Chapter 2

Clinical and Pathological Effects

CONTENTS

Oral Mucosal Lesions: Clinical Findings In Relation to Smokeless Tobacco Use Among U.S. Baseball Players[1]

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ABSTRACT We have been conducting a 3-yr study of the health effects of smokeless tobacco use by members of Major League and minor league baseball teams. Findings from the first year (1988), involving 1,109 players, were previously reported. This report presents data about ST usage and associated oral mucosal lesions among 894 additional players who first entered the study in 1989 (n=523) and 1990 (n=371). The 1989 and 1990 data were obtained by the same study team, using the same study methods, as in 1988. Findings in the two sets of data are highly consistent. There was a significant association between the prevalence of oral mucosal lesions and the frequency, amount, and recency of ST use and the type and brand used. Players who used ST year round had a much higher prevalence of lesions (67 percent) than those who used it primarily during the baseball season (32 percent). Among those who used snuff year round, 72 percent had lesions, compared to 44 percent of seasonal users. Year-round snuff users who used four or more cans per week had a lesion prevalence of 88 percent.

INTRODUCTION We previously reported findings from the first year (1988) of a 3-yr study of the health effects of smokeless tobacco use among members of Major League and minor league baseball teams (Ernster et al., 1990; Grady et al., 1990 and 1991). Our primary purpose has been to study the association of ST use with various oral health and other health measures: oral mucosal lesions; gingival inflammation and recession; dental caries, erosion, attrition, and staining; pulse and blood pressure; and total and high-density lipoproteins. The study was designed to detect the effects of the type and brand of ST used and the amounts and length of use, controlling for potentially confounding variables such as age, alcohol use, cigarette smoking, and oral hygiene practices. For efficiency, we chose an adult population known to have a high prevalence of ST use—professional baseball players.

Ernster and coworkers (1990) described the overall study design and provided an overview of findings involving 1,109 players examined during the first year. Grady and colleagues (1990) presented a more extensive analysis of the mucosal lesion findings from the same study as they relate to a number of variables. Because the long-term use of ST has been strongly associated with oral cancer in previous studies (IARC, 1985; US DHHS, 1986; Winn et al., 1981), the oral mucosal lesions that are associated with ST use are of special interest and concern.

In this paper we present new data on oral mucosal lesions from 894 players examined for the first time in the second and third years of the study. We also examine the effects of seasonality of ST use on the prevalence and severity of oral mucosal lesions.

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METHODS A detailed description of the study methods is presented by Ernster and coworkers elsewhere in this volume. The study population consisted of professional baseball players and other male team personnel from seven Major League teams and their associated minor league teams who participated in spring training in and around Phoenix, Arizona. People who agreed to take part in the study were asked to complete a questionnaire; a study staff member was present to assist in filling out the questionnaire, if needed. Players then were asked to rinse their mouths with water to remove any traces of tobacco, and to undergo an oral examination. The participants were instructed not to inform the examiners of their ST use status. The examination consisted of an inspection of the oral mucosa and a standardized clinical examination of the periodontium and the dentition by trained examiners calibrated for diagnostic uniformity. Unless otherwise indicated, the present analysis is confined to players who first entered the study in 1989 (n=523) and 1990 (n=371).

Because self-reports of ST use were more than 90 percent accurate, according to serum cotinine and thiocyanate verification in year 1, the smokeless tobacco use status reported here is derived from questionnaire data. On the basis of self-reports, the players were classified as never or minor users, former users, current-month users, or current-week users. Abnormalities of the oral mucosa were categorized and recorded as (1) leukoplakia/erythroplakia—any white, opaque, or leathery-appearing plaque not clinically characteristic of another type of white lesion, or any red patch not clinically identifiable as another type of red lesion, or (2) other mucosal abnormalities—i.e., interdental hyperkeratosis, retromolar hyperkeratosis, other white changes, aphthous ulcer, other mucosal ulcers, pigmented lesions, fibroma, or tongue changes. The leukoplakia/erythroplakia lesions were also classified into four degrees of severity: (1) no or slight color change, with a change in texture; (2) color and texture change, but no thickening; (3) color and texture change, with mild to moderate thickening; (4) no normal color, severe texture change, and heavy thickening. These categories are similar to the ones developed by Greer and Poulson (1983) from a system published by Axéll and coworkers (1976). Because erythroplakia was not noted in the current study group, we refer to the oral mucosal lesions either as leukoplakia or, simply, as lesions.

Study participants were asked to indicate the type and brand of ST they usually used. They were also asked when they first started using ST and how long they had been using it. Participants' exposure to ST was recorded in terms of cans per week for snuff, and pouches per week for chew. When a player reported using both snuff and chew, but used one type usually and the other only occasionally, the exposure was counted as the dominant type for purposes of determining dose-response. We calculated hours per day that either snuff or chew was kept in the mouth as a combined measure of ST use. Recency of use was reported in hours since ST was last used. Because recency of use was not recorded in the third year of the study, multivariate logistic regression analysis of recency data was performed on second-year data only.

During the first year of the study, it became obvious that some players used ST only during the regular baseball season, whereas others used it year round. Therefore, seasonality of use was included in the questionnaire for the second and third years, and the effects of this variable are reported here. Seasonality analyses include all current-week ST users examined in year 2 (n=389), some of whom had been examined for the other variables in year 1 and are not otherwise included in this report, and all current-week users examined in year 3 but not in year 2 (n=156).

RESULTS Baseline demographic and other characteristics (age, race, educational level, cigarette smoking, alcohol consumption) of the 894 study participants were very similar in percentages to those of the entire study population from all 3 yr combined (see Ernster et al., this volume). Most of the study subjects (76 percent) were between the ages of 20 and 29, nearly 70 percent were white, and 77 percent had some college education. Only 2.0 percent of subjects reported that they were current smokers, and only 4.7 percent reported heavy alcohol consumption. Approximately two-thirds indicated that they visited their dentist to have their teeth cleaned at least once every 12 mo.

As we reported earlier (Ernster et al., 1990; Grady et al., 1990), the prevalence of ST use among professional baseball players is very high. Data on ST use status were available for 879 players in this study. Nearly 37 percent of these players (323) reported using ST within the 7 d prior to the examination and thus were classified as current-week users. Most of those had used ST in the previous 24 h. Oral mucosal lesions were found in 51.7 percent of the current-week users, 3.5 percent of former users, and 2.9 percent of non-users (odds ratio = 36.0 for current-week users vs. non-users). The association between severity of the most severe lesion and ST use status is shown in Table 1. Most of the lesions (76.7 percent) were degree 1 or 2 in severity. All of the degree 3 and degree 4 lesions were found in current-week users.

The prevalence of lesions according to characteristics of ST use in current-week users is shown in Table 2. Prevalence of lesions increased with earlier first use, with longer duration of use, and with more recent use. Lesion prevalence increased as the exposure to ST increased, whether measured by the amount used per week or by hours ST was held in the mouth each day. Among snuff users, 40 percent of those who used one can or less than two cans per week had lesions, whereas 84.7 percent of those who used more than three cans per week had lesions. A similar trend was seen among tobacco chewers: 8 percent of those who used up to one pouch per week had lesions, while 27 percent of those who used more than three pouches per week had lesions. When prevalence of lesions was analyzed according to the number of hours of ST use per day, 30 percent of those using ST for 0.5 h/d or less had lesions, whereas 85 percent of those using ST for more than 4.0 h/d had lesions.

The prevalence of lesions in current-week users, according to the type and brand of ST used, is shown in Table 3. Most of the ST users usually used snuff, and the snuff users had a much higher risk of developing oral lesions

Table 1
Severity of most severe lesion, by ST use status

	Degree of Severity of Lesions[a]								
	1		2		3		4		
	n	(%)	n	(%)	n	(%)	n	(%)	Total
Use Status									
Never/minor	10	(83.3)	2	(16.7)	0	(0.0)	0	(0.0)	12
Former	3	(75.0)	1	(25.0)	0	(0.0)	0	(0.0)	4
Current month	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	2
Current week	63	(37.7)	61	(36.5)	40	(24.0)	3	(1.8)	167
Total	78		64		40		3		185

[a] *Lesions were graded from 1 to 4 by increasing degree of severity.*

than chew users (61.2 percent vs. 14.8 percent). Of the players who used Copenhagen brand snuff, 72 percent had a lesion at the time of the examination, compared with only 11 percent of the Hawken brand users.

Multivariate logistic regression analyses were based on year 2 data only, because recency of use was not recorded in year 3. Variables included in the analysis were duration, recency, and amount of use; age when ST use began; type and brand of ST; and seasonality of use. Amount of use (hours per day), recency of use, and use of Copenhagen and Skoal brands were independently and significantly associated with the presence of oral lesions (Table 4). Lesion prevalence increased with increasing amounts of use (odds ratio [OR]=1.46 per hour of use per day; p=0.009). Lesion prevalence decreased with time since last use of ST (OR=0.96 for each additional hour since last use; p=0.0012). The odds of having a lesion were eightfold higher for users of Copenhagen snuff than for users of chew (OR=8.05; $p < 0.0001$), and more than sixfold higher for users of Skoal than for users of chew (OR=6.78; p=0.0003). Duration of use and age when ST use was begun showed no independent effect on lesion prevalence. The odds ratio for lesion prevalence among year-round users compared to seasonal users was elevated but was not statistically significant (OR=1.46; p=0.23).

