 years; during this period prevalence of malignant transformation in leukoplakia

was found 4.4% whereas Banocz and Csibareported 6% transformation in their study of 670 patients observed over a period ranging from 1 to 30 years [73].

10. Erythroplakia: Erythroplakia is also a clinical term which appears as a red patch/eroded area. It occurs most commonly on the floor of the mouth. Biopsy is required to see the degree of atypical changes in oral mucosa or to exclude oral cancer. There may be mixed lesion white and red patch termed as erythro-leukoplakia.

11. Prognosis: Majority of oral leukoplakia lesions will behave benign and often regress after stoppage of smokeless tobacco product or irritating agent. [74] But total response is unpredictable; it also depends on degree of associated dysplasia, duration, frequency and type of tobacco product used. Verrucous or nodular lesions, red lesions (erythroplakia) and mixed red and white lesions (erythro-leukoplakia) have a higher risk of cancerous change than homogenous leukoplakia [75].

12. Tobacco pouch keratosis: It is a focal, ill-defined area of white patch which develops at the site where tobacco chewers placed the tobacco each time. Most commonly, it involves the mandibular labial and buccal mucosal folds. The continued use of tobacco causes the affected areas to become opaque white and corrugated. Microscopically, tobacco pouch keratoses show hyperkeratosis and acanthosis of the mucosal epithelium. But epithelial dysplasia is uncommon and if present usually mild type [76]. The condition usually disappears once the tobacco habit is stopped. It is associated with slightly increased risk of mouth cancer [77].

13. Quid /Areca nut induced lichenoid reaction: Areca nut is group I carcinogen and is one of the culprit to cause oral cancer in smokeless tobacco users. Areca nut also causes various precancerous to cancerous lesion of oral cavity. Areca-induced lichenoid lesions are mainly seen at the sites of quid application like buccal mucosa and tongue. [48] It is seen as fine wavy keratotic (white) lines/striae which are radiating from a central red/atrophic area. These lines do not criss-cross and are parallel to each other. The histological picture shows hyperkeratosis. The lesion usually resolves following cessation of areca use.

4.3.2. Proliferative Verrucous Leukoplakia (PVL)

It is a clinical term and previously was termed as oral florid papillomatosis. It is a rare form of oral leukoplakia (non-homogeneous type) which is irreversible, slow growing with highest potential of malignant transformation and resistant to treatment. It starts initially as a white plaque of hyperkeratosis that eventually becomes a multifocal disease with confluent, exophytic, papillary and proliferative features.

Aetiology of PVL is uncertain but some viruses and tobacco association is described by some authors [78,79]. Biopsy examination of such lesions may show spectrum of histo-

pathological lesions from simple hyperkeratotic lesion to verrucous hyperplasia or verrucous carcinoma or verrucous hyperplasia with dysplasia or well differentiated squamous cell carcinoma. The buccal mucosa, gingiva, and alveolar ridges were most commonly affected sites. The average age of presentation of PVL is above 60 years and more common in women. Risk of malignant transformation is highest which can range between 60-100% [79-80].

Malignant lesions of oral cavity developed by chewing tobacco: Chewing tobacco can also leads to development of various malignant lesions which are

1. Verrucous carcinoma (VC): It is a rare warty variant of squamous cell carcinoma frequently seen in smokeless tobacco chewers or those who frequently use snuff orally; hence it is sometimes referred to as "Snuff dipper's cancer. Verrucous carcinoma is slow-growing tumor shows keratotic exophytic surface comprising of sharp/blunt hyperplastic epithelial projections with keratin plug and also these projections pushing downward into sub-epithelial tissue. It behaves as locally invasive tumour and rarely metastasizes. Clinically, these tumors present as, slow growing, painless, white-gray, warty lesions. It should be differentiated carefully from close simulating benign and malignant lesions like verrucushyplasia/ proliferating verrucus leukoplakia, well differentiated squamous cell carcinoma. It commonly seen in men over the age of 65 years. It most commonly affects the buccal, gingival and alveolar mucosa hard palate, floor of the mouth, larynx but any oral segment can be affected. Secondary infections are frequent, and may lead to an unpleasant odor and reactive lymphadenopathy [81]. *Aetiology*: Various causative factors have been suggested for verrucous carcinoma and Human Papilloma Virus (HPV) has been considered one of them. [82] Smoking tobacco is highly associated with the development of mucosal verrucous carcinoma of the neck and head while poor oral hygiene, presence of oral lichenoid and leukoplakic lesions may act as enhancing effect. In Asia it has also been found that leukoplakia has a synergistic effect which is associated with smoking, smokeless and chewing tobacco habits. [62] Shear and Pindborg in their study reported that out of 28 patients with verrucous lesions, 24 (86%) used tobacco and one was an areca quid chewer [83]. Chen et al in a study found that tobacco appears to be a major factor in causation of verrucous lesions [84]. In Taiwan, a study of verrucous carcinoma, areca quid chewing was reported 97.3% of cases [85].

