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Sister Chromatid Exchanges in Betal and Tobacco Chewers and Tobacco Smokers

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INTRODUCTION

The sister chromatid exchange (SCE) analaysis has been extensively used for the assessment of the DNA damaging potential of various physical and chemical environmental agents (Latt et al., 1980). However, neither the mechanism of production nor the biological consequences are fully understood. The present study was undertaken:

1. to investigate the frequency of SCEs in human amnion cells.

2. to investigative the effect of betel and tobacco chewing on the frequency of SCE.

3. to investigate the effect of bidi and cigarette smoking on the frequency of SCE in human lymphocyte chromosomes.

4. to investigate the SCE levels in the peripheral blood of patients with oral leukoplakia and to correlate them with the habits of different forms of tobacco usage.

5. to investigate SCE levels in the peripheral blood of patients with submucuous fibrosis and to correlate them with the habits of different forms of tobacco usage.

6. to investigate the effect of betel chewing on the frequency of SCE in pregnant women and women using oral contraceptives, in whom the hormonal profile is known to be different from normal women (For details see, Ghosh and Ghosh, 1984, 1986, 1987, 1988; Ghosh et al., 1988, 1990).

MATERIALS AND METHODS

The material collected and analysed for various investigations were as follows:

1. to study the frequency of SCEs in human amnion cells, the number of pregnant women participating in the study was 27. This consisted of 12 smokers who smoked at least 10 cigarettes per day and 15 non-smoking controls. The average age of smoking women was 36.4 years (range 35-38 years) and those of non-smoking control was 35.7 years (range 34-39 years) (For details see Ghosh and Ghosh, 1986).

2. to study will effect of betel and tobacco chewing on the frequency of SCE. Peripheral blood samples were obtained from 53 healthy adult males who were not occupationally exposed to mutagens, they were non-smokers and had no previous history of drug consumption. 18 were betel chewers, 15 chewed betel-with-tobacco, and the remaining 20 individuals were apparently normal non-chewing males who served as controls.

3. to study the effect of bidi and cigarette smoking in the SCE samples were obtained from 62 healthy adult males belonging to lower socio-economic status who were not occupationally exposed to mutagens and who had no known disease or recent viral, chemical, or radiation exposure. Twenty-four were bidi smokers, 18 smoked cigarettes, and the remaining 20 individuals were apparently normal, nonsmoking males who served as controls (For details see Ghosh and Ghosh, 1987).

4. to study the SCE levels in the patients with oral leukoplakia and to correlate that with habits of different forms of tobacco usage, peripheral blood sampels were collected from 59 cases of oral leukoplakia. Leukoplakia was defined as a white patch that cannot be removed by scraping and cannot be attributed to any other diagnosable disease. This definition has no histologic connotation. The control group consisted of 65 age-matched normal individuals with no history of tobacco usage, radiation, or viral exposure. The mean age was 38.42 years (range 23-54 years) for individuals with oral leukoplakias and 36.53 years (range 21-52 years) for the controls (For details see Ghosh et al., 1988).

5. to study SCE in will patients with oral leukoplakia and to correlate them with the habits of different forms of tobacco usage, peripheral blood samples were collected from 45

cases of oral submucous fibrosis. Submucous fibrosis was diagnosed solely on clinical grounds and only when the patients exhibited the presence of palpable fibrous bands. The control group consisted of 56 age-matched normal individuals with no history of tobacco usage or radiation or viral exposure. The mean age was 41.53 years (range 24 to 58 years) for individuals with oral submucous fibrosis and 39.25 years (range 22-55 years) for the controls (For details see Ghosh et al., 1990).

6. to study the effect of betel chewing on the SCE frequency, peripheral blood samples were obtained from 129 healthy adult females who were not occupationally exposed to mutagens. They were non-smokers without recent viral infections or radiation exposure. The first group included non-chewers consisting of 20 normal women, 24 pregnant women, and 27 women using oral contraceptives. The second group included betel chewers consisting of 16 control women, 19 pregnant women, and 23 oral contraceptive users. All pregnant women were in their last trimester of pregnancy. A combination of ethenyl estradiol and d-norgestrel has been taken by women using oral contraceptives for periods ranging from 5 to 26 months (mean, 17 months). Chewing habits were 15.2±6.4 (range, 6-29) betel leaves per day for normal women, 14.5±5.8 (range, 8-24) betel leaves per day for pregnant women, and 14.8±6.1 (range, 8-31) betel leaves per day for women using oral contraceptives (For details see Ghosh and Ghosh, 1988).

RESULTS AND DISCUSSION

1. Sister Chromatid Exchanges in Human Amnion Cells

There was a marked difference in the SCE frequency between smoking pregnant women ($\bar{X}$=8.27) and their amnion cells ($\bar{X}$=6.46) (p<0.01). In 10 out of 12 lymphocyte-amnion comparisons, a t-test produced p values <0.01 indicating a highly significant difference in SCE frequency (Table 1.1).

The group mean of the non-smoking pregnant women is 8.58 SCEs per cell compared to 6.84 for the amnion cells (Table 1.2). This difference was also highly significant (p<0.01). In 12 out of 15 lymphocyte-amnion comparisons, a t-test produced p values that were significant at the 1 per cent level. A comparison of the mean SCE frequency of smoking pregnant women ($\bar{X}$=8.27) and that of the non-smoking pregnant women ($\bar{X}$=8.58) showed no significant difference (p<0.50). This agrees well with certain earlier findings (Ardito et al., 1980; Sutherland et al., 1980) but contradicts other (Husgafvel et al., 1980; Lambert et al., 1978; Livingstone and Fineman, 1983; Lundberg and Livingston, 1983; Murthy, 1979) who observed higher SCE levels in smokers than in non-smokers (Table 1.2).

The difference in the SCE frequency of amnion cells and the lymphocytes of pregnant women from whom these amnion cells are derived may correspond to different growth kinetics as a relationship between SCE frequen-

Table 1.1: SCE frequency in smoking pregnant women and their amnion cells (in years)

Case No.	Tissue	Y	SCE per cell Mean±S.D.	t-statistics
1.	Lymphocyte	9	6.41±1.98	3.76*
	Amnion		5.13±1.31	
2.	Lymphocyte	5	9.64±2.87	2.94*
	Amnion		8.11±2.29	
3.	Lymphocyte	11	7.92±2.17	5.60*
	Amnion		5.68±1.79	
4.	Lymphocyte	10	7.86±2.32	5.21*
	Amnion		5.64±1.91	
5.	Lymphocyte	5	8.13±2.56	0.47*
	Amnion		7.91±2.11	
6.	Lymphocyte	6	8.47±2.45	7.22*
	Amnion		5.29±1.82	
7.	Lymphocyte	4	8.38±2.33	5.21*
	Amnion		6.14±1.91	
8.	Lymphocyte	10	7.46±1.87	3.77*
	Amnion		6.14±1.72	
9.	Lymphocyte	8	9.56±2.96	1.03*
	Amnion		8.98±2.67	
10.	Lymphocyte	12	10.74±3.26	4.51*
	Amnion		8.17±2.42	
11.	Lymphocyte	8	6.95±2.11	4.69*
	Amnion		5.12±1.79	
12.	Lymphocyte	7	7.78±2.27	6.27*
	Amnion		5.21±1.84	
Mean Lymphocyte SCE			8.27±1.15	3.54*
Mean Amnion SCE			6.46±1.35	

* Significant (p>0.01).