Of the current-week users, 277 (51.2 percent) used ST only during the baseball season, and 264 (48.8 percent) used it year round. Of the seasonal users, about one-third (32.1 percent) had lesions, in contrast to two-thirds (66.7 percent) of the year-round users. The distribution of seasonal and year-round ST users with lesions according to various ST use characteristics is shown in Table 5. The percentage of participants with lesions was greater for year-round users than for seasonal users at virtually all levels of each use characteristic.

Table 6 shows the prevalence of lesions in seasonal and year-round current-week users, according to type and brand of ST usually used. Except for Hawken brand snuff, for which there were only 5 year-round users,

Table 2
Prevalence of leukoplakia, by ST use characteristics in current-week users (n=323)

	n[a]	Percentage With Leukoplakia
Age at First Use		
< 10 yr	13	69.2%
10-14	75	57.3
15-19	185	51.9
≥ 20	49	36.7
Duration of Use		
≤ 3 yr	98	37.8
4-6	122	52.5
7-9	45	68.9
≥ 10	52	59.6
Time Since Last Used[b]		
> 24 h	26	11.5
> 12-24	36	22.2
> 1-12	37	51.4
≤ 1	57	79.0
Amount of Use		
Snuff[c] (cans/wk)		
≤ 1	95	40.0
> 1-3	89	65.2
> 3	72	84.7
Chew[d] (pouches/wk)		
≤ 1	26	7.7
> 1-3	17	17.6
> 3	11	27.3
Hours ST in Mouth/Day		
0.0-0.5	76	30.3
> 0.5-1.0	65	36.9
> 1.0-1.5	37	54.0
> 1.5-2.0	26	61.5
> 2.0-4.0	66	74.2
> 4.0	26	84.6

[a] *Category totals differ because of missing values.*
[b] *Information not collected in year 3, so these values are for year 2 subjects only.*
[c] *Includes only subjects who usually used snuff.*
[d] *Includes only subjects who usually used chew.*

prevalence was greater in the year-round user group for each type and brand usually used. Nearly three-fourths (72 percent) of those who used snuff year round had lesions, compared to 44 percent of seasonal snuff users. Players who used chew were more likely to be seasonal users, while those who used snuff were more likely to be year-round users.

Table 3
Prevalence of leukoplakia, by type and brand of ST used, current-week users (n=323)

	n[a]	Percentage With Leukoplakia
Type of ST Usually Used		
Snuff	258	61.2%
Chew	54	14.8
Brand of ST Usually Used		
Snuff		
Copenhagen	177	72.3
Skoal	54	42.6
Hawken	18	11.1
Chew		
Red Man	21	14.3
Levi Garrett	24	16.7

[a] *Category totals differ because of missing values.*

Table 4
Association of various risk factors with the presence of oral lesions in current ST users[a]

	Risk per:	Odds Ratio	p
Duration	Year of ST use	1.00	0.91
Amount	Hours/day of ST use	1.46	0.0093
Initiation	Year of age ST use begun	1.03	0.49
Recency	Hours since ST last used	0.96	0.0012
Type/Brand			
Chew	(referent)	1.00	—
Copenhagen		8.05	< 0.0001
Skoal		6.78	0.0003
Hawken		3.24	0.16
Seasonality			
Seasonal	(referent)	1.00	—
Year round		1.46	0.23

[a] *This multivariate regression analysis includes all current-week users seen in year 2 for whom data on all variables were available; n=290.*

Table 5
Prevalence of leukoplakia in current-week ST users (n=545),[a] by seasonality of use and use characteristics

	Seasonal Current-Week ST Users		Year-Round Current-Week ST Users	
	n[b]	Percentage With Leukoplakia	n[b]	Percentage With Leukoplakia
All Current-Week ST Users[c]	277	32.1%	264	66.7%
Age at First Use				
< 10 yr	3	66.7	12	75.0
10-14	34	20.6	72	65.3
15-19	174	32.8	137	67.2
≥ 20	65	33.8	43	65.1
Duration of Use				
≤ 3 yr	104	36.5	46	54.4
4-6	105	31.4	102	64.7
7-9	28	28.6	47	76.6
≥ 10	34	26.5	64	70.3
Time Since Last Used[d]				
> 24 h	46	10.9	14	21.4
> 12-24	47	27.8	33	30.3
> 1-12	36	34.0	46	71.7
≤ 1	47	51.1	64	87.5
Hours ST in Mouth/Day				
0.0-0.5	111	26.1	42	40.5
> 0.5-1.0	70	35.7	48	56.2
> 1.0-1.5	25	40.0	41	65.8
> 1.5-2.0	15	33.3	26	76.9
> 2.0-4.0	21	52.4	64	79.7
> 4.0	5	20.0	28	82.1
Amount of Use				
Snuff[e] (cans/wk)				
≤ 1	112	33.9	55	52.7
> 1-3	55	61.8	91	70.3
> 3	13	61.5	88	87.5
Chew[f] (pouches/wk)				
≤ 1	47	4.3	8	0.0
> 1-3	26	11.5	6	50.0
> 3	9	22.2	8	25.0

[a] Includes some players examined in year 1 who are not otherwise included in this report.
[b] Category totals differ because of missing values.
[c] Seasonality data were not available for four current-week users.
[d] Information not collected in year 3, so these values are for year 2 subjects only.
[e] Includes only persons who usually used snuff.
[f] Includes only persons who usually used chew.

Table 6
Prevalence of leukoplakia in current-week users (n=545), by seasonality of use and by type and brand of ST used

	Seasonal Current-Week ST Users		Year-Round Current-Week ST Users	
	n[a]	Percentage With Leukoplakia	n[a]	Percentage With Leukoplakia
Type of ST Usually Used				
Snuff	184	44.0%	236	72.0%
Chew	82	8.5	22	22.7
Brand of ST Usually Used				
Snuff				
Copenhagen	117	52.1	179	76.5
Skoal	39	33.3	46	63.0
Hawken	23	17.4	5	0.0
Chew				
Red Man	24	12.5	6	16.7
Levi Garrett	42	4.8	10	30.0
Other	16	12.5	6	16.7

[a] *Category totals differ because of missing values.*

Univariate analysis showed that lesion prevalence was significantly increased among year-round ST users (OR=4.17; $p < 0.0001$), compared to seasonal users. However, as shown in Table 4, seasonality showed no statistically significant independent effect on lesion prevalence.

Finally, lesions found in year-round ST users were more likely to be severe (29.6 percent were degree 3 or 4 leukoplakia) than those in seasonal users (6.7 percent degree 3 or 4 leukoplakia).

DISCUSSION Questionnaire and clinical examination data from 894 new participants in the second and third years of this 3-yr study of professional baseball players yielded findings that were highly consistent with the previously published results from the first year (Ernster et al., 1990; Grady et al., 1990). The prevalence of current-week ST use in this new group of players was 36.7 percent, and oral lesions were found in 51.7 percent of current-week users, yielding an odds ratio of 36.0 for users compared with non-users. The prevalence of oral lesions increased with the amount used (whether expressed in hours per day or in cans or pouches per week), with recency of last use, with use of snuff instead of chew, and with use of certain brands of snuff. All of these trends are very consistent with and confirmatory of our earlier reports on first-year findings from our study of 1,109 different players.

That ST users are at increased risk of oral lesions has been shown by other investigators (US DHHS, 1986). However, no other group has had as large a sample of adults or examined the effects of so many variables related to patterns of use.

Lesion prevalence associated with the use of Copenhagen and Skoal brands of snuff continued to be several times greater than prevalence with the use of chew, as we have noted previously. Our previously published first-year results were the first to show an increased risk of oral lesions associated with recency of use and with type and brand used. Other investigators have previously reported an increased risk of oral lesions with increasing amounts of ST used (Greer and Poulson, 1983; Hirsch et al., 1982; Poulson et al., 1984; Wolfe and Carlos, 1987).

Information on the prevalence and severity of oral mucosal lesions related to seasonality of ST use has not previously been reported. The data gathered on seasonality of use in years 2 and 3 of this study show that about half of the current ST users in this population are year-round users, while the other half use ST only during the baseball season. Year-round users were much more likely to have lesions (66.7 percent) than were seasonal users (32.1 percent). It might be expected that, because the oral exams were made at the beginning of the season, seasonal users would naturally be less likely to have developed a white keratotic mucosal response. However, when the effects of other variables are taken into account, seasonality was not a statistically significant predictor of lesion prevalence in the multivariate model shown in Table 4; in that model the ST use variables of amount, recency, and type of ST were shown to have statistically significant independent effects. These findings are not unexpected, because there is no real reason to believe that seasonality should have an effect on lesion prevalence apart from the amount and type of ST used. It is interesting to note that of those who reported using ST year round, 91.5 percent used snuff, while of those who used ST only during the baseball season, 69.2 percent used snuff. Furthermore, of the year-round snuff users, 77.9 percent used Copenhagen, whereas 65.4 percent of the seasonal snuff users did so.