2. Prognosis: Most patients with verrucous carcinoma have a good prognosis. Surgical excision or laser therapy is possible treatments.

3. Oral intraepithelial dysplasia: These lesions denote the various stages of cancer progression from initial to invasive form and can be associated with long standing premalignant conditions like leukoplakia, erythroplakia, oral submucous fibrosis etc. Clinically, this may look like above premalignant entities or with some atypical changes within them as ulceration, bleeding, rapid change in shape and appearance or pain. In these cases diagnosis and manage-

ment of lesions should be decided by histopathological examination. In epithelial dysplsia, there is disorganisation and abnormal proliferation of epithelial cells; if they remain within boundary of epithelium it is term as intra epithelial dysplasia when these abnormal proliferating cells cross the boundary and goes into underlying subepithelial loose tissue it is termed invasive tumor/*squamous cell carcinoma.*

4. Oral squamous cell carcinoma: It is most common cancer of oral cavity and accounts for 90% of all oral cancers. Oral cancer ranks the eighth position in the cancer incidence ranking worldwide and in India it ranks third most common malignancy [86-88]. Along with cancers in the head and neck region; it is one of the leading causes of death in developing countries of South East Asia [89].

5. Aetiology: Smokeless tobacco is one of the major risk factors associated with the high prevalence of head and neck and oral cancer in this region. It is estimated that over 90% of the global smokeless tobacco use burden is in South East Asia. [16,17] In a systemic meta-analysis Khan et al reported that the pooled odds ratio for chewing tobacco and risk of oral cancer was 4.7 whereas the pooled odds ratio for chewing paan/betel quid and risk of oral cancer was 7.1 [90]. Some other causes of oral cancer include smoking, poor oral hygiene, irritation due to ill-fitting dentures, alcohol, rough edges on the teeth and some chronic infections caused by fungi, bacteria or viruses. It is found in middle and older age (50-70 years) but in tobacco users it may see in younger age (35-40 years). Squamous cell carcinoma can arise from any part of the mucosal lining of oral cavity, pharynx and larynx; however alveolar gingiva, floor of mouth, lip, tongue, hard palate, base of tongue is the most common site **[Figure 15]** [88]. At early stage, it may present red or white patches, nodule, a painless non-healing ulcer, progressive increase in size, sudden tooth mobility, unusual oral bleeding, epistaxis and prolonged hoarseness of voice. In advance stage, it may spread to lymph nodes in neck region or metastasizes to distant sites in the body. In majority of cases it precedes the premalignant condition like leukoplakia, erythroplakia, oral submucous fibrosis and various degree of oral intra-epithelial dysplasias. Azad et al in their study emphasized that squamous cell carcinoma in tobacco users showed more expression of GLUT-1 which marks the more aggressive nature of tumor [91]. Diagnosis of suspected lesion is confirmed by biopsy and its histopathological examination.

6. Types: Gross appearance: cauliflower like (solid outgrowth), ulcero-proliferative, diffuse infiltrative (hard to firm area) Histological: according to tumor cell morphology and arrangement it can be, well differentiated, moderately and poorly differentiated

Prognosis is depending on the site, size of tumor, stage of disease and overall health of patient. Treatment of oral cancer involves a multidisciplinary team with specialists from the realms of radiation, surgery, chemotherapy, nutrition, dentistry, and all possibly involved with diagnosis, treatment, rehabilitation, and patient care.