Table 1.2: SCE frequency in non-smoking pregnant women and their amnion cells

Case No.	Tissue	SCE per cell Mean±S.D.	t-statistics
1.	Lymphocyte	10.45±3.14	4.03*
	Amnion	8.19±2.38	
2.	Lymphocyte	7.78±1.93	4.43*
	Amnion	6.14±1.85	
3.	Lymphocyte	8.84±2.71	5.70*
	Amnion	6.18±1.93	
4.	Lymphocyte	9.19±2.68	0.88
	Amnion	8.73±2.44	
5.	Lymphocyte	6.43±2.14	1.15
	Amnion	5.97±1.93	
6.	Lymphocyte	7.48±1.73	5.51*
	Amnion	5.66±1.59	
7.	Lymphocyte	8.36±2.29	4.47*
	Amnion	6.35±2.18	
8.	Lymphocyte	6.95±2.21	2.86*
	Amnion	5.72±2.14	
9.	Lymphocyte	8.5±2.18	3.9?*
	Amnion	6.72±1.91	
10.	Lymphocyte	9.78±2.92	3.21*
	Amnion	8.11±2.23	
11.	Lymphocyte	6.47±2.12	0.9
	Amnion	6.11±1.89	
12.	Lymphocyte	9.91±3.28	5.40*
	Amnion	7.13±1.64	
13.	Lymphocyte	10.38±3.12	5.08*
	Amnion	7.69±2.24	
14.	Lymphocyte	10.79±3.12	4.52*
	Amnion	8.17±2.43	
15.	Lymphocyte	7.62±2.18	4.5*
	Amnion	5.73±1.95	
Mean Lymphocyte SCE SCE frequency		8.58±1.41	3.86*
Mean Amnion Frequency		6.84±1.03	

* Significant (p>0.01).

cy and cell proliferation kinetics has been observed (Lamberti et al., 1983; Lindbald and Lambert, 1981). The reduced frequcency of SCEs in human amnion cells as compared to adults may be attributed to several factors. The elevated SCE frequency in adults may be due to their exposure to potential DNA-damaging agents in the environment. The lack of antenatal effect could be due to insufficient exposure, inability of metabolic products to cross the placenta or placental detoxification of DNA-damaging agents. Further studies with known mutagens and carcinogens in amnion cells and lymphocytes may tell us whether there exists a differential response in terms of DNA damage in these cells.

2. Sister Chromatid Exchanges in Betel and Tobacco Chewers

Data on the mean frequency of SCE per cell are presented in table 2.1. Betel chewers had a mean SCE per cell of 7.95±1.53 whereas betel-with-tobacco chewers had a mean SCE per cell of 9.62±2.32. These values are significantly higher than the mean value of 5.32±1.21 found in normal controls. The mean SCE frequency in those who chewed more than 10 betel leaves per day, irrespective of duration, was 9.84±2.37, while those who chewed less than 10 betel leaves per day had a mean SCE frequency of 7.53±1.49. This difference was highly significant ($p<.01$). Similarly, the light betel-with-tobacco chewers had a mean SCE frequency of 8.17±2.14 which was significantly different from the mean SCE frequency of heavy betel-with-tobacco chewers 12.13±3.58 ($p<0.01$). The mean SCE frequencies in those who had chewed habitually for over 10 years, irrespective of number of betel leaves chewed, were 10.61±2.85 betel chewers and 12.56±3.47 for betel-with-tobacco chewers (Table 2.2). These values were significantly different from the mean SCE frequencies in those who had chewed for less than 10 years; the frequency being 7.15±1.16 for betel chewers, and 7.98±1.81 for betel-with-tobacco chewers ($p<0.001$). The correlation coefficient was also calculated for the 2 variables (betel chewing *versus* SCE frequency) and tested by the *t*-test. There was significant correlation between the number of betel leaves chewed (with or without tobacco) and the mean SCE frequency ($r=0.61$; $p<0.001$), and the duration of chewing and mean SCE frequency ($r=0.63$, $p<0.01$). The degree of exposure to betel chewing may be an important determinant of frequency of occurrence of SCE, as the difference in SCE frequency between betel chewers and betel-with-tobacco chewers is only significant in the group who had chewed for more than 10 years ($p<0.05$). Moreover, chewing of betel-with-tobacco may have a synergistic effect compared to chewing betel alone, since there are

indications that betel extract definitely accelerate the promotion of tobacco carcinogenesis (Ranadive et al., 1976). Our data indicate that a dose-response relationship may exist between the level of chromosome damage, as reflected by SCE frequency, and the degree of exposure to betel and tobacco chewing.

The significance of the increased frequency of SCE in betel and tobacco chewers is not clear. In India, the buccal cavity and pharynx are frequently affected by cancer (Jussawalla et al., 1968). The incidence of oral cancer has been reported to be as high as 34.9% of all cancers reported in Indian males (Paymaster, 1964). This high incidence of oral cancer has been attributed to the habit of betel and tobacco chewing (Atkinson et al., 1964; Hirayama, 1966; Mehta et al., 1969; Sanghvi et al., 1955; Schonland and Bradshaw, 1969). The risk of developing oral cancer in chewers of betel-with-tobacco was found to be 4.8 times higher than in non-chewers, and those who chewed the betel quid without tobacco showed a 3-fold greater risk of developing such cancer than the non-chewers (Jussawalla and Desphande, 1971). Thus the carcinogenic potetial of betel is enhanced by tobacco. Studies of SCE *vis-a-vis* oral cancer in betel and tobacco chewers, may help us to understad the epidemiology of buccal and pharyngeal carcinogenesis.

Table 2.1: Mean Frequencies of SCE in chewers

S. No.	Group	Number of subjects	Mean age (in years)	SCE per cell Mean±S.D.	Range
1.	Non-chewers	20	38.6	5.32±1.21	0-12
2.	All betel Chewers	18	27.4	7.95±1.53	0-19
	(a) Light	10	36.8	6.53±1.49	0-18
	(b) Heavy	8	37.1	9.84±2.37	2-21
3.	All betel-with-tobacco chewers	15	39.1	9.62±2.32	3-22
	(a) Light	9	39.4	8.17±2.14	1-19
	(b) Heavy	6	38.9	12.13±3.58	4-24

Differences between 1 and 2 and between 1 and 3 are significant ($p<0.01$) by the paired *t*-test.
Differences between 2a and 2b and between 3a and 3b are significant ($p<0.01$) by the paired *t*-test.

Table 2.2: Mean Frequencies of SCE in 'HIGH' and 'LOW' chewers

S. No.	Group	Number of subjects	Mean age (in years)	SCE per cell Mean±S.D.	Range
1.	Non-chewers	20	38.6	5.32±1.21	0-12
2.	Betel chewers				
	(a) More than 10 years	9	37.5	10.61±2.85	3-19
	(b) Less than 10 years	9	36.9	7.15±1.16	0-17
3.	Betel-with-tobacco chewers				
	(a) More than 10 years	7	38.8	12.56±3.47	3-22
	(b) Less than 10 years	8	39.2	7.98±1.81	0-17

Differences between 2a and 2b and between 3a and 3b are significant ($p<0.01$) by the paired *t*-test.
Difference between 2a and is 3a are significant ($p<0.05$) by the paired *t*-test.