Thus, the greater prevalence of lesions among year-round users compared to seasonal users appears to result largely from increased and recent exposure to certain types and brands of ST. In keeping with this, the lesions found in year-round users tended to be more severe. From other evidence gathered in this study (Ernster et al., this volume), it appears that year-round users are more likely to have difficulty quitting and to be addicted. For these reasons, year-round ST users should be preferentially targeted for documenting long-term health effects of such use and for cessation intervention.

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Smokeless Tobacco Use in India: Effects on Oral Mucosa[1]

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ABSTRACT Smokeless tobacco is used in diverse forms in India for chewing, sucking in the mouth until it becomes bland, or applying over the teeth and gums. Tobacco is chewed most commonly in betel quid. ST use results in oral cancer, precancer, and other oral lesions. Tobacco smoking and other factors such as nutrition and viruses like HPV and HSV may modify the effect of ST use. There is a need to study the exact role of other factors in the causation of oral cancer. Long-term studies have demonstrated that in most instances oral cancer arises from precancerous lesions or conditions. The methods currently available to identify cancer potential in precancer, however, have limited usefulness. Therefore, research should be extended in that direction.

INTRODUCTION Tobacco was introduced to India by Portuguese traders about 1600. Although tobacco was initially smoked, it later was used in smokeless form as well. Of the 400 million individuals aged 15 and over in India, 47 percent use tobacco and 16 percent use tobacco in smokeless forms; of the 250 million kg of tobacco consumed each year, 86 percent is used for smoking, 13 percent in smokeless forms other than snuff, and 1 percent as snuff (Sanghvi, 1989).

Smokeless tobacco is used in diverse forms in different regions of India for chewing, holding in the mouth, or applying over teeth and gums. ST is chewed, more often in betel quid (*pan*), consisting of betel leaves (*Piper betle*), areca nut (*Areca catechu*), slaked lime, and catechu (*Acacia catechu*); with areca nut and slaked lime (e.g., *mainpuri* tobacco, *mawa*, *pan masala*); and less commonly by itself. A mixture of tobacco and slaked lime (*khaini*) is kept in the mouth and sucked. Other products like roasted and powdered tobacco (*mishri*), dry snuff (*bajjar*), or tobacco paste with molasses (*gudhaku*) are applied over teeth and gums. Creamy snuff is used initially as a dentifrice but soon turns into an addiction.

Betel quid chewing is widespread all over India, whereas most other uses are popular in specific geographic regions. A link between betel quid chewing and oral cancer was suspected as early as 1908 (Bentall, 1908; Fells, 1908), and by the late 1960's, several studies had demonstrated the association between betel quid chewing and other forms of tobacco use with oral cancer in India (Orr, 1933; Sanghvi et al., 1955; Shanta and Krishnamurthy, 1959; Wahi, 1968).

Several oral lesions are associated with ST use, of which oral cancer and precancer are the most serious. Since 1966 we have conducted several cross-sectional and prospective epidemiological studies on oral cancer and

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precancer (Bhonsle et al., 1976; Daftary et al., 1980; Gupta et al., 1980; Mehta et al., 1971 and 1972) in different geographic areas chosen for specific tobacco habits. In this paper, we review oral mucosal lesions associated with some common forms of ST use in India.

This paper discusses oral cancer and precancerous lesions and conditions associated with chewing betel quid with tobacco in Ernakulam district in Kerala State. The effects of chewing *mainpuri* tobacco, *mawa*, the use of tobacco-plus-lime mixture, and other habits practiced elsewhere in India are also described briefly.

PREVALENCE OF ST USE

There are no nationwide surveys on the prevalence of tobacco use in India. Table 1 gives the prevalence of ST use reported among 255,194 individuals in 9 areas in some major studies: The reported prevalence ranges from 11 to 49 percent. Except in Singhbhum, Pune, and Mainpuri districts, chewing betel quid with tobacco was widespread. Chewing tobacco without any other ingredient was practiced in two areas by 3 and 7 percent of the individuals. However, there are differences in the selection of samples, methodology, and the age groups of the individuals in these studies.

EFFECTS OF BETEL QUID CHEWING

Chewing of betel quid (betel leaf, areca nut, slaked lime, sweeteners, and flavoring) is an Indian habit dating back more than 2,000 years. Sometime after its introduction, tobacco became an important constituent of betel quid, and currently most habitual users chew betel quid with tobacco (Figure 1). Various tobacco preparations (e.g., *zarda* or *kiwam*) that can be used in the betel quid are commercially available. Regional differences in the choice of material and the way in which betel quid is chewed abound.

Betel quid chewing inevitably stains the mucosa bright red as a result of the formation of *o*-quinone from water-soluble polyphenols, notably leucocynidins, at an alkaline pH of 8 to 9 via secondary reactions (Jayalakshmi and Mathew, 1980). These stains can be washed clean or will disappear if the habit is discontinued. It is not uncommon, however, to find habitual chewers with perpetually stained mucosa.

***Pan* Chewer's Lesion**

Among heavily addicted betel quid chewers, a thick, brownish-black encrusted lesion was observed on the buccal mucosa and the mandibular groove corresponding to the site of the quid placement. This could be scraped off with a piece of gauze. The annual age-adjusted incidence rate of this lesion was 28.0 per 1,000 male betel quid chewers and 17.4 per 1,000 among females (Gupta et al., 1980).

These lesions show a pale-staining, parakeratin-like surface layer of epithelium containing round nuclear remnants, ballooning vacuolated cells, and consistent epithelial hyperplasia. *Pan* chewer's lesion is a thick encrustation, but it disappears when the habit is discontinued. The lesion does not seem to exhibit any cancer potential. Of the 532 lesions observed over a 3-yr period, 26 percent remained persistent, 45 percent regressed spontaneously, and 29 percent recurred (Gupta et al., 1980).

Table 1
Prevalence of some ST habits in India, by area

	n	Prevalence	Predominant Habit[a]
Ernakulam	10,287	37%	Betel quid
Srikakulam	10,169	11	Betel quid
Singhbhum	10,048	28	Tobacco-plus-lime
Darbhanga	10,340	16	Betel quid
Bhavnagar	10,071	13	Betel quid, dry snuff
Pune	101,761	49	Tobacco-plus-lime, *mishri*
Mainpuri	35,000	30[b]	*Mainpuri* tobacco, *pattiwala*
Lucknow	10,000	13[c]	Betel quid
Ahmedabad	57,718	47[d]	Betel quid

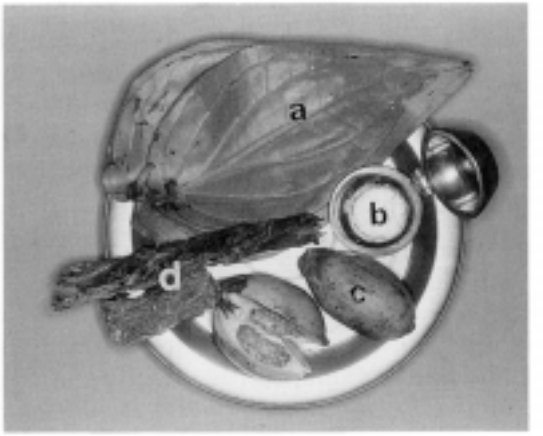
[a] *Many smoked as well.*
[b] *7 percent* mainpuri *tobacco.*
[c] *3 percent tobacco alone.*
[d] *7 percent tobacco alone.*
Source: Mehta et al., 1971 and 1972; Wahi, 1968; Pindborg et al., 1972; Smith et al., 1975.

Epithelial Dysplasia Epithelial dysplasia is generally assumed to indicate the malignant potential of a lesion. Dysplastic lesions exhibited malignant transformation rates that were 15 times higher than nondysplastic lesions (Gupta et al., 1980). In a 10-yr followup study of 16 dysplastic lesions in betel quid chewers, 8 dysplastic lesions in those who chewed and smoked, and 18 dysplastic lesions in smokers only, 19 percent, 25 percent, and 6 percent, respectively, progressed to cancer, and 25 percent, 50 percent, and 22 percent, respectively, regressed. The remaining dysplastic lesions either remained stationary or changed their clinical characteristics.

Leukoplakia Leukoplakia is the most common precancerous lesion, initially hypothesized as precancerous mainly because of its coexistence with cancer. In a study of 650 oral cancers in India, 32 percent had coexistent leukoplakia (Paymaster, 1956). Furthermore, leukoplakias and oral cancers share the same etiologic agent, tobacco, and they occur at strikingly similar locations. For example, oral cancer and leukoplakias among betel quid chewers generally occur on the buccal mucosa. The most important evidence of leukoplakia's precancerous nature, however, is its excess risk for cancer as demonstrated in prospective studies (see below).

Epidemiology The prevalence of leukoplakia in Ernakulam district was 17 per 1,000. Prevalence was 18 per 1,000 among betel quid chewers and 61 per 1,000 among those who chewed and smoked (Mehta et al., 1969). The annual age-adjusted incidence rate was 2.1 per 1,000 person-years among men and 1.3 per 1,000 among women; among betel quid chewers, the incidence was 2.5 per 1,000 among men and 3.0 per 1,000 among women. The incidence rate was highest (6.0) in those who smoked and chewed (Gupta et al., 1980).

Figure 1
Constituents of betel quid in Kerala: (a) betel leaf; (b) shell lime; (c) raw areca nut; (d) tobacco. Note that catechu is not used in Kerala.