[B] Systemic effects:

Besides nicotine smokeless tobacco and its products contain various harmful substances including tobacco-specific nitrosamines (TSNAs) and toxic metals which enter into systemic circulation and producing various deleterious effects on human health. The metabolites of TSNAs can be detected in urine of smokeless tobacco users and can be used as biomarkers for assessments of a person's exposure to specific TSNAs. Raised TSNAs levels and its metabolites increase the risk for development of various cancer of oral cavity as well as distant organs [6,92].

1. Nicotine Dependence/Addiction: During tobacco chewing its contents mixed saliva when comes with contact of oral mucosa; nicotine and others soluble substances gets absorb and goes to blood circulation. Nicotine present in smokeless tobacco is a potent addictive agent and other substances like flavouring agent and sweetener may further enhance its effects and causing dependence and addiction. Smokeless tobacco consumers feel pleasurable psychoactive effects, but after long term use they continue to crave and use despite of its harmful effects on body. They may sometimes switch to other products with higher nicotine levels and frequently relapse occurs upon cessation. Tobacco users also promote other near ones or friends to tobacco products for enjoyment and celebrations. It has been found that addiction to ST is related to age at initiation, amount and frequency of nicotine ingested per day, and years of use. Nicotine addiction can lead to an artificially increased heart rate and blood pressure. In addition, it can constrict the blood vessels that are necessary to carry oxygen-rich blood throughout the body. Athletic performance and endurance levels are decreased by this reaction [93].

2. Other organ cancer: Beside the oral cavity and head and neck cancer smokeless tobacco use can cause cancer of other organ of body including esophagus, pancreas, uterine cervix, lung, kidney etc. studies from Asia, Scandinavia Sweden shows epidemiologic evidence of a causal association between esophageal, pancreatic, and lung cancer and use of smokeless tobacco including chewing tobacco, snus and snuff [1,6,43,94]. Few research has detected higher levels of carcinogen (TSNAs) in human cervical cells of smokers than in those of non-smokers [44]. also studies have confirmed that smoking is an independent risk factor for cervical squamous cell carcinoma. In a case-control study in India showed a significant dose-response relationship between the number of betel quids with and without tobacco chewed per day and increased risk of invasive cervical cancer [1,40].

3. Reproductive system: various constituents of ST like nicotine, areca nut, PAHs, and several metal including arsenic, cadmium, lead, and mercury in smokeless tobacco products have deleterious effects on reproductive system, causing hormonal irregularity and infertility. They act like developmental toxicants. Metals may cause oxidative stress in cells and interfere with fetal nutrition. Studies suggest that infants born to mother who use tobacco and its products

during pregnancy have a higher risk of several adverse outcomes such as pre-term birth and fetal growt

4. Diabetes and Insulin Resistance: Nicotine in tobacco products increases circulating levels of insulin-antagonistic hormones and impairs insulin sensitivity, hence tobacco users have an increased risk of developing type-2 diabetes [6,96].

5. Cardiovascular system: Nicotine present in tobacco and its product can lead to an artificially increased heart rate and blood pressure. Toxic agents present in tobacco products like nicotine, PAHs, and heavy metals such as cadmium cause damage to the vessels, insulin resistance, hyperinsulinemia, vasoconstriction and inflammation leading to the development of endothelial dysfunction and atherosclerosis. Smokeless tobacco user is associated with an increased risk of ischemic heart disease, hypertension and stroke. Some studies suggest an increased risk of non-fatal cardiovascular disease associated with use of ST including snuff, chewing tobacco, betel quid with tobacco, and mishri/ pan masala.Overall athletic performance and endurance levels are decreased in chronic tobacco/ tobacco product user [97-99].

5. Others

Environment and surrounding: Tobacco cultivation and its processing consume lot of water and pesticide which is not eco-friendly. Further small plastic pouches /sachets of packing material of ready to use tobacco products thrown by users here and there creates unsightly appearance and also increase burden of plastic garbage, as it is too small to pick-up by rag pickers, and blow away with wind.