3. Sister Chromatid Exchanges in Tobacco Smokers

Data on the mean frequency of SCE per cells are presented in table 3.1. Bidi smokers had a mean SCE per cell of 10.12±2.41, whereas, the cigarette smokers had a mean SCE per cell of 8.15±1.62. These values are significantly higher than the mean value of 5.48±1.29 found in controls. The mean SCE frequency in those who smoked more than ten bidis per day irrespective of duration, was 12.25±3.43, whereas, those who smoked less than ten bidis per day had a mean SCE frequency of 8.23±2.16. This difference was highly significant ($p<0.01$). Similarly, the light cigarette smokers had a mean SCE frequency of 6.72±1.53, which was significantly different from the mean SCE frequency of heavy cigarette smokers (*i.e.*, 9.56±2.28; $p<0.01$). The mean SCE frequencies in those who had smoked habitually for over 10 years, irrespective of the number of bidis or cigarettes smoked, were 12.71±3.52 for bidi smokers and 10.48±2.63 for cigarette smokers (Table 3.2). These values were significantly different from the mean SCE frequencies in those who had smoked for less than 10 years; the frequency being 8.03±1.49 for bidi smokers and 7.86±1.12 for cigarette smokers ($p<0.01$). The correlation coefficient was also calculated for the two variables (smoking *versus* SCE frequency) and tested by the *t*-test. There was significant correlation between the number of bidis or cigarettes smoked and the mean SCE frequency ($r=0.67$; $p<0.001$), and the duration of smoking and mean SCE frequency ($r=0.69$; $p<0.01$). The degree of exposure to tobacco smoking may be an import-

ment determinant of the frequency of occurrence of SCE, as the difference in SCE frequency between bidi smokers and cigarette smokers is only significant in the group who had smoked for more than 10 years ($p<0.01$). Our data indicates that a dose-response relationship may exist between the level of chromosome damage, as reflected by the SCE frequency, and the degree of exposure to tobacco smoking.

The significance of the increased frequency of SCE in bidi smokers compared with cigarette smokers is not clear. The amount of tobacco content of the "Indian bidi" varies from 0.2 to 0.3 g in comparison with 1 g of tobacco contained in a standard sized cigarette (Jussawala and Deshpande, 1971). In a comparative chemical analysis of the mainstream smoke of Indian bidi and U.S. nonfilter cigarette, it was found by Hoffman et al. (1974) that bidi smoke has a higher content of several toxic agents, such as carbon monoxide (7.7 *vs* 3.5 vol/dl), ammonia (284 *vs* 180 µg), hydrogen cyanide (903 *vs* 445 µg), phenol (250 *vs* 150 µg), other volatile phenols (264 *vs* 173 µg), and carcinogenic hydrocarbons, benz(a)anthracene (117 *vs* 81 ng) and benzo(a)pyrene (78 *vs* 47 mg). These chemical data suggest that the smoke of bidi has more carcinogenic activity compared with the smoke of cigarettes.

Table 3.1 : Mean frequencies of SCE in smokers

S. No.	*Group*	*Number of subjects*	*Mean age (in years)*	*SCE per cell* Mean±S.D.	Range
1.	Nonsmokers	20	37.4	5.48±1.29	0-11
2.	All bidi smokers	24	36.7	10.12±2.41	2-19
	(a) Heavy	10	35.8	12.25±3.43	3-21
	(b) Light	14	37.5	8.23±2.16	1-17
3.	All cigarette smokers	18	38.2	8.15±1.62	2-16
	(a) Heavy	11	38.9	9.56±2.28	3-20
	(b) Light	7	39.1	6.72±1.53	0-14

Differences between nonsmokers and bidi smokers, between nonsmokers and cigarette smokers are significant ($p<0.01$) by the paired *t*-test.
Difference between heavy bidi smokers and light bidi smokers and between heavy cigarette smokers and light cigarette smokers are significant ($p<0.01$) by the paired *t*-test.

Table 3.2: Mean frequencies of SCE in high and low smokers

S. No.	*Group*	*Number of subjects*	*Mean age (in years)*	*SCE per cell* Mean±S.D.	Range
1.	Nonsmokers	20	37.4	5.48±1.29	0-11
2.	Bidi smokers	11			
	(a) More than 10 years	13	36.9	12.71±3.52	3-23
	(b) Less than 10 years		37.2	8.03±1.49	1-17
3.	Cigarette smokers				
	(a) More than 10 years	8	37.8	1048±2.63	3-19
	(b) Less than 10 years	10	38.3	7.86±1.12	1-16

Differences between >10 yr bidi smokers and between > 10 yr cigarette smokers and < 10 yr cigarette smokers are significant ($p<0.01$) by the paired t-test.
Difference between > 10 yr bidi smokers and > 10 yr cigarette smokers are significant ($p<0.01$) by the paired *t*-test.

The tobacco smoke condensates produce DNA lesions that result in SCE and there may be qualitative similarities between the respsonse to tobacco tar of lymphocytes *in vitro* and bronchial epithelium *in vivo*. The level of carcinogenic ingredients that can react with cellular DNA may be partiularly high in the respiratory airways of inhaling cigarette smokers (Hopkin and Evans, 1978). Epidemiologic studies indicate an association between the habit of bidi and cigarette smoking and lung cancer (Mehta et al., 1969; Notani and Sanghvi, 1974) and there is increasing evidence to support the development of cancer as a result of somatic mutation (Hopkin and Evans, 1980). The risk of developing lung cancer in bidi smokers was found to be 3.38 times higher than nonsmokers and those who smoked cigarettes showed 2.36 times greater risk of developing such cancer than nonsmokers (Notani et al., 1977). However, factors besides smoking may also play an important role in the etiology of these cancers (Jayant et al., 1971). The bidi and cigarette smokers of the present study belonged to lower socio-economic status where nutritional deficiencies may exist. Several studies have shown that the level of nutrition affects the frequency of SCE (Balabrishna et al., 1980, 1982) and that nutritional deficiency is an important underlying factor in the etiology of human cancer (Wynder and Mabuchi, 1972). Studies of SCE *vis-a-vis* lung cancer in bidi and cigarette smokers belonging to the higher socio-economic classes, where the occurrence of nutritional deficiency is low, may be helpful to a better understanding of the mechanism by which smoking leads to cancer.

4. *Sister Chromadit Exchanges Among Oral Leukoplakia Patients*

Data on the mean frequency of SCE per cell in patients with oral leukoplakia and normal controls are presented in table 4. Patients with oral leukoplakia had a mean SCE frequency of 8.61±1.89, which was significantly higher than the mean SCE value of 5.58±1.26 observed in normal controls ($p<0.001$). The mean SCE frequency in patients with oral leukoplakia who were addicted to the single habit of betel with tobacco chewing, bidi/cigarette smoking, or combined habits of chewing and smoking tobacco were found to be 7.95±1.63, 8.17±1.66, and 9.23±2.14, respectively. These values were also significantly higher as compared to the SCE values observed in normal controls ($p<0.001$). There was significant correlation between the number of betel leaves with tobacco chewed and the mean SCE frequency ($r=-.62$; $p<0.001$) and also the number of cigarettes or bidis smoked and the mean SCE frequency ($r=0.65$; $p<0.001$).

Table 4: Mean frequencies of SCE in patients with oral leukoplakia in relation to different habits of tobacco usage

S. No.	*Group*	*Tobacco habits*	*Number of subjects*	*Mean age (in years)*	*SCE per cell*	
					Mean±S.D.	*Range*
1.	Normal controls	No tobacco habits	65	36.53	5.58±1.26	0-12
2.	Oral leukoplakias		59	38.42	8.61±1.89	1-19
		(a) Betel with tobacco chewing	17	37.64	7.95±1.63	1-16
		(b) Bidi/cigarette smoking	19	38.19	8.17±1.66	2-17
		(c) Combined habits[2]	23	39.71	9.23±2.14	3-19

1. Differences between 1 and 2, 1 and 2a, 1 and 2b, 1 and 2c are significant ($p<0.001$) by student's t-test.
2. Persons addicted to both tobacco chewing and smoking.