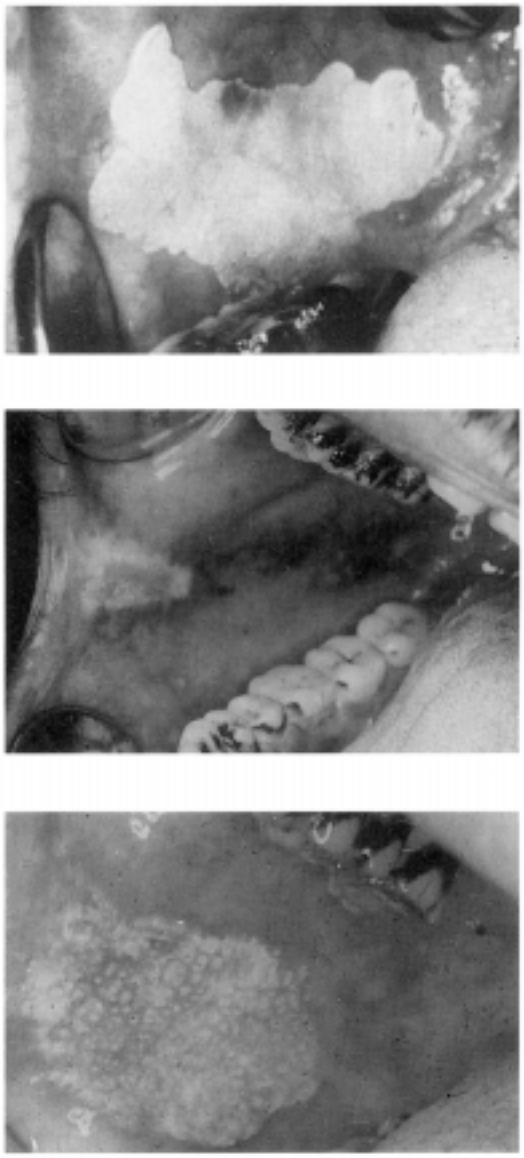


Clinical Aspects Leukoplakia is a raised white patch on the oral mucosa that measures 5 mm or more and that cannot be scraped off and cannot be attributed to any other diagnosable disease (Mehta et al., 1969). Leukoplakias are classified as homogeneous, ulcerated, or nodular types. Homogeneous leukoplakia is characterized by raised plaque formation consisting of plaque (Figure 2, top) or groups of plaques varying in size and with irregular edges. Ulcerated leukoplakia consists of an area of ulceration that is sometimes surrounded by keratinized areas, pigmentation, or both (Figure 2, middle) and generally occurs among smokers of *bidi* (a kind of cheap smoking stick). Nodular leukoplakia consists of many small white nodules on an erythematous base (Figure 2, bottom). About 94 percent of the leukoplakias among betel quid chewers are the homogeneous type. The risk for cancer varies with different clinical types of leukoplakia (see below).

In Ernakulam district, leukoplakias occurred more often among men, with the male-to-female ratio being 4:1 (Mehta et al., 1971). The average age of individuals with this lesion was 47.8 yr. Generally the location of leukoplakias varies according to where tobacco is held in the mouth. For example, those associated with betel quid chewing occurred more often on the posterior part of the buccal mucosa and the mandibular groove, whereas leukoplakias associated with tobacco-plus-lime and *mishri* occur in the premolar region of the buccal mucosa and the labial mucosa.

Histology Leukoplakias associated with betel quid chewing showed hyperorthokeratosis in 82 percent and hyperparakeratosis in 12 percent. Hyperparakeratosis was seen somewhat more frequently in those who chewed betel quid and smoked (23 percent) or who smoked only (24 percent) (Mehta et al., 1969). Epithelial dysplasia was observed in 15 percent of the betel quid-associated leukoplakias compared with 5 and 8 percent in those associated with the combined habit of chewing and smoking or smoking only. In regard to epithelial thickness, atrophy was observed in 75 percent of the

Figure 2
Homogeneous leukoplakia in a betel quid chewer (top); ulcerated leukoplakia in a *bidi* smoker (middle); nodular leukoplakia in a betel quid chewer (bottom).



betel quid-associated leukoplakias compared with 40 and 31 percent, respectively, in those associated with the combined habit or with smoking only. Orthokeratinization occurred generally in combination with epithelial atrophy, whereas parakeratinization was more commonly associated with hyperplastic epithelium.

Natural History In a 10-yr followup study of 94 betel quid-associated leukoplakias, 7 percent progressed to oral cancer, in contrast to only 4 percent of the 85 leukoplakias associated with combined betel quid chewing and smoking and none of the 45 leukoplakias associated with smoking only (Gupta et al., 1980). The higher rate of malignant transformation in betel quid-associated leukoplakias may be caused by epithelial atrophy. The malignant transformation rate was highest in nodular leukoplakias (21.0 percent) and in homogeneous leukoplakias (1.7 percent) (Gupta et al., 1980). Leukoplakias associated with betel quid chewing were less persistent (25 percent), regressed more often (57 percent), and showed higher recurrence rates than did smoking-associated leukoplakias. In another 8-yr followup study, the relative risk (RR) for the development of oral cancer among all oral precancerous lesions and conditions was highest (RR=3,243.2) for nodular leukoplakia (Gupta et al., 1989); the RR for homogeneous (RR=25.6) and ulcerated (RR=43.6) leukoplakia were also high and significant.

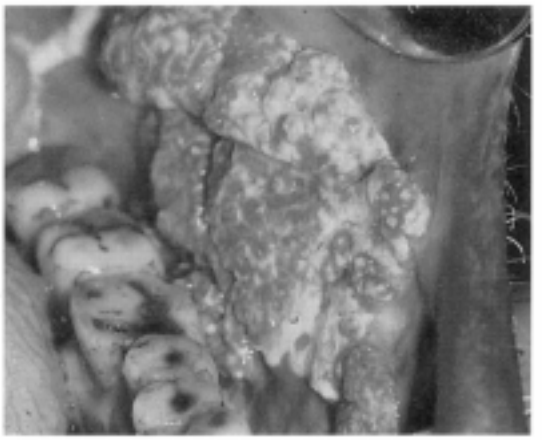
Oral Cancer Oral cancer is common in India. According to weighted averages from six cancer registries in India, oral cancer is the second and third most common cancer among men and women, respectively (Indian Council of Medical Research, 1989). Contributing to the problem of high morbidity, patients with this disease often seek medical attention in a late clinical stage, consequently having a poor prognosis.

Epidemiology In a cross-sectional study among 10,287 villagers in Ernakulam district, all 12 oral cancers occurred among those who chewed betel quid; 6 of these patients smoked as well (Mehta et al., 1969). In a 10-yr followup study of the above sample, the overall age-adjusted incidence was 16 per 100,000 person-years, 23 per 100,000 among betel quid chewers, and 32 per 100,000 in those who chewed and smoked (Gupta et al., 1980). Several extensive independent assessments have confirmed the causal role of betel quid chewing and oral cancer (IARC, 1985; National Institutes of Health, 1986; US DHHS, 1986; WHO, 1984). About 30 percent of the oral cancers in this part of the world are attributable to betel quid chewing and an additional 50 percent to the combined habit of chewing and smoking (WHO, 1984).

In India, most of the habitual betel quid chewers include tobacco. Betel quid, however, may be chewed without tobacco. As several investigators in the past were not explicit about this, there was some confusion about the carcinogenicity of betel quid with and without tobacco. The question has been extensively reviewed, and the conclusions are that the relative risk for oral cancer in those who chewed betel quid without tobacco was insignificant or significantly lower than for those who chewed betel quid with tobacco (Gupta et al., 1982). Overall, there is inadequate evidence that the habit of chewing betel quid without tobacco is carcinogenic for humans (IARC, 1985).

Clinical Aspects Oral cancer occurs more frequently among men than among women. In a sample of 2,007 oral cancers seen at the main cancer treatment center in Kerala, the male-to-female ratio was 1.8:1 (Nair et al., 1988); the mean age for men was 57.1 yr and for women, 58.6. The bucccal mucosa is the most commonly involved location (Figure 3), and in a sample of 2,007 oral

Figure 3
Buccal carcinoma in a betel quid chewer



cancers, 50 percent were located in the buccal mucosa and 24 percent on the tongue. The frequency of buccal mucosal involvement among men was slightly higher (53 percent) than that among women (45 percent). This may be because men smoke and chew, whereas women generally chew betel quid only. The mean ages of patients with cancer of the buccal mucosa and the tongue were quite similar, 56.9 and 55.8 yr, respectively, among men, and 58.5 and 56.5 yr among women.

Squamous cell carcinoma is the most common cancer, accounting for 95 to 98 percent of all oral malignancies in India. In Kerala, 7 percent of the 2,007 oral cancers reported were verrucous carcinomas (Nair et al., 1988). This is a variant of squamous cell carcinoma that is broad based and locally invasive; generally it does not metastasize.

Natural History In most instances oral cancer arises from precancerous lesions or conditions. In a 10-yr followup study in Ernakulam district, all 12 oral cancers arose from a precancerous condition (Gupta et al., 1980), whereas in another 8-yr followup study, 15 of the 19 oral cancers developed from precancer (RR=69.2) (Gupta et al., 1989).