6. Conclusion

It is clear from the various studies from different parts of world, that betel nut alone or smokeless tobacco and ready to use products have significant deleterious effects on dental hard and soft tissues. They are carcinogenic and on chronic use they can cause deadly cancer of oral cavity as well as cancer of pharynx, larynx, esophagus, pancreas, lung, and uterine cervix. It also affects the heart and reproductive system. Thus, there is an urgent need to ban and stop the marketing and advertisement of tobacco and its products as well as areca nut. Legislation against open sale and use of such products should be stricter and more countries should be encouraged to bring out such legislations. Public health programs regarding the harmful effects of smokeless tobacco and betel nuts, along with increased awareness by healthcare professionals of the signs and symptoms of this disease, can inhibit the user from tobacco habit.

These programmes should specially target school going adolescents and young population or they must be taught about side effects to help them to escape from tobacco habit. No street vendor or retail/general shop should be permitted to sell these products as these places

are easily approachable to adolescents. In addition to educating consumers about the health risks of tobacco and betel nut use, we should encourage them to de-addiction. At global level there is need to reduce tobacco cultivation as it consumes large quantity of water, pesticides and at some places involve child and women labour. Instead, other more useful plants can be cultivated for generation of revenue and this will save money in long term as it reduces cancer burden, cost of medicine and hospitalisation.

Figures

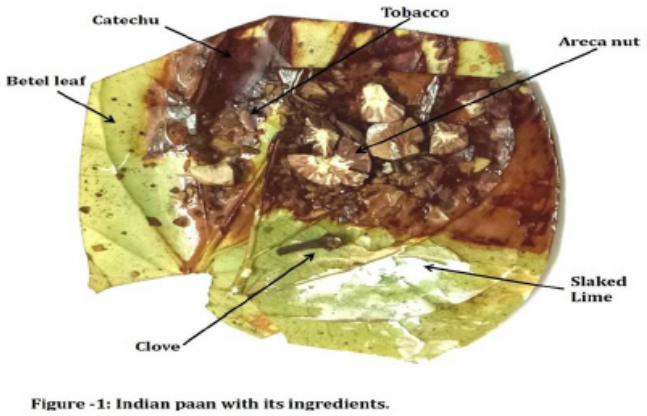

Figure 1: Indian pan with its Ingredients

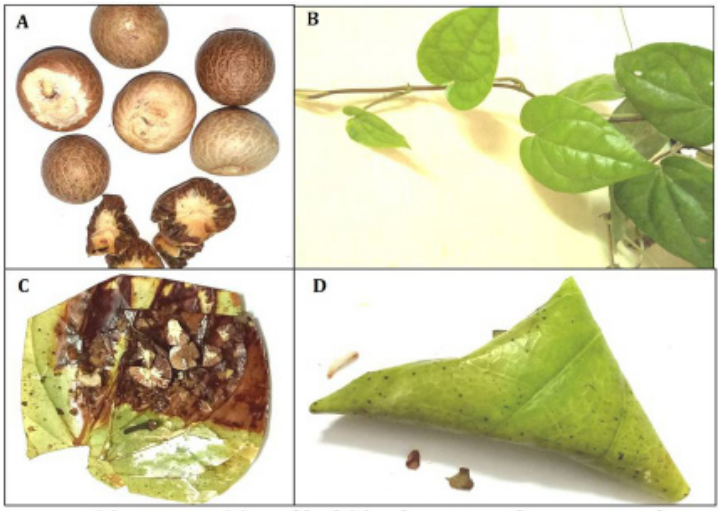

Figure 2: (A) Areca nut, (B) Betel leaf, (C) Indian paan with various ingredients and (D) Folded paan with ingredients ready to chew (Betel Quid)

Figure 3: (A) Guta mixed with jarda (tabacco), (B) Gutka/pan masala Zarda/tobacco, (C) Gulka/pan masala sachet and (D) Tobacco/zarda in can.