The most striking finding of the study is the association of leukoplakia with tobacco usage. All the patients with oral leukoplakia were habituated to tobacco usage, and not a single individual without this habit was encountered in the series. Of the patients with leukoplakia, 28.8% were addicted to betel with tobacco chewing, 32.20% smoked tobacco in the form of bidi or cigarettes, and 38.98% were habituated to the combined usage of tobacco chewing as well as tobacco smoking. In the present study, an increase in the frequency of SCEs was observed in patients with oral leukoplakia, which may be attributed to their addiction to tobacco habits. Several studies have indicated that the habit of tobacco smoking and tobacco chewing enhances the frequency of SCEs (16-23) (Ghosh and Ghosh, 1984, 1987, 1988; Lambert et al., 1978; Livingstone and Fineman, 1983; Meiying et al., 1982; Obectal, 1982; Wulf et al., 1983). However, the extent of SCE induction in patients with oral leukoplakia by different types of tobacco usage was not identical. The mean elevation of SCE frequency in patients with oral leukoplakia induced by single habits of betel with tobacco chewing, bidi/cigarette smoking and the combined habits of chewing and smoking of tobacco was found to be 2.37, 2.59, and 3.65 SCEs, respectively, as compared to the SCE values observed in normal controls. This finding may imply that combined habits of tobacco smoking and chewing induces more chromosomal instability as compared to the single habits of betel with tobacco chewing or tobacco smoking alone. Epidemiologic studies have reported an increased frequency of oral leukoplakia as well as oral cancer in people addicted to the combined habits of tobacco chewing and smoking as compared to those addicted to the single habits of tobacco chewing or smoking (Mehta et al., 1969a,b, 1972 a,b, 1976). It may be possible that simultaneous chewing and smoking of tobacco has a synergistic effect, that is, chewing of tobacco may cause more damage to the lymphocyte chromosomes of tobacco smokers as compared to nonsmokers. Experimental studies with animals have shown that chronic exposure to tobacco smoke results in impaired immune responsiveness

and decreased resistance to tumor challenges. (Esber et al., 1973; Jacob et al., 1980; Roszam and Rogers, 1973; Thomas et al., 1973, 1974 a,b). Michalek et al., (1985), based on his observation of patients with bladder cancer, speculated that cigarette smoking may decrease the body's resistance to tumor challenge, thereby accelerating tumor growth. Allison and Law (1968) and Allison and Taylor (1967) claimed that tumor induction and promotion by carcinogens may be related to the immunosuppressive property of tobacco smoking. Shubick (1985) observed a decrease in the natural killer cell activity in tobacco smokers, resulting in a lowered defence against cancer. Since the ingredients of the betel nut, which is invariably chewed with betel leaf and tobacco, have been shown to induce tumors in the hamster buccal pouch (Suri et al., 1971) it may be possible that betel chewing enhances the process of malignant transformation in smokers as compared to nonsmokers. This may explain the increased incidence of precancerous lesions of oral cavity (leukoplakia) *vis-a-vis* increased SCE frequency in people addicted to the combined habits of tobacco chewing and tobacco smoking as compared to people addicted to single tobacco habits of either chewing or smoking.

In the 1978-1979 period, the quantity of raw tobacco cleared for home consumption in India was 41 million kg. Sixty-five per cent of this tobacco was cleared for smoking, 11% for chewing, 1% for snuff use, and 21% for other miscellaneous purposes including use in agriculture and industry. Due to the implementation of stronger legislation regarding the licensing of land under tobacco cultivation, the quantity of raw tobacco cleared for consumption was reduced to 35 million kg in 1982 and 1983 (Tobacco in India, 1983). This is a welcome trend since the usage of tobacco seems to be an important factor in determining the biologic behavior of precancerous lesions of oral cavity like leukoplakia in terms of their progression to cancer. The reduction of tobacco consumption may thus be one of the contributory factors in lowering the incidence rate of oral cancer.

It has been observed that the leukoplakias regressed more often among tobacco chewers, whereas among smokers, the leukoplakia was found to be more persistent (Pindborg et al., 1967). Long-term epidemiologic studies have revealed the rate of malignant transformation of oral leukoplakia to be around 0.9-1.4% (Mehta et al., 1972b; Pindborg, 1969). Two assumptions prevail in the literature on leukoplakia: that the condition is precancerous, and that its prevalance (like that of oral carcinoma) is correlated with tobacco habits. It would seem useful to test both these assumptions in controlled epidemiologic studies. A long-term controlled follow-up of leukoplakia patients for possible malignant changes should establish more clearly the nature of the lesion. Further, an assessment of the prevalence of leukoplakia in the general population, especially tobacco users, may be helpful in early cancer detection campaigns. Since most oral cancers are preceded by precancerous lesions, education on tobacco habits should be a feasible and effective method for primary prevention of oral cancer. Recent long-term intervention studies in South India have already pointed to the success of such an approach (Mehta et al., 1982; Gupta et al., 1986).

5. Sister Chromatid Exchanges in Oral Susmucous Fibrosis Patients

Data on the mean frequency of SCE per cell in patients with oral submucous fibrosis and normal controls are shown in table 5. Patients with oral submucous fibrosis had a mean SCE frequency of 9.26±2.15, which was significantly higher than the mean SCE value of 5.49±1.24 observed in normal controls ($p<0.001$). The mean SCE frequency in patients with oral submucous fibrosis who were addicted to the single habit of betel with tobacco chewing or bidi/cigarette smoking or to combined habits of chewing and smoking tobacco was 8.12±1.69, 9.43±1.87, and 10.06±2.28, respectively. These values were also significantly higher as compared with the SCE values observed in normal controls ($p<0.001$). There was significant correlation between number of betel leaves with tobacco chewed and mean SCE frequency ($r=0.65$; $p<0.001$) and also the number of cigarettes or

bidis smoked and the mean SCE frequency (r=0.68; p<0.001).

Oral cancer presents a major health problem in India. The buccal cavity and pharynx are the areas most frequently involved by cancer and constitute 34.9% of the total cancers in India (Paymaster, 1964). Tobacco usage is an important etiological factor in development of cancer of oral epithelium Sanghvi (1981). In India, tobacco is smoked in the form of cigarettes in urban areas, whereas in rural areas, particularly in the lower socio-economic classes, the prevalent habit is bidi smoking. The other important form of tobacco usage in India is betel chewing, which is practiced in a variety of ways. Every patient with oral submucous fibrosis studied in the present investigation was addicted to either one form of tobacco usage or the other: 24.4% of the patients with oral submucous fibrosis were addicted to betel with tobacco chewing, 28.9% smoked tobacco in the form of bidi or cigarettes, and 46.7% were habituated to the combination of tobacco chewing and tobacco smoking. Thus, the increase in frequency of SCEs observed in patients with oral submucous fibrosis may be attributed to their addiction to tobacco habits, as has been widely reported (Ghosh and Ghosh, 1984, 1987, 1988; Ghosh et al., 1988; Livingstone and Fineman, 1983; Meiying et al., 1982). The SCE frequencies in patients with oral submucous fibrosis varied according to the mode of tobacco usage. The single habits of betel/tobacco chewing and bidi/cigarette smoking and the combined habits of chewing and smokng tobacco induced a mean elevation of 2.63, 3.94, and 4.57 SCEs, respectively, as compared with the SCE values observed in normal controls. Thus, the combined habits of tobacco smoking and chewing may induce more chromosomal instability as compared with the single habits of betel with tobacco chewing or tobacco smoking alone.

Table 5: Mean frequencies of SCE in patients with oral submucous fibrosis in relation to different habits of tobacco usage

S. No.	Group	Number of subjects	Mean age (in years)	SCE per cell	
				Mean±S.D.	Range
1.	Normal controls/no tobacco habits	56	39.25	5.49±1.24	0-11
2.	Oral submucous fibrosis	45	41.53	9.26±2.15	1-20
	(a) Betel with tobacco chewing	11	39.95	8.12±1.69	1-16
	(b) Bidi/cigarette smoking	13	41.26	9.43±1.87	2-18
	(c) combined habits	21	42.18	10.06±2.28	3-20

Persons addicted to both tobacco chewing and smoking. Differences between series 1 and 2, 1 and 2a, 1 and 2b, 1 and 2c were significant (p<0.001) by Student's t-test.