Unfortunately, patients in India seek medical attention in the later stages of disease. Of the 2,007 oral cancers described above, only 12 percent were localized lesions and most of the remaining ones were extensive (Nair et al., 1988).

Intervention Oral cancer is amenable to primary and secondary prevention. In Ernakulam district, after 5 yr of educational intervention among 12,000 tobacco users, 10 percent of the men and 15 percent of the women discontinued their betel quid chewing, and 26 percent of men and 31 percent of women reduced betel quid use (Gupta et al., 1986). This resulted in a significant drop in the incidence of leukoplakia among betel quid chewers. The rate ratios that indicate the protective effect of educational intervention were 0.51 for men and 0.19 for women. After 8 yr the cessation rate

increased to 13 percent among men and 18 percent among women, and the rate of reduction increased to 35 percent among men and 39 percent among women (Gupta et al., 1990). Correspondingly, the number of observed leukoplakias among betel quid chewers was 27.0 among men vs. 79.7 predicted, and 49.0 among women vs. 163.9 predicted. A further increase in the rates of cessation and reduction in tobacco use and a decrease in the incidence of leukoplakia also were observed after 10 yr of intervention.

Submucous Fibrosis

Submucous fibrosis is a chronic condition marked by mucosal rigidity of various intensity caused by a fibroelastic transformation of the juxtaepithelial layer that results in progressive inability to open the mouth (Figure 4). When the tongue is involved, it is shrunken, and its mobility may be restricted. Occasional pharyngeal and esophageal involvement also has been observed. Diagnosis of the condition is based on the presence of palpable fibrous bands. Submucous fibrosis occurs predominantly among Indians at home and abroad and, to a lesser extent, other Asiatics. Areca nut chewing in any form is currently believed to be involved in the pathogenesis of submucous fibrosis (Bhonsle et al., 1987; Mehta et al., 1972; Sinor et al., 1990). Because areca nut, like tobacco, is an ingredient in betel quid, and tobacco is known to contain carcinogens, this condition is discussed here. Furthermore, submucous fibrosis is a high-risk precancerous condition (Gupta et al., 1989) in which the malignant potential is the result of epithelial atrophy and the action of carcinogens (Pindborg et al., 1984).

Epidemiology

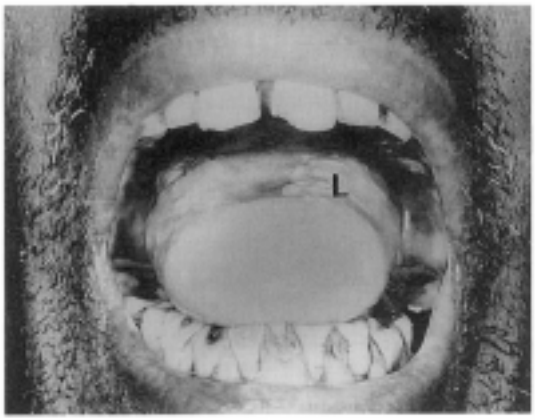
The prevalence and the incidence rates of submucous fibrosis are high in Ernakulam district relative to rates in other areas of India. The overall prevalence was 351 per 100,000, and prevalence was highest among betel quid chewers (1,090 per 100,000) (Pindborg et al., 1968). This condition was not seen among people who do not chew betel quid. The overall age-adjusted incidence rate was 7 per 100,000 person-years among men and 17 per 100,000 among women; all new cases of submucous fibrosis developed among betel quid chewers (Gupta et al., 1980). In comparison to the incidence rates, the prevalence rates seem too large (prevalence = incidence x duration).

Submucous fibrosis affects both sexes, but a definite female predominance was observed in Ernakulam district (Gupta et al., 1980; Pindborg et al., 1968). Such predominance, however, seemed to depend on the type and extent of areca nut chewing habits among men and women in different areas (Bhonsle et al., 1987). Although there is some regional variation, this condition generally occurs between the ages of 20 and 40 yr. The mean age of patients in Ernakulam district (51 yr) was higher than the mean age of patients in Pune district (37 yr) (Bhonsle et al., 1987).

Clinical Aspects

Submucous fibrosis commonly affects the buccal mucosa, retromolar areas, and the soft palate. The frequency of their involvement, however, varies from one geographic area to another, probably depending on the variations in the method of areca nut chewing (Bhonsle et al., 1987). The earliest and most common sign of this condition is blanching of the oral mucosa that imparts a marblelike appearance (Pindborg et al., 1980). When the disease is fully developed, palpable fibrous bands develop in the buccal

Figure 4
Limited oral opening in a patient with submucous fibrosis. Note the shrunken appearance of the tongue, the absence of lingual papillae, and associated leukoplakia (L).



mucosa, soft palate, and the rima oris. The bands run vertically in the buccal mucosa and are circular around the rima oris. As the disease progresses, the mucosa becomes stiff and the oral opening may be restricted. Petechial spots resulting from the breakdown of connective tissue support to the vasculature were observed in 11 percent of patients, according to Bhonsle and coworkers (1981). Furthermore, submucous fibrosis is often associated with leukoplakia (Figure 4), oral cancer, and pigmentation changes (Pindborg et al., 1968 and 1984). Most of the patients complained of a burning sensation, often aggravated by spicy food; another common complaint is excessive or decreased salivation.

Histology Epithelial atrophy, juxtaepithelial hyalinization, and varying density of collagen are the common features in submucous fibrosis. A notable feature is the presence of epithelial dysplasia in 26 percent of the cases (Pindborg et al., 1984).

Natural History Unlike other precancerous lesions, submucous fibrosis does not regress, either spontaneously or with cessation of betel quid chewing. The most serious aspect of this condition is its malignant potential. In a 17-yr followup study of 66 patients with submucous fibrosis, malignant transformation was observed in 0.4 percent at the end of 10 yr (Gupta et al., 1980), which increased to 4.5 percent at the end of 15 yr (Pindborg et al., 1984) and to 7.6 percent at the end of 17 yr (Murti et al., 1985). All five individuals in whom cancer developed were women who chewed betel quid with tobacco. In another 8-yr followup study of 12,000 tobacco users, the RR of malignant transformation for submucous fibrosis, compared with that for tobacco users without any oral mucosal lesion or condition, was 397.3 (Gupta et al., 1989). There is no effective cure for submucous fibrosis; however, the condition appears to be amenable to primary prevention (Murti et al., 1990).

Oral Lichen Planus Lichen planus is primarily a dermatologic disorder. Mucosal surfaces may be affected along with cutaneous lesions; the mucosal lesions may occur alone; or one may precede the other. The oral mucosa is more commonly affected, and oral lesions are suspected of having some cancer potential. In Ernakulam district, oral lichen planus was found to be strongly associated with betel quid chewing (Bhonsle et al., 1979; Gupta et al., 1980; Pindborg et al., 1972). This paper describes oral lichen planus as a result of betel quid use and its malignant potential.

Epidemiology Oral lichen planus was diagnosed on the basis of presence of Wickham's striae. The overall prevalence of oral lichen planus was 1.5 percent; it was highest (3.2 percent) among betel quid chewers, and lowest (0.3 percent) in non-users of tobacco (Pindborg et al., 1972). The annual age-adjusted incidence rate per 1,000 person-years was 2.1 among men and 2.5 in women who chewed betel quid vs. 0.6 among men and 0.9 in women who were not users of tobacco (Gupta et al., 1980). The RR for oral lichen planus among betel quid chewers was 6.2 for men and 4.9 for women (Bhonsle et al., 1979). Oral lesions thus showed a strong association with betel quid chewing, although tobacco use is not regarded as an etiologic factor for oral lichen planus.

Clinical Aspects Oral lichen planus occurred predominantly among women. The buccal mucosa was the most favored location; the lesions occurred in diverse morphological forms such as reticular, annular, linear, erosive or ulcerated, and pigmented forms. Of these, 20 percent were erosive or ulcerated lesions (Pindborg et al., 1972).

Natural History The malignant potential of oral lichen planus over a 10-yr period (mean, 5.1 yr) was assessed in 722 individuals with the condition. Oral cancer developed in three patients (0.4 percent) who had erosive (atrophic) lesions; two of them were betel quid chewers and the third was a smoker (Murti et al., 1986). In another 8-yr followup study of 344 individuals with oral lichen planus, the RR for malignant transformation was 15.8, which was not significant ($p > 0.05$) (Gupta et al., 1989). Oral lichen planus often regressed and sometimes recurred. The regression rates were highest in non-users of tobacco and lowest in those with the combined habits of chewing and smoking (Gupta et al., 1980). These observations support the hypothesis that tobacco plays some role in this disease.

OTHER ST USE AND EFFECTS

Chewing tobacco without any other ingredient does not seem to be common in India. In two cross-sectional studies, the prevalence of leukoplakia among tobacco chewers was 7.3 percent in Lucknow (Pindborg et al., 1972) and 12.7 percent in Ahmedabad (Smith et al., 1975).

Tobacco Chewing

Mainpuri Tobacco *Mainpuri* tobacco contains tobacco, slaked lime, finely cut areca nut, cloves, camphor, and other flavoring agents. This product is widely used in Mainpuri district in Uttar Pradesh and nearby areas, where it is known by different names, and is strongly associated with leukoplakia and oral cancer.