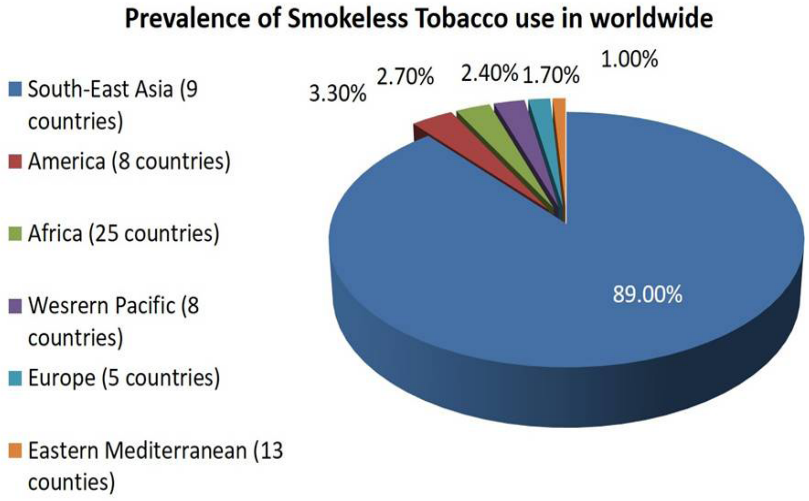

Figure 4: Prevalence of smokeless of tobacco in worldwide.

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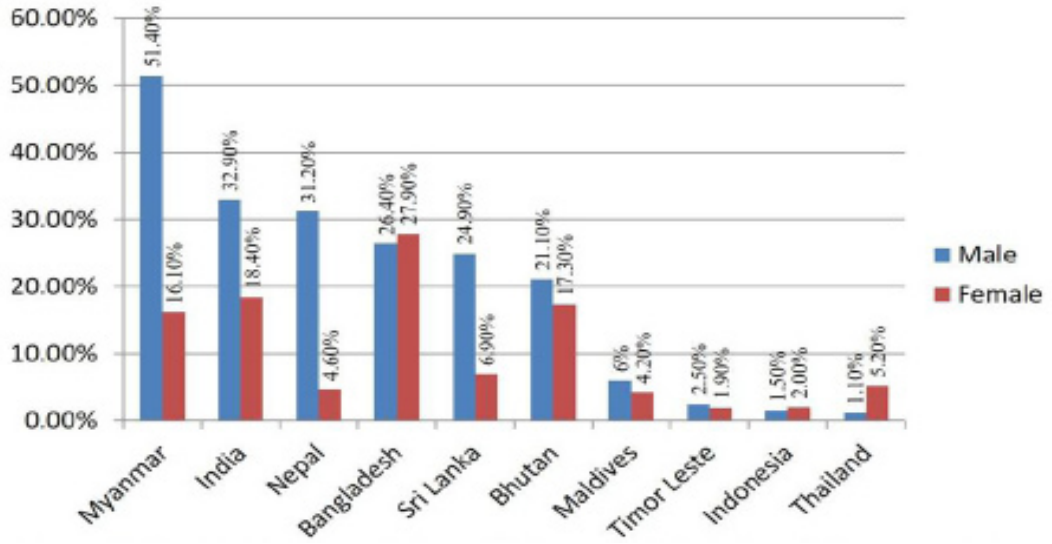

Figure 5: Prevalence of smokeless of tobacco in South-East Asia among male and female. (data source:http://www.searo.who.int/mediacentre/releases/2013/pr1563/en/)

Figure 6: Guta / Pan masala in street vendor.