About 250,000 persons in India are believed to have submucous fibrosis. Epidemiological studies on the prevalence of submucous fibrosis have been conducted by Pindborg et al. (1965) and Zachariah et al. (1966), who examined 35,000 urban Indians seeking admission to clinics of dental colleges of various hospitals of India and reported the frequency of submucous fibrosis to range from 0.2% to 1.2%. The etiology of submucous fibrosis is still unknown. The most likely explanation is that chilli, a spicy ingredient of Indian food, may play a role in its onset. Sirsat and Khanolkar (1962) demonstrated experimentally that painting rat palates with capsicum, the active ingredient of chili, causes a connective tissue response similar to the changes observed in humans with submucous fibrosis.

Patients with oral submucous fibrosis are often characterized by epithelial atypia. Lemmer and Shear (1968) reported the incidence of atypia to be 16.6% in patients with submucous fibrosis. In a series of surveys, Pindborg (1966, 1972) and Pindborg et al. (1970) found the frequency of epithelial atypia to range from 7% to 23.8% in patients with oral submucous fibrosis. Similar figures were reported by Seedat (1986) who found atypia in 23% of 49 specimens of submucous fibrosis. The extent to which this high rate of atypia indicates the premalignant potential of oral submucous fibrosis is not precisely known, especially because atypia may not be a permanent feature. In a follow-up of patients with gastric dysplasia, Andersson et al. (1987) reported that atypia disappeared after 10 to 63 months. Furthermore, submucous fibrosis has been reported in patients who never chewed tobacco/betel nut (Paissat, 1981; Seedat and Van Wyk, 1988a). Some patients showed severe early changes in the mucosa with minimal be-

tel nut contact, whereas in others this change was not induced despite prolonged betel nut chewing (Seedat and Van Wyk, 1985). This indicates a varying susceptibility among chewers which may be genetically determined. That this may be true was recently reported by Cannif et al. (1985), who performed HLA typing in 50 patients with oral submucous fibrosis. They reported that haplotypes-A10, B7, and DR3 showed a significant elevation and haplotype DRT was substantially decreased in these patients. A systematic long-term follow-up of patients with oral submucous fibrosis with and without the habit of tobacco/betel nut chewing with respect to epithelial atypia in relation to chromosomal instability may help us understand the processes involved in initiation of oral carcinogenesis.

6. *Sister chromadit Exchanges Among Pregnant Women and Women Using Oral Contraceptives Who are Chewing Betel*

Data on mean frequency of SCE per cell in betel chewing and non-chewing normal women, pregnant women, and oral contraceptive users are shown in table 6. Non-chewing pregnant women had a mean SCE per cell of 7.82±0.24, whereas, non-chewing oral contraceptive users had a mean SCE per cell of 8.27±0.27. This value was significantly higher as found in normal non-chewing women (5.21±0.18). Betel chewing pregnant women and oral contraceptive users, on the other hand, had a mean frequency of SCE of 11.79±0.38 and 12.51±0.44, respectively, which was significantly higher than the mean SCE frequency of 6.28±0.21 found in betel chewing normal women. There was significant correlation between the number of betel leaves chewed and the mean SCE frequency (r=0.523, p<0.01).

Changes in the level of certain sex hormones, such as progesterone and estrogen are known to occur during pregnancy, especially in the last trimester (Simmer, 1968; Brotherton, 1976; McArther and Powell, 1976). Sex hormones, when administered *in vivo*, have been shown to induce chromosome aberrations (Carr, 1967; Wippiams et al., 1968; Badr et al., 1972; Prasad and Haider, 1982). Women using an estrogen-progesterone combination contraceptive exhibited either an increased frequency in SCE (Murthy and Prema, 1979) or no change (Husum et al., 1982). SCE frequency has been found to be enhanced in pregnant women in the last trimester of pregnancy and also in the lymphocyte cultures of normal women to which the sex hormones progestrone estrogen, and human chroionic gonadotrophin have been exogenously added (Sharma and Das, 1980). The increased frequency of spontaneous SCE in pregnant women and women using oral contraceptives observed in the present study, thus, may be due to the altered hormonal profile in these women, compared with normal controls.

Table 6: Mean frequencies of SCE in betel chewing and non-chewing normal women, pregnant women, and women using oral contraceptives

S. No.	Group	Number of subjects	Mean age (in years)	SCE per cell Mean±S.D.	SCE per cell Range
1.	Normal women				
	Non chewers	20	36.9	5.21±0.18	0-11
	Chewers	16	35.8	6.28±0.21	0-16
2.	Pregnant women				
	Non chewers	24	32.5	7.82±0.24	0-17
	Chewers	19	33.2	11.79±0.38	2-21
3.	Oral contraceptive users				
	Non chewers	27	34.1	8.27±0.27	1-19
	Chewers	23	34.7	12.51±0.44	3-22

Differences between non-chewing normal women and pregnant women, between non-chewing normal women and oral contraceptive users are significant (p<0.001) by Student's t-test.

Differences between non-chewing normal women and betel chewing normal women, between non-chewing pregnant women and betel chewing pregnant women, between non-chewing oral contraceptive users and betel chewing oral contraceptive users are significant (p<0.001) by Student's t-test.

Differences between betel chewing normal women and betel chewing pregnant women, between betel chewing normal women and betel chewing oral contraceptive users are significant (p<0.001) by Student's t-test.

The observation of increased frequency of SCE in betel chewing normal women, pregnant women, and women using oral contraceptives indicates that betel induces SCE. This is in agreement with our previous study where an increase in the frequency of SCE were observed in betel chewers (Ghosh and Ghosh, 1984). However, the extent of SCE increase induced by betel chewing in the present study was found to be much more pronounced in pregnant women and women using oral con-

traceptives, compared with normal women. Betel chewing pregnant women and oral contraceptive users showed an elevation of the mean SCE frequency of 3.97 and 4.24 SCE compared with non-chewing females in contrast to an increase of mean values of only 1.07 SCE in betel chewing control women. This finding may imply that chromosomes from pregnant women and women using oral contraceptives are more susceptible to DNA damage induced by betel chewing compared with normal women. This observation may be related to the different hormonal environment. There are indications that a higher level of sex hormones in pregnant women and those using oral contraceptives may increase the sensitivity of lymphocytes to genetic damage (Beaconsfied and Ginsberg, 1968; McQuarrie et al., 1970). The frequency of X-ray induced chromosome aberrations was found to be significantly higher in pregnant muntjac compared with normal controls (Das and Sharma, 1983). Lymphocytes of pregnant women and oral contraceptive users have been found to be more susceptible to SCE induction by diethylstilbestrol and mitomycin-C (Hill and Wolf, 1982; Murthy and Prema, 1983).

The incidence of oral cancer has been found to be as high as 34.9% of all cancers reported from India (Paymaster 1964). This high incidence of oral cancer has been ascribed to the habit of betel chewing (Atkinson et al., 1964; Mirayama, 1966; Mehta et al., 1969; McMichad, 1984; Sanghir et al., 1955; Schonpand and Bradsham, 1969; Winn et al., 1981). According to Jussawalla and Deshpande (1971), the betel chewers showed a three-fold greater risk of developing oral cancer than non-chewers. Presence of high levels of sex hormones during pregnancy may also initiate processes leading to an enhanced growth of certain cancers due to increased sensitivity of lymphocytes to mutagens/carcinogens (Siiteri et al., 1981; Urmancheeva et al, 1981; MacMohan et al., 1983; Pike et al., 1983). SCE are good indicators of genetic damage and there exists a relationship between increased SCE rates and altered mutation frequency (Carrano et al., 1975). Increased SCE frequency observed in betel chewing pregnant women and oral contraceptive users may be due to the mutagenic activity of betel on the one hand and estrogen or its metabolites on the other. It seems that the carcinogenic potential of betel is enhanced by the elevated hormonal profile in pregnant women and oral contraceptive users. Epidemiologic studies may tell us whether there is an increased likelihood of developing cancer in betel chewing pregnant women and oral contraceptive users due to the increased sensitivity of their lymphocytes to genetic damage compared with non-chewing pregnant women and oral contraceptive users.