Leukoplakia The overall prevalence of leukoplakia in Mainpuri district was 5,160 per 100,000; it was 26,740 per 100,000 in *mainpuri* tobacco users and 15,160 among those who used *pattiwalla* tobacco (sun-cured tobacco chewed with or without slaked lime and without areca nut), which is another popular brand of ST used in that region. Among those who used both types of tobacco, the prevalence was 80,000 per 100,000 (Wahi et al., 1970).

Oral Cancer The prevalence of oral cancer in Mainpuri district was 99 per 100,000. Prevalence was 781 per 100,000 among *mainpuri* tobacco users, 117 per 100,000 in *pattiwalla* tobacco users, and 413 per 100,000 in those who used both forms. Prevalence was 36 per 100,000 in non-users of tobacco (Wahi, 1968).

***Mawa* Chewing** *Mawa* consists of areca nut shavings sprinkled with watery slaked lime. A little sun-cured tobacco is added, and the product is packed in a cellophane paper and sold. In recent years *mawa* chewing has gained considerable popularity in Bhavnagar district and adjoining areas in Gujarat.

Mawa chewing is strongly associated with oral submucous fibrosis (Sinor et al., 1990). For example, the RR for submucous fibrosis for all forms of areca nut chewing was 109.6, 106.4 for *mawa* chewing, and 780.0 for chewing *mawa* and betel quid. Clinically, submucous fibrosis in *mawa* chewers differs in regard to age, sex, and location from those observed among betel quid chewers.

Tobacco-Plus-Lime Sun-cured tobacco and slaked lime is used in Maharashtra and other states in northern and eastern India where it is known as *khaini*. A small quantity of tobacco and slaked lime is held in the palm and rolled with the thumb. In Maharashtra, the mixture is placed in the premolar region of the mandibular groove (Bhonsle et al., 1979); in Bihar and Uttar Pradesh, the mixture is held in the lower labial groove. It is sucked from time to time until it becomes bland.

Tobacco-Plus-Lime User's Lesion A thick, yellowish-white lesion, occasionally with loose tags of tissue, occurs where tobacco-plus-lime is held in the mouth by patients in Maharashtra. Prevalence among 101,761 villagers in Maharashtra was 2.9 percent (Bhonsle et al., 1979), and use occurred more often among men. As this lesion is similar in appearance to leukoplakia, it may be misdiagnosed. However, unlike leukoplakia this lesion can be scraped off; it will disappear if tobacco-plus-lime use is discontinued.

The histological characteristics include pale, parakeratin-like surface layers of epithelium containing round nuclear remnants, ballooning vacuolated cells, and epithelial hyperplasia (Bhonsle et al., 1979).

Tobacco-plus-lime user's lesion as a specific entity appears to be a counterpart of the *pan* chewer's lesion (described above). This lesion does not show any malignant potential.

Leukoplakia Tobacco-plus-lime use is associated with both leukoplakia and oral cancer among patients in Maharashtra (Mehta et al., 1972). The prevalence of leukoplakia was 1,442 per 100,000 among tobacco-plus-lime users and

6,337 per 100,000 among those who also smoked. Generally, leukoplakias associated with this habit occurred in the premolar region of the mandibular groove where the tobacco-plus-lime mixture is held.

Oral Cancer Oral cancer was also observed among tobacco-plus-lime users. Prevalence was 28 per 100,000 among tobacco-plus-lime users and 186 per 100,000 in those who also smoked. As with leukoplakia, cancer generally occurred at the site of placement of the tobacco-plus-lime, and sometimes leukoplakia coexisted with it.

Mishri Compared with use of betel quid or tobacco-plus-lime, *mishri* use produced fewer leukoplakias and no oral cancers. The prevalence of leukoplakia among *mishri* users in Maharashtra was 190 per 100,000. Although no oral cancers were detected among *mishri* users, short-term experimental tests have indicated mutagenic potential of *mishri* (Kulkarni et al., 1987).

Bajjar In Gujarat, dry snuff known as *bajjar* (described above as *mishri*) is applied over teeth and gums by 8 percent of 10,071 individuals (Mehta et al., 1971). Very few leukoplakias were observed among *bajjar* users; the prevalence was 280 per 100,000, and these leukoplakias did not show any cancer potential.

Gudhaku *Gudhaku* is a tobacco paste with molasses. Initially, it is used to clean teeth, but the product becomes addictive. *Gudhaku* was used by 8.3 percent of the 10,048 individuals in Singhbhum district in Bihar, but no oral lesions were observed among them.

Miscellaneous Products Among other ST products, *pan masala* and creamy snuff are increasingly becoming popular all over the country. *Pan masala* contains pieces of areca nut, slaked lime, catechu, and powdered tobacco. *Pan masala* comes in attractive tins and foil packs, making it appealing and convenient to use. Undoubtedly the aggressive advertising in the print and the electronic media contribute to its popularity. Animal experimental studies indicated the genotoxic nature of *pan masala*. Creamy snuff is initially used as a dentifrice but becomes an addictive substance. There are, however, no studies to date on the effects of these products on human oral mucosa.

DISCUSSION Betel quid chewing is the most common form of ST use in India. Extensive investigations in several cross-sectional and prospective epidemiological studies showed a strong association between many forms of ST use, oral cancer, precancer, and other oral mucosal lesions. Of the several ST forms, *mawa* and *pan masala* are of relatively recent origin, but there is an upswing in their use. *Mawa* chewing was demonstrated to cause oral submucous fibrosis (Sinor et al., 1990). There is practically no information, however, on the effects of *pan masala* on human oral mucosa.

Smokeless tobacco is an independent risk for oral cancer and precancer. There is also an additive effect of using more than one type of ST product and a synergistic effect with smoking (Gupta et al., 1980; Mehta et al., 1969; Pindborg et al., 1984; Sinor et al., 1990; Wahi et al., 1968 and 1970; WHO, 1984).

Although the pathogenic effects of ST in oral cancer and precancerous lesions are clear, the role of other factors that may modify the effect of ST use in India is not fully understood. Such factors may include nutrition, viruses, especially HPV and HSV, and the role of oncogenes. Furthermore, several animal studies and epidemiological studies have shown that vitamins A, C, and E, retinoic acid, carotenoids, riboflavin, selenium, and zinc may influence the risk of oral cancer independently or by modifying the effect of tobacco. There are several hospital-based studies with short-term followup on chemoprevention with vitamin A and beta-carotene. Population-based studies with long-term followup, however, are highly desirable and will be helpful in the development of practical chemopreventive measures.

The finding that oral cancer is generally preceded by precancer facilitates the early detection of cancer. Although nodular leukoplakia and submucous fibrosis are demonstrated to be a high-risk lesion and condition, respectively, the difficulty lies in identifying which particular lesion would progress to cancer. The conventional approach of using epithelial dysplasia as a marker has been very helpful. There is, however, a need to develop other markers based on advanced methodologies. In recent times flowcytometric methods have been tried and reported to be promising, but additional long-term studies are essential to demonstrate the potential of such methods.

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Smokeless Tobacco-Associated Epithelial And Langerhans Cell Changes[1]

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ABSTRACT Smokeless tobacco-associated oral mucosal lesions from professional baseball players were studied for histopathologic changes and changes in Langerhans cell (LC) density and antigen expression. Four types of epithelial change were found: hyperparakeratosis, hyperorthokeratosis, pale surface staining, and basal cell hyperplasia. These changes were associated with the type of ST used (snuff or chewing tobacco) but not with the duration (years) or amount (hours per day) of ST use. The thickness of the hyperkeratotic layer in a specimen correlated directly with the amount of ST use. Immunohistochemical analysis with four monoclonal antibodies detected fewer LC in lesion specimens than in autologous control specimens. The average reduction in LC was 58 percent (range, 3 to 95 percent). There were no significant differences in the LC reduction identified by the four different marker antigens. Specimens with all types of epithelial change showed similar reductions in LC. Our data indicate that snuff causes a greater variety and severity of epithelial change than does chewing tobacco, and that smokeless tobacco reduces the number of LC at its site of contact with the oral mucosa.

INTRODUCTION Smokeless tobacco contains carcinogens (Hoffmann et al., 1987), and an association between snuff use and oral carcinoma has been observed (Peacock et al., 1960; Sundström et al., 1982; US DHHS, 1986; Winn et al., 1981). ST use results in oral mucosal lesions, usually described as leukoplakia (Grady et al., 1990; Pindborg and Renstrup, 1963), that can become dysplastic (Andersson et al., 1989; Greer et al., 1986; Hirsh et al., 1982; Jungell and Malmström, 1985; Kaugars et al., 1989; Roed-Petersen and Pindborg, 1973; Smith et al., 1970) and can undergo malignant transformation (Roed-Petersen and Pindborg, 1973; Sundström et al., 1982). Histopathologic studies of ST-associated oral mucosal lesions have found hyperkeratosis in most specimens and various frequencies of dysplasia and carcinoma (Andersson et al., 1989; Axéll et al., 1976; Greer et al., 1986; Hirsh et al., 1982; Jungell and Malmström, 1985; Kaugars et al., 1989; Pindborg and Renstrup, 1963; Pindborg et al., 1980; Roed-Petersen and Pindborg, 1973; Smith et al., 1970).