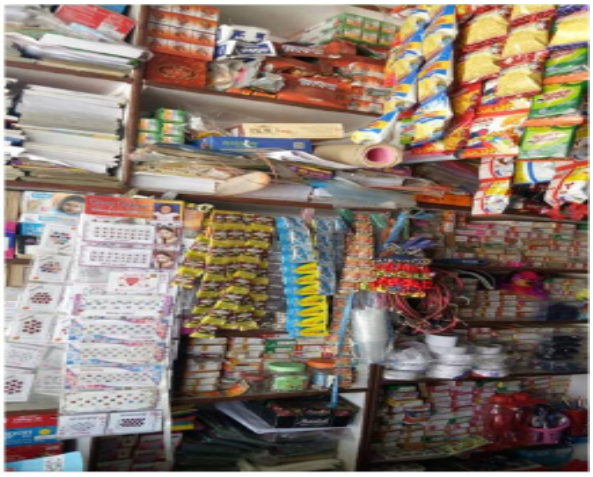

Figure 7: Guta / Pan masala at retail shop

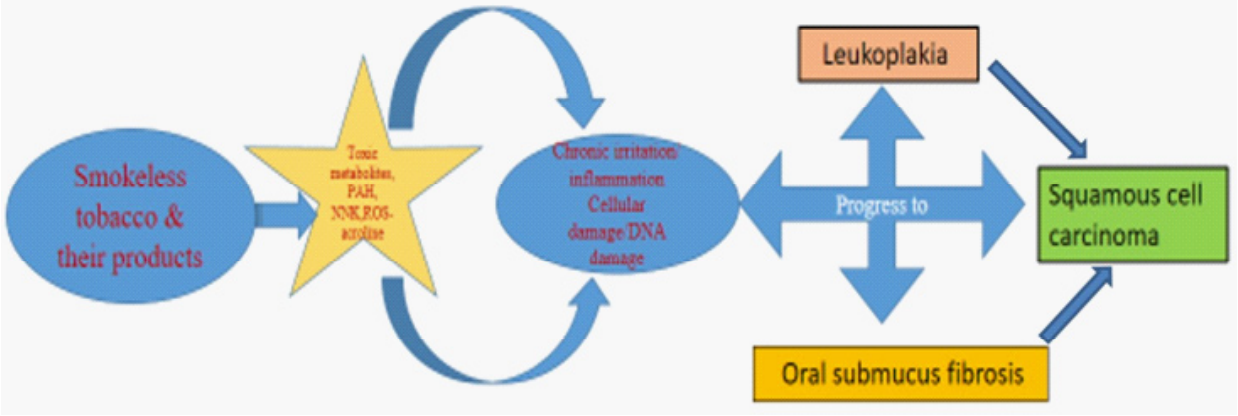

Figure 8: Mechanism implicated in induction of cancer due to use of smokeless tobacco.

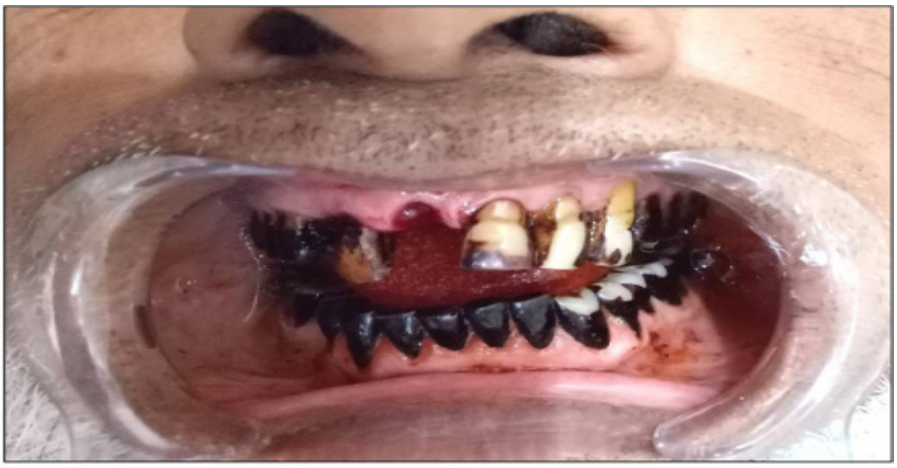

Figure 9: Staining of teeth and fractured front teeth in chronic tobacco chewer.