KEY WORDS Sister Chromatid Exchange. Tobacco Chewers or/and Smokers. Betel Chewers.

ABSTRACT The frequency of sister chromatid exchange (SCE) is now believed to be a more sensistive indicator of genetic damage than chromosome aberrations. In the present study an attempt has been made to study the effect on individuals who are chewing and/or smoking tobacco and chewing betel. The findings are as follows:

1. The incidence of sister chromatid exchange (SCE) in human amnion cells and the lymphocytes of women from whom these amnion cells are derived was determined using the BrdU-Giemsa technique. The results showed that the SCE rate in the amnion cells is significantly less than the lymphocytes of women from whom these amnion cells are derived. These SCE frequencies were not affected by the smoking habit of the pregnant women.
2. The incidence of sister-chromatid exchange (SCE) among betel chewers and betel-with-tobacco chewers showed higher yields of SCE than normal controls. Higher frequencies of SCE were also observed in individuals who chewed more than 10 betels leaves, or betel leaves-with-tobacco, per day, compared to people who chewed less than 10 betel leaves, or betel-with-tobacco, per day, respectively. Subjects who had chewed betel leaves and betel leaves + tobacco for more than 10 years showed an elevated frequency of SCE.
3. The incidence of sister chromatid exchange (SCE) in bidi and cigarette smokers had a mean SCE per cell of 10.12±2.41 and 8.15±1.62, respectively, which were significantly higher than the mean value of 5.48±1.29 found in controls. Higher frequencies of SCE were also observed in individuals who smoked more than ten bidis or cigarettes per day, compared with people who smoked less than ten bidis or cigarettes per day, respectively. Individuals who smoked bidis or cigarettes for more than 10 years also showed an increased frequency of SCE as compared with those who smoked bidis or cigarettes for less than 10 years.
4. The frequency of SCE was found to be 8.61±1.89

in patients with oral leukoplakia, which was significantly higher than the mean SCE value of 5.58±1.26 observed in normal controls. The frequency of SCE in patients with oral leukoplakia addicted to the single habit of betel with tobacco chewing, bidi/cigarette smoking, and combined habits of chewing and smoking of tobacco were found to be 7.95±1.63, 8.17±1.66, and 9.23±2.14, respectively. These values were also significantly higher as compared to the SCE values observed in normal controls.

5. The frequency of SCE was 9.26±2.15 in patients with oral submucous fibrosis, which was significantly higher than the mean SCE value of 5.49±1.24 observed in normal controls. The frequency of SCE in patients with oral submucous fibrosis addicted to the habit of betel with tobacco chewing, "bidi"/cigarette smoking and combined habits of chewing and smoking of tobacco were 8.12±1.69, 9.43±1.87, and 10.06±2.28, respectively. These values were also significantly higher as compared with the SCE values observed in normal controls.

6. The frequency of SCE was found to be 7.82±0.24 and 8.27±0.27 in non-chewing pregnant women and women using oral contraceptives respectively, which were significantly higher than the mean value of 5.21±0.18 observed in non-chewing normal women. Betel chewing induced higher SCE in pregnant women and women using oral contraceptives, the frequencies being 11.79±0.38 and 12.51±0.44, respectively, which were significantly higher than the SCE frequency of 6.28±0.21 found in normal betel chewing females.

REFERENCES

Allison, A.C. and Law, L.W.: The effects of antilymphocyte serum on viral oncogenesis. *Proc. Soc. Exp. Biol. Med.*, **127**: 207-212 (1968).

Allison, A.C. and Taylor, R.B.: Observations on thymectomy and carcinogenesis. *Cancer Res.*, **27**: 707 (1967).

Andersson, A.P., Lavritsen, K.B., West, F. and Johansen, A.: Dysplasia in gastric mucosa: Prognostic significance. *Acta Chir. Scand.*, **153**: 29-31 (1987).

Ardito, G., Lamberti, L., Ansaldi, E. and Ponzett, P.: Sister chromatid exchanges in cigarette-smoking human females and their newborns. Mutat. Res., **78**: 209 (1980).

Atkinson, L., Chester, I.C., Smyth, G.D. and Seldam, R.E.I.: Oral cancer in New Guinea, *Cancer*, **17**: 1289-1298 (1964).

Badr, E.M., Badr, R.S., Ibrahim, A. and Shaaba, A.: Chromosome studies on women using oral and injectble contraceptive drugs. *Med. J. Cairo Univ.*, **4**: 37-43 (1972).

Balakrishna, Murthy, P., Abdul, Rahiman, M. and Tulpule, P.C.: Lymphocyte proliferation kinetics in malnourished children measured by differential chromatid staining. *Br. J. Nutr.*, **47**: 445-450 (1982).

Balakrishna, Murthy, P., Bhaskaram, P. and Srikantia, S.G.: Sister chromatid exchanges in protein-energy malnutrition. *Hum. Genet.*, **55**: 105-106 (1980).

Beaconsfied, P. and Ginsberg, J.: Oral contraceptives and cell replication. *Lancet*, **i**: 592 (1968).

Brotherton, J.: Pregnancy. pp. 248-288: In: *Sex Hormone Pharmacology*. N.Y. (1976).

Cannif, J.P., Batchelor, J.R., Dodi, I.A. and Harvey, W.: HLA-typing in oral submucous fibrosis. *Tissue Antigens*, **26**: 138-142 (1985).

Carr, D.H.: Chromosomes after oral contraceptives. *Lancet*, **ii**: 830-832 (1967).

Carrano, A.V., Thompson, L.H., Lindl, P.A. and Minkler, J.C.: Sister chromatid exchange as an indicator of mutagenesis. *Nature*, **271**: 551-553 (1978).

Das, B.C. and Sharma, T.: Influence of age on the frequency of sister chromatid exchanges and X-ray induced chromosome aberrations in muntjac. *Mutat. Res.*, **109**: 53-63 (1983).

Esber, H.J., Menninger, F.F. and Bogden, A.E.: Immunological deficiency associated with cigarette smoke inhalation, by mice. *Arch. Environ. Health*, **27**: 99-104 (1973).

Eulg, H.C., Husum, B. and Nielbuhr, E.: SCE in smokers of high-tar cigarettes, low-tar cigarettes, cheroots and pipe tobacco. *Hereditas*, **98**: 225-228 (1983).

Ghosh, P.K. and Ghosh, R.: Effect of betel chewing on the frequency of sister chromatid exchanges in pregnant women and women using oral contraceptives. *Cancer Genet. Cytogenet.*, **32**: 211-216 (1988).

Ghosh, P.K. and Ghosh, R.: Sister chromatid exchanges in human amnion cells. *Nucleus*, **29**: 45-47 (1986).

Ghosh, P.K., Madvi, R. Mohan Guntur and Ghosh, R.: Sister chromatid exchanges in patients with oral submucous fibrosis. *Cancer Genet. Cytogenet.*, **44**: 197-201 (1990).

Ghosh, R. and Ghosh, P.K.: Sister chromatid exchanges in betel and tobacco chewers. *Mutat. Res.*, **139**: 79-81 (1984).