Langerhans cells are dendritic cells that migrate from the bone marrow to reside in epithelium throughout the body. They perform important immunologic functions by participating in cutaneous and mucosal immune reactions that can have both local and systemic effects. Changes in the number of LC can affect systemic immune responses (Toews et al., 1980), and the localized absence of LC in the mucosa may be associated with colonization by viruses and fungi (Daniels et al., 1987). Localized reductions in the number of LC might impair skin or mucosal immunologic

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protection against mutagens or opportunistic viruses and have a role in epithelial dysplasia and carcinoma (Viac et al., 1990).

A 3-yr study of the oral and systemic effects of ST use on professional baseball players provided us an opportunity to examine patterns of histopathologic change, and possible effects on LC numbers and antigen expression, in oral epithelial lesions associated with the use of ST (Ernster et al., 1990). This paper summarizes the results of studies presented in detail elsewhere (Daniels et al., 1992 and in press).

SUBJECTS AND METHODS

We conducted oral examinations among 1,632 members of seven Major League and minor league professional baseball teams during spring training in 1988 (Ernster et al., 1990) and 1989. Participants were asked to identify all ST products they used and to indicate the types and brands they used most often. Of the players examined, 40 percent had used ST within the past week and were classified as current ST users. Previous detailed analysis of the 1988 data showed that 75 percent of current users reported a brand of snuff as their usual ST product, 21 percent reported chewing tobacco as their usual product, and the remainder did not indicate a preference (Ernster et al., 1990).

At oral examination, 350 of the players (21 percent) had mucosal lesions, usually consisting of an ill-defined white area on the upper or lower labial, alveolar, or buccal mucosa; 94 percent of the lesions were in mucosal sites adjacent to the lower jaw. There were 138 biopsy specimens from 129 current ST users that were satisfactory for morphological evaluation.

The average age of the players who had biopsies was 25 yr. By self-report, 126 of the players who had biopsies stated their usual brand of ST as follows: 6 (5 percent) used chewing tobacco; 90 (71 percent) used Copenhagen brand snuff; 27 (21 percent) used other brands of snuff; and 3 (2 percent) used a combination of ST products. The average duration of ST use by the players whose lesions were biopsied was 7.3 yr. Other details about these players and methods of histopathologic examination and grading are described elsewhere (Daniels et al., in press).

We selected 17 pairs of lesion and autologous control specimens for LC studies. LC were identified by the use of monoclonal antibodies against the T6 antigen, usually considered to be intrinsically expressed by LC, and against three class II *HLA-D* gene products: *HLA-DR*, *HLA-DP*, and *HLA-DQ*. We expressed mean LC counts as number of LC per millimeter of epithelial surface (LC/mm) and as number of LC per square millimeter of epithelial cross-section (LC/mm^2). The calculation in terms of sectional area of epithelium compensates for the difference in thickness between some of the lesion specimens and their autologous controls. We expressed the numbers of LC in the paired lesion and control specimens from each individual as a percentage reduction of LC in the lesion specimen compared with its control. The selection of cases and immunohistochemical methods are described elsewhere (Daniels et al., 1989 and 1992).

EPITHELIAL CHANGES Each specimen had a predominant pattern of epithelial change:

- Hyperparakeratosis (HPK), characterized by increased epithelial thickness and keratinization, with nuclei remaining in most of the surface epithelial cells;
- Hyperorthokeratosis (HOK), characterized by increased keratinization, the presence of a granular cell layer, and absence of nuclei in the surface epithelial cells;
- Pale surface staining (PSS) of the epithelium, characterized by a pale staining zone of hyperkeratosis having ill-defined epithelial cell boundaries with few nuclei, no granular cell layer, and frequent vacuoles; or
- Basal cell hyperplasia (BCH), characterized by cells with a basilar pattern extending through a larger-than-usual portion of the epithelium, widened rete processes, thinning of the epithelium over the connective tissue papillae, and little or no hyperkeratosis.

Of the 132 specimens from snuff users, 56 percent had HPK, 35 percent had PSS, 5 percent had HOK, and 4 percent had BCH. The thickness of hyperkeratosis was grade 1 (slight) in 32 percent of the lesions, grade 2 (moderate) in 50 percent, and grade 3 (severe) in 18 percent. Of the six specimens from chewing tobacco users, all had HPK and the hyperkeratosis was grade 1 in five and grade 2 in the sixth. The grade of hyperkeratosis and the amount of ST use, in estimated hours per day, were highly correlated ($p=0.005$, one-way analysis of variance). No significant correlations were observed between the four patterns of epithelial change in the specimens and the age of the players, the duration of ST use, or the number of hours of ST use per day.

None of the HPK, PSS, or HOK specimens showed any signs of dysplasia. Among the five BCH specimens, we observed slight epithelial dysplasia (BCH and cellular atypia) in only one, taken from a subject who had used snuff for 3 yr. Of the remaining BCH specimens, two were from players who reported using snuff for 3 yr or less, and two were from players who used snuff for 5 yr. All five used Copenhagen brand snuff.

In addition to the changes in epithelial maturation, we observed koilocytic cells (Koss and Durfee, 1956) in 12 percent of the specimens, which resemble those seen in papillomavirus-infected epithelium of the uterine cervix (zur Hausen and Schneider, 1987). We also observed a chevron pattern of keratinization (Pindborg et al., 1980) in 6 percent of the specimens, all of the PSS type.

LC CHANGES Langerhans cells were readily identified within the epithelium by their intense staining, dendrites, and generally suprabasal location. Those identified by their *HLA-DP* and *-DQ* antigens tended to have shorter, blunter dendritic processes than those identified by their T6 and *HLA-DR* antigens. Epithelial cell expression of *HLA-DR* antigen was not observed in either lesion or control specimens.

The number of LC expressing each of the four antigens was fairly uniform in each of the four types of pathological lesions. Comparison of the mean number of LC in all types of lesions identified by all four antigens showed that there were fewer LC in the lesion specimens (means of 6 LC/mm and 10 LC/mm^2) than in the control specimens (means of 14 LC/mm and 30 LC/mm^2).

The 17 pairs of lesion and autologous control specimens showed an average reduction in LC per millimeter of 58 percent (range, 3 to 95 percent). That each pair showed a difference in the same direction is highly significant ($p < 0.0001$ by the sign test) (Conover, 1980). In all but one pair of specimens, there were fewer LC per square millimeter in each lesion specimen than in its corresponding control, by an average of 60 percent. Control specimens averaged 0.4 mm in thickness. The lesion specimens with PSS were the thickest at 0.7 mm, and those with HPK were the next thickest at 0.6 mm. Both of those types showed slightly greater reductions in LC per square millimeter than in LC per millimeter, as a result of differences in epithelial thickness between lesion and control specimens. However, similar reductions in LC were seen in HOK specimens, which were not thicker than their autologous controls, and in BCH specimens, which do not have hyperkeratosis.

DISCUSSION

Epithelial Changes

Our findings suggest that the epithelial response in snuff users is different from that in chewing tobacco users. Snuff was associated with a greater variety and severity of epithelial change than chewing tobacco. Snuff has been reported to be more likely than chewing tobacco to cause oral lesions and to cause more severe lesions (Grady et al., 1990), possibly because of differences in pH, chemical composition, or moisture content (Hoffmann et al., 1977). However, caution should be used in interpreting our findings about possible differences between snuff and chewing tobacco, because there were only six chewing tobacco users among the subjects who had biopsies.

Chewing tobacco users have a lower rate of mucosal lesions than snuff users, and a smaller percentage of players use chewing tobacco than use snuff (Ernster et al., 1990). Previous histopathologic studies of ST lesions in Europe and North America have mainly examined snuff-associated lesions. Two studies (Greer et al., 1986; Kaugars et al., 1989) included snuff-associated and chewing tobacco-associated lesions but did not analyze the findings separately (Table 1).

Some of these previous histopathologic studies showed the prevalence of epithelial dysplasia to range from 2 to 18 percent (Andersson et al., 1989; Greer et al., 1986; Hirsch et al., 1982; Jungell and Malmström, 1985; Roed-Petersen and Pindborg, 1973; Smith et al., 1970), whereas other studies did not note dysplasia (Axéll et al., 1976; Pindborg et al., 1980; Pindborg and Renstrup, 1963), and one included only specimens having dysplasia (Kaugars et al., 1989). Several authors have described ST-associated oral carcinomas (Peacock et al., 1960; Roed-Petersen and Pindborg, 1973; Smith et al., 1970; Sundström et al., 1982; Winn et al., 1981) (Table 1). The prevalence of dysplasia in our study was < 1 percent. It is not known,

Table 1
Data summary: histopathologic studies of snuff-associated oral lesions

Authors	Year	Country	n	Mean Age	Epithelial Chevrons	Epithelial Dysplasia	Carcinoma
Pindborg and Renstrup	1963	Denmark	12	60		0	0
Smith et al.	1970	United States	657	62		12 (2%)	2 (< 1%)
Roed-Petersen and Pindborg	1973	Denmark	31	58	yes	1 (3%)	1 (3%)
Axéll et al.	1976	Sweden	114	50	yes	0	0
Pindborg et al.	1980	Denmark	12	59	12 (100%)	0	0
Sundström et al.	1982	Sweden	23	76		0	23 (100%)
Hirsch et al.	1982	Sweden	50	41		9 (18%)	0
Jungell and Malmström	1985	Finland	21	19	1 (5%)	1 (5%)	0
Greer et al.[a]	1986	United States	45	41	42 (93%)	1 (2%)	0
Kaugars et al.[a]	1989	United States	108	56		108 (100%)	0
Andersson et al.	1989	Sweden	252	36	yes	"few"	0
Daniels et al.[a]	1991	United States	129	25	8 (6%)	1 (< 1%)	0

[a] *Includes chewing tobacco use.*

however, whether similar criteria were used for the diagnosis of dysplasia in the other studies. Differences in rates of dysplasia might also be attributable to differences in the populations studied, types of ST used, duration and amount of ST use, placement site of ST, and variations in concurrent smoking and alcohol use.

Although the establishment of consistent and reproducible morphological criteria for epithelial dysplasia is not yet possible, evidence of disturbed epithelial maturation and the presence of atypical cells are considered risks for malignant transformation (WHO Collaborating Centre for Oral Precancerous Lesions, 1978). BCH, as observed alone in four of the five BCH specimens in this study, does not by itself define dysplasia but is a commonly observed feature in dysplastic epithelium. All of the BCH specimens in this study were associated with a single brand of snuff, but the significance of this observation is not yet established.

We have demonstrated a variety of morphological changes in the epithelium that contacts ST. Although the lesions from this group of generally young men were all benign and only one exhibited signs of dysplasia, that specimen was from a player who had been using snuff for only 3 yr. Our data indicate that of the various forms of ST, snuff is more frequently associated with development of oral mucosal lesions and with a greater variety and severity of epithelial changes than chewing tobacco.

Langerhans Cells Our present data are consistent with previous observations that mucosal or cutaneous lesions that are or may develop into carcinoma are associated with a significant reduction in T6+ (CD1+) Langerhans cells (Barton et al., 1989; Meissner et al., 1986; Pitigala-Arachchi et al., 1989; Smolle et al., 1986; Viac et al., 1990). In vivo chemical carcinogenesis experiments have suggested that loss of LC during tumor promotion may impair immunologic protection against skin tumors (Halliday et al., 1988).

We have shown that the number of LC per millimeter in ST-associated oral lesions is on average 58 percent less than the number in autologous control tissue. This reduction in the number of LC in lesional tissue is not accounted for by differences in epithelial thickness, because the number of LC is similarly reduced when measured per square millimeter. Nonkeratinized areas of the oral mucosa generally have more LC than keratinized areas have (Cruchley et al., 1989; Daniels, 1984), but no study has investigated changes in the number of LC at sites undergoing increasing keratinization.

Epidemiological studies have shown that cigarette smoking is associated with an increased risk for cancer of the uterine cervix (Winkelstein, 1990). A recent study (Barton et al., 1988) observed a dose-response relationship between the number of cigarettes smoked daily and reduction in LC counts in the cervical epithelium. The counts were reduced in both clinically normal and lesional epithelium in the cervix. The amounts of reduction noted in the above studies were similar to those noted in our study.

The pathogenesis of oral mucosal carcinoma is unknown, but viruses and immunologic changes may be cofactors. There is clear evidence of participation by a few types of human papillomavirus (HPV) in the

development of most cervical carcinomas (zur Hausen and Schneider, 1987), but a consistent relationship has not been established for oral carcinoma (Scully, 1988). Koilocytic epithelial cells (Koss and Durfee, 1956) are associated with HPV infection (zur Hausen and Schneider, 1987), and those cells have been observed in some of the specimens from previous studies of ST-associated lesions (Andersson et al., 1989; Daniels et al., in press; Greer et al., 1986; Hirsch et al., 1982; Pindborg and Renstrup, 1963) but without examination for HPV. Viral DNA from diverse types of HPV has been identified by in situ hybridization methods in only small proportions of ST lesions, oral mucosal dysplasias and carcinomas (Greer et al., 1990), and by polymerase chain reaction methods in less than half of oral carcinomas (Palefsky et al., 1991). LC are intraepithelial antigen-processing cells that are crucial in epithelial immunity, and their number is greatly reduced at the site of contact with ST (Daniels et al., 1989 and 1992). This localized reduction or loss of immunologic function may contribute to colonization of the mucosal sites by opportunistic viruses as noted in oral hairy leukoplakia (Daniels et al., 1987).

ST reduces the number of LC at the site of its contact with the mucosa. This illustrates one form of host response to the constituents of that complex material. More knowledge is needed about the constituents of various types of ST that are responsible for morphological epithelial changes, the immunologic sequelae of ST-induced LC reduction, and the presence of cofactors that may lead to the development of malignant neoplasia.

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The Use of Smokeless Tobacco In Sweden

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ABSTRACT Twenty-five years of observation of increasing consumption of moist snuff in Sweden have undoubtedly offered some experience. Use of moist snuff is inevitably associated with a more or less severe mucosal lesion, with a typically wrinkled appearance at the site of placement of the tobacco quid. The rate of aggravation and the risk for malignant change are conclusions not readily deduced from the information presently available. For a better understanding of such complex problems, prospective studies are warranted.

INTRODUCTION Smokeless tobacco in the form of moist snuff seems to be a variety of tobacco that by tradition is related to Sweden. From a historical point of view, the use of smokeless tobacco or snuff has been known in Sweden since the early 1600's. Snuff seems to have been appreciated since, in a report dated 1638, it is stated that "there is nobody neither man nor woman who does not snuff or drink tobacco" (P. Brake, report to Axel Oxenstierna, Royal Library, Stockholm). At that time, however, snuffing tobacco probably meant to inhale it into one's nose, as was the custom elsewhere in Europe. Later on, from the early 1800's and onward, moist snuff for intraoral use became a specifically Swedish fashion.

At first, the use of moist oral snuff had social implications: whereas nasal snuff was used among the upper class and the bourgeoisie, oral moist snuff was the habit of the simple people. Later, however, use of the former variety declined, while the latter increased and became a widespread habit, remaining so into the 1920's when it was gradually replaced by cigarette smoking. In some parts of the country, snuff dipping remained a frequent habit. In the mid-1960's there was a revival of the habit, starting among young people, especially high school students and team athletes. It is not well known why this old habit reemerged, but it may be speculated that it replaced cigarette smoking among those who were either theoretically aware of the risks of smoking (students) or practically aware that smoking actually interfered with their physical performance (sportsmen).

EPIDEMIOLOGICAL ASPECTS Since 1965 there has been a steady increase in the use of moist snuff in Sweden. It is further evident that the increase has been concentrated among young men. According to a study among Stockholm schoolchildren in 1970, 4 percent of 12- and 13-yr-olds, 10 percent of 15- and 16-yr-olds, and 35 percent of students aged 16 to 18 dipped snuff (Bergström et al., 1975); all of the snuff dippers were male. Furthermore, 80 percent of the students exhibited typical lesions of the mucosa—sometimes including gingival recession at the site of placement.

According to the Swedish National Smoking and Health Association, 20 to 25 percent of Swedish men used snuff daily or occasionally during the period 1985 to 1988 (Ramström, 1985; Ramström and Tibblin, 1986, 1987, and 1988). The most recent report, based on 1988 data, shows that 35 percent of males in the 16 to 24 age group are regular or occasional users

(Table 1). The frequency of snuff use decreases with increasing age. Still, the habit is nearly totally limited to males, with a predominance among the lower educational stratum of the population (Table 2). As observed by Nordgren and Ramström (1990), the trend in Sweden over the past two-and-a-half decades clearly indicates an emerging pattern of oral snuff use among young Swedish males.

According to reports over the past 5 yr, snuff dipping among young men is usually not associated with regular smoking. Only 13 percent are regular consumers of both smoked and smokeless tobacco. Interestingly, it appears that snuff dipping has, to some extent, replaced smoking: 37 percent of the 18- to 34-yr-old and 61 percent of the 35- to 70-yr-old dippers claim to be former smokers. Ramström (1990) found that, among smokers who turn to snuff dipping, nearly twice as many quit smoking as compared to "pure smokers." Thus there seems to be some evidence that snuff dipping in Sweden may be a result of shifting tobacco habits, serving as a substitute for smoking and thus as a means of smoking cessation. The number of young dippers who later turn to smoking seems to be relatively small. Some 15 percent of current smokers report that they started their tobacco use as snuff dippers. Among older smokers, the percentage of those starting as snuff dippers is even smaller (4 percent).

EFFECTS ON ORAL HEALTH

The most common adverse oral effect associated with the use of moist snuff is a lesion at the site of the quid placement, which has been described by several investigators (Andersson and Axéll, 1989; Axéll et al., 1976; Frithiof et al., 1983; Mörnstad and Axéll, 1989). The clinical severity of the lesion is usually scored on a four-point scale based mainly on the wrinkling pattern of the mucosa. There is some evidence that the severity of lesions associated with moist snuff increases with age, with duration of use, with exposure, and to some extent with brand (some brands seem to be more hazardous than others) (Axéll et al., 1976; Hirsch et al., 1982). A variety of snuff available on the Swedish market is the portion-packed snuff in paper bags. The bag allows diffusion of the active components, without being dissolved itself. Although presently only 10 percent of dippers