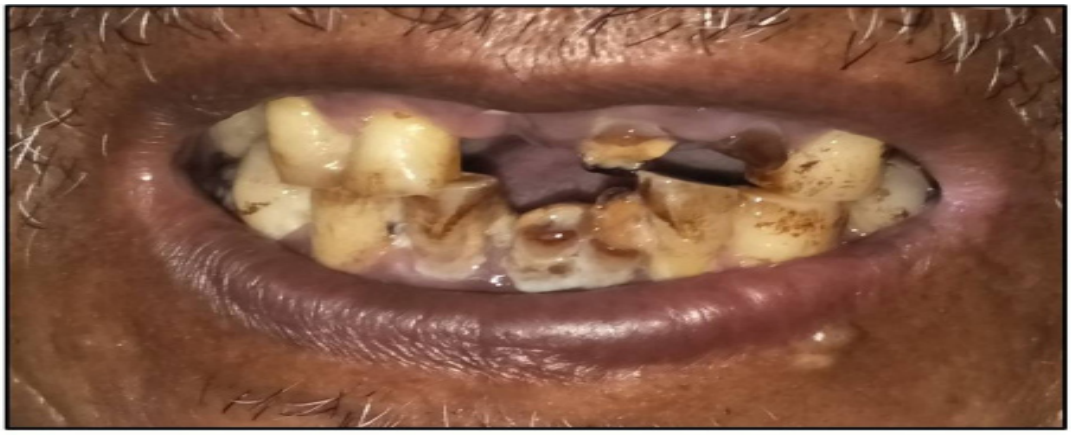

Figure 10: Poor oral hygiene and teeth loss due to chronic tobacco chewing.

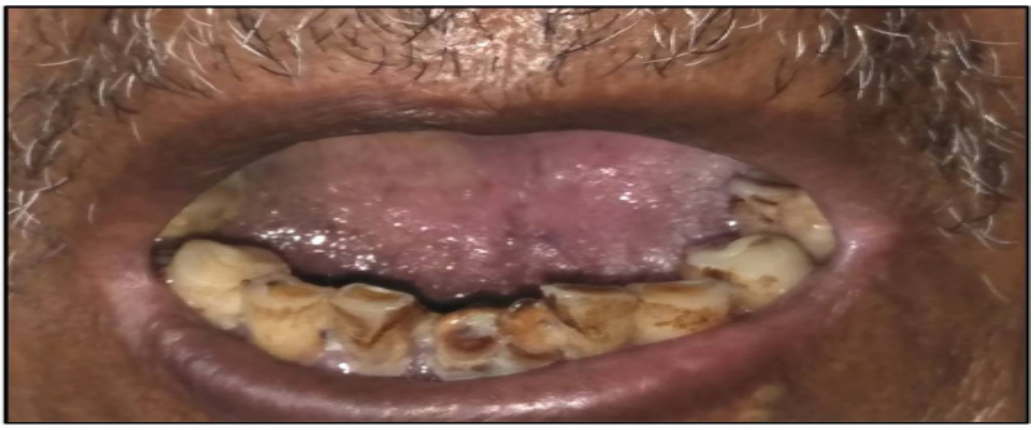

Figure 11: oral hygiene and teeth loss due to chronic tobacco chewing.