Ghosh, R. and Ghosh, P.K.: The effect of tobacco smoking on the frequency of sister chromatid exchanges in human lymphocyte chromosomes. *Cancer Genet. Cytogenet.*, **27**: 15-19 (1987).

Ghosh, R., Sharma, J.K. and Ghosh, P.K.: Sister chromatid exchanges in the lymphocytes of patients with oral leukoplakia. *Cancer Genet. Cytogenet.*, **36**: 177-182 (1988).

Gupta, P.C., Pindborg, J.J., Bhonsle, R.B., Munti, P.R., Mehta, F.S., Aghi, M.B., Daftary, D.K. and Shah, H.T.: Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet*, **i**: 1235-1239 (1986).

Hill, A. and Wolff, S.: Increased induction of sister chromatid exchanges by diethylstilbestrol in lymphocytes from pregnant and premenopsusal women. *Cancer Res.*, **42**: 893-896 (1982).

Hirayama, T.: An epidemiological study of oral and pharyngeal carcinoma in Central and South East Asia, *Bull W.H.O.*, **34**: 41-69 (1966).

Hoffmann, D., Sanghvi, L.D. and Wynder, E.L.: Comparative chemical analysis of Indian bidi and American cigarette smoke. *Int. J. Cancer*, **14**: 49-53 (1974).

Hopkin, I.M. and Evans, H.J.: Cigarette smoke condensates damage DNA in human lymphocytes. *Nature*, **279**: 241-242 (1979).

Hopkin, J.M. and Evans, H.J.: Cigarette smoke induced

DNA damage and lung cancer risk. *Nature*, **283**: 388-390 (1980).

Husgafvel, Pursiainen, K., Maki Paakkanen, J. Norppa, H. and Sorsa M.: Smoking and sister chromatid exchange. *Hereditas*, **92**: 247, (1980).

Husum, B., Wulf, H.C. and Niebuhs, E.: Normal sister chromatid exchanges in oral contraceptive users. *Mutat. Res.*, **103**: 161-164 (1982).

Jacob, C.V., Stelzer, G.T. and Wallace, J.H.: The influence of cigarette smoke products on the immune response. *Immunology*, **40**: 621-627 (1980).

Jayant, K., Balkrishnan, V. and Sanghvi, L.D.: A note on the distribution of cancer in some endogenous groups in Western India. *Br. J. Cancer*, **25**: 611-619 (1971).

Jussawalla, D.J. and Deshpande, V.A.: Evaluation of cancer risk in tobacco chewers and smokers: An epidemiologic assessment. *Cancer*, **28**: 244-252 (1971).

Jussawalla, D.J., Haenszel, W., Desphande V.A., and Natekar, M.V.: Cancer incidence in Greater Bombay: Assessment of cancer risk by age. *Br. J. Cancer*. **22**: 623-636 (1968).

Lambert, B., Lindbald, A., Nordenskjold, M. and Werelins, B.: Increased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas*, **88**: 147-149 (1978).

Lamberti, L., Ponzelto, P.B. and Ardito, G.: Cell kinetics and sister-chromatid exchange frequency in human lymphotyes. *Mutat. Res.*, **120**: 193 (1983).

Latt, S.A., Schreck, R.R. Loveday, K.S., Dougherty, C.P. and Shuler, C.F.: Sister chromatid exchanges In: H. Harris, and K. Hirschhorn (Eds.): *Advances in Human Genetics*, **10**: 267. Plenum Press, New York (1988).

Lemner, J. and Shear, M.: Precancerous and cancerous lesions of the mouth. *J. Dent. Assoc. S. Afr.*, **23**: 274-285 (1968).

Lindbald, A. and Lambert, B.: Relation between sister chromatid exchange, cell proliferation and proportion of B and T cells in human lymphocyte culture. *Hum. Genet.*, **57**: 31 (1981).

Livingstone, G.K. and Fineman, R.M.: Correlation of human lymphocyte SCE frequency with smoking history. *Mutat. Res.*, **119**: 59-64 (1983).

Lundberg, M.s. and Livingstone, G.K.: Sister chromatid exchange frequency in lymphocytes of smoking and non-smoking mothers and their newborn infants. *Mutat. Res.*, **121**: 241 (1983).

MacMohan, B., Cole, P., Brown, J.B., Paffenberger, R., Trichopoulos, D. and Stella, Y.: Uterine estrogens, frequency of ovulation and breast cancer risk: Case control study in premenopausal women. *Cancer Res.*, **70**: 749-750 (1983).

McArther, J.W. and Powell, D.A.: Assays of chroionic hormones. pp. 517-555. In: *Hormones in Human Blood*. H.H. Anotoniades (Ed.). Harvard University Press, Boston (1976).

McMichael, A.J.: Oral cancer in the third world: Time for preventive intervention. *Int. J. Epidemiol.*, **13**: 403-405 (1984).

McQuarrie, H.G., Scott, C., Ellsworth, H.S., Harris, J.W. and Stone R.A.: Cytogenetic studies on women using oral contraceptives and their pregnancy. *Am J. Obstet. Gynecol.*, **108**: 659-662 (1970).

Mehta, F.S., Pindborg J.J. and Gupta P.C.: Oral leukoplakia among Indian villagers. The association with smoking habits. *Br. Dent. J.*, **127**: 73-77 (1969b).

Mehta, F.S., Pindborg, J.J., Gupta, P.C. and Daftary, D.K.: Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer*, **24**: 832-849 (1969).

Mehta, F.S., Aghi, M.B., Gupta, P.C., Pindborg J.J., Bhonsle, R.G., Jalanwalla, P.N. and Sinor, P.N.: An intervention study of oral cancer and precancer in rural Indian populations: A preliminary report. *Bull. WHO*, **60**: 441-446, (1982).

Mehta, F.S., Gupta, P.C., Daftary, D.K., Pindborg, J.J. and Choksi, S.K.: An epidemiologic study of oral cancer and precancerous conditions among 101, 761 villagers in Maharashtra, India. *Int. J. Cancer*, **10**: 134-141 (1972a).

Mehta, F.S., Pindborg, J.J. and Bhonsle, R.B.: The incidence of oral leukoplakias among 20358 Indian villagers in a 7-year period. *Br. J. Cancer*, **33**: 549-554 (1976).

Mehta, F.S., Shroff, B.C., Gupta, P.C. and Daftary, D.K.: Oral leukoplakia in relation to tobacco habits. *Oral Surg.*, **34**: 426-433 (1972b).

Meiying, C., Jivjin, X. and Xianting, Z.: Comparative studies on spontaneous and mitomycin C induced sister chromatid exchanges in smokers and non-smokers. *Mutat. Res.*, **105**: 195-200 (1982).

Michalek, A.M., Cummings, K.M. and Pontes, J.E.: Cigarette smoking, tumor recurrence and survival from bladder cancer. *Prev. Med.*, **14**: 92-98 (1985).

Morgen, W.F. and Crossen, P.E.: The incidence of sister chromatid exchange in cultured human lymphocytes. *Mutat. Res.*, **42**: 305 (1977).

Murthy, P.B.K.: Frequency of sister chromatid exchange in cigarette smokers. *Hum. Genet.*, **52**: 343 (1979).

Murthy, P.B.K. and Prema, K.: Further studies on sister chromatid exchange frequency in users of hormonal contraceptives. *Mutat. Res.*, **119**: 351-354 (1983).

Murthy, P.B.K. and Prema, K.: Sister chromatid exchanges in oral contraceptive users. *Mutat. Res.*, **68**: 149-152 (1979).

Notani, P. and Sanghvi, L.D.: A retrospective study of lung cancer in Bombay. *Br. J. Cancer*, **29**: 477-482 (1974).