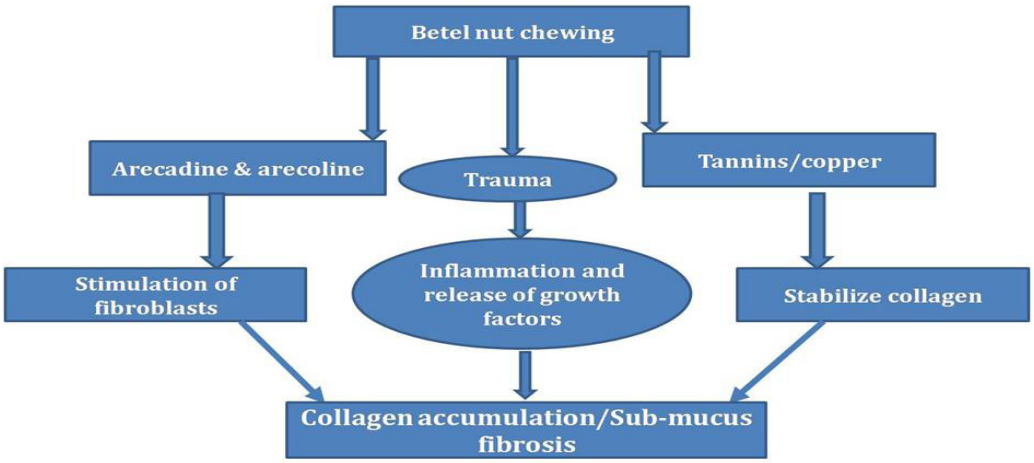

Figure 12: Mechanism of formation of sum-mucous fibrosis

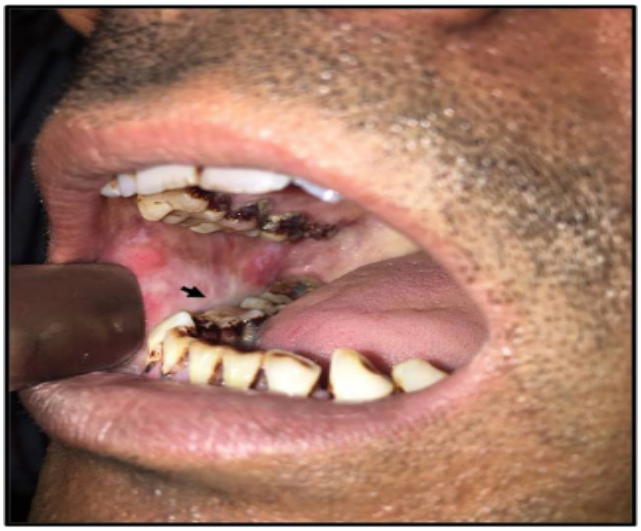

Figure 13: Leucoplakia in chronic tobacco chewer (arrow)

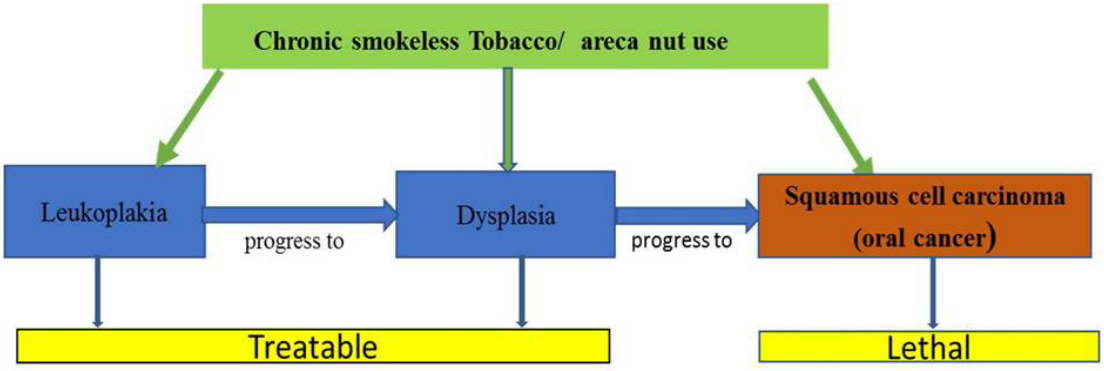

Figure 14: progression of leukoplakia

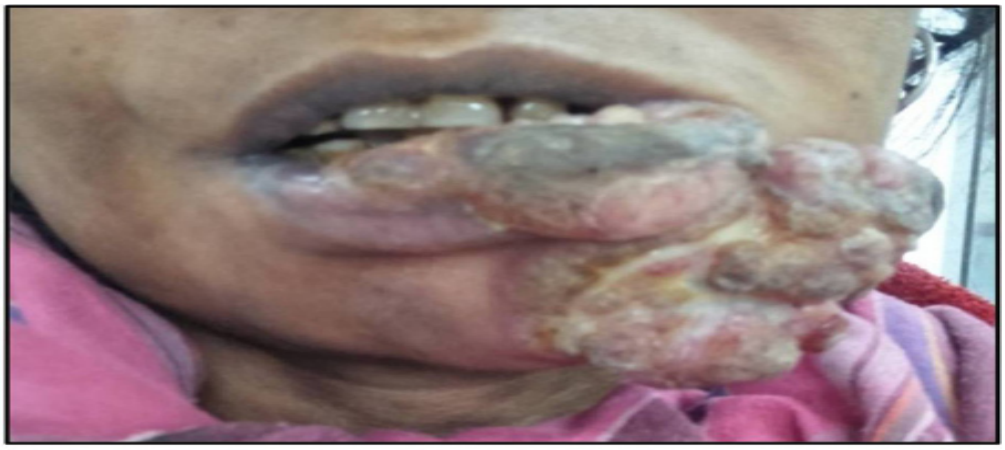

Figure 15: Oral cancer in chronic tobacco chewer.

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