Notani, P., Rao, D.N., Sirsat, M.V. and Sanghvi, L.D.: A study of lung cancer in relation to bidi smoking in different religious communities of Bombay. *Ind. J. Cancer*, **14**: 115-121 (1977).

Obe, G., Vogt, H.J., Madle, S., Fahning, A. and Heller, W.D.: Double blind study on the effect of cigarette on the chromosomes of human peripheral blood lymphocytes. *Mutat. Res.*, **92**: 309-319 (1982).

Paissat, D.K.: Oral submucous fibrosis. *Int. J. Oral. Surg.*, **20**: 307-312 (1981).

Paymaster, J.C.: Cancer and its distribution in India. *Cancer*, **17**: 1026-1034 (1964).

Pike, M.C., Krailo, M.D., Kenderson, B.E., Casergrande, J.T. and Hoel, D.G.: Hormonal risk factors, breast tissue age and the age incidence of breast cancer. *Nature*, **303**: 767-770 (1983).

Pindborg J.J.: Oral submucous fibrosis as a precancerous condition. *J. Dent. Res.* **45**: 546-553, (1966).

Pindborg, J.J. Chawla, T.N., Misra, R.K., Nagpaul, R.K.

and Gupta, V.K.: Frequency of oral carcinoma, leukoplakia, leukokeratosis, leukoedema, submucous fibrosis and lichen planus in 10,000 Indians in Lucknow, Uttar Pradesh, India: Preliminary report. *J. Dent. Res*, **44**: 615 (1965).

Pindborg, J.J., Kiaer, J., Gupta, P.C. and Chawla, T.N.: Studies on oral leukoplakias. Prevalence of leukoplakia among 10,000 persons of Lucknow, India with special reference to use of tobacco and betel nut. *Bull WHO*, **37**: 109-116 (1967).

Pindborg, J.J., Mehta, F.S. and Daftary, D.K.: Occurrence of epithelial atypia in 51 Indian villagers with oral submucous fibrosis. *Br. J. Cancer*, **24**: 253-257 (1970).

Pindborg, J.J., Renstrup, G., Jolst, O. and Roed-Petersen, B.: Studies in oral leukoplakia. A preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow up study of 240 patients. *J. Am. Dent. Ass.*, **76**: 767-771 (1969).

Pindborg, J.J.: Is submucous fibrosis a precancerous condition in the oral cavity ? *Int. Dent. J.*, **22**: 474-480 (1972).

Prasad, P.R. and Haider, Z.A.: Cytomorphology of steroid-induced root tumours. *Mutat. Res.*, **103**: 25-28 (1982).

Ranadive, K.J., Gothoskar, S.V., Rao, A.R., Tezabwalla B.U. and Ambaye, R.Y.: Experimental evidence of betel nut and tobacco carcinogenitiey. *Int. J. Cancer*, **17**: 469-476 (1976).

Roszam, T.L. and Rogers, A.S.: The immunosuppressive potential of products derived from cigarette smoke. *Amer. Rev. Resp. Dis.*, **108**: 1158-1163 (1973).

Sanghvi, L.D., Rao, K.C.M. and Khanolkar, V.R.: Smoking and chewing of tobacco in relation to cancer of the upper alimentary tract. *Br. J. Cancer*, **23**: 670-689 (1955).

Sanghvi, L.D., Rao, K.C.M. and Khanolkar, V.R.: Smoking and chewing of tobacco in relation to cancer of the upper alimentary tract. *Br. Med. J.*, **1**: 111-114 (1955).

Sanghvi, L.D.: Cancer epidemiology: The Indian scene. *J. Cancer Res. Clin. Oncol.*, **99**: 1-14 (1981).

Schonland, M. and Bradshaw, E.: Upper alimentary tract: Cancer in Natal Indians with special reference to the betel chewing habit. *Br. J. Cancer*, **23**: 670-689 (1969).

Seedat, H.A. and Van Wyk, C.W.: The oral features of betel nut chewers without submucous fibrosis. *J. Biol. Buccale*, **16**: 123-138 (1988b).

Seedat, H.A.: *Oral Submucous Fibrosis in Durban, Natal: A Study of Its Epidemiology, Aetiology and Morphological Features*. Thesis, University of Stellenbosh (1986).

Sharma, T. and Das, B.C.: Higher incidence of spontaneous sister chromatid exchanges (SCEs) and X-ray induced chromosome aberrations in peripheral blood lymphocytes during pregnancy. *Mutat. Res.*, **174**: 27-33 (1986).

Shubick, V.M.: The impact of alcohol and smoking on immune reactions (Abstract). *Smoking and Health Bulletin*. U.S. Govt. Priming Office. DHHS, Washington, D.C., ISSN: 0081-0363 (1985).

Siiteri, P.K., Hammond, G.L. and Nisker, J.A.: Increased availability of serum estrogens in breast cancer: A new hypothesis. pp.87 In: *Hormones and Breast Cancer*, M.C. Pike, P.K. Siiteri, C.W. Webch, (Eds.). Banbury Report No. 8. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1981).

Simmer, H.H.: Placental hormones pp 290-335. In: *Biology of Gestation*, N.S. Assali (Ed.). Academic Press, NY (1968).

Sirsat, S.M. and Khanolkar, V.R.: Submucous fibrosis of the palate and pillars of the Fauces. *Ind. J. Med. Sci.*, **16**: 189-197 (1962).

Suri, K., Goldman, H.M. and Herbert, W.: Carcinogenic effect of a dimethyl sulphoxide extract of betel nut on the mucosa of the hamster buccal pouch. *Nature*, **230**: 383-384 (1971).

Sutherland, G.R., Baker, E., Seshadri, R.S. and Black, A.: Increased sister chromatid exchange in multiple sclerosis. *New Engl. J. Med.*, **303**: 1126 (1980).

Thomas, W.R., Holt, P.G. and Keast, D.: Recovery of immune system after cigarette smoking. *Nature*, **248**: 358-359 (1974).

Thomas, W.R., Holt, P.G. and Keast, D.: Cellular immunity in mice chronically exposed to fresh cigarette smoke. *Arch. Environ. Health*, **27**: 327-375 (1973).

Thomas, W.R., Holt, P.G. and Papadimitriou, J.M.: The growth of transplanted tumours in mice after chronic inhalation of fresh cigarette smoke. *Br. J. Cancer*, **30**: 459-462, (1974b).

Tobacco in India: *A Handbook of Statistics*. Published by the Directorate of Tobacco Development, Ministry of Agriculture. Govt. of India, New Delhi (1983).

Urmancheeva, A., Nakikova, A.I. and Anisimov, V.N.: Simulating effect of pregnancy in growth of cancer of cervix uteri. *Akush Ginekol.*, **1**: 53-54 (1989).

Williams, D.L., Rungan, J.W. and Hagen, A.A.: Meiotic chromosome alterations produced by progesterone. *Nature*, **220**: 1145-1146 (1968).

Winn, D.M., Blot, W.J., Shy, C.M., Pickele, L.W., Toledo, A. and Fraumeni, J.F.: Snuff dipping and oral cancer among women in the Southern United States. *N. Engl. J. Med.*, **304**: 745-749 (1981).

Wulf, H.C., Husum, B. and Nielbuhr, E.: SCE in smokers of high-tar cigarrettes, low-tar cigarettes, cheroots and pipe tobacco. *Hereditas*, **98**: 225-228 (1983).

Wynder, E.L. and Mabuchi K.: Etiological and preventive aspects of human cancer. *Prev. Med.*, **1**: 300-334 (1972).

Zachariah, J., Mathew, B., Verma, N.A.R., Iqbal, A.M. and Pindborg, J.J.: Frequency of oral mucosal lesions among 5000 individuals in Tribandrum, South India. *J. Ind. Dent. Assoc.*, **38**: 290; 290-294 (1966).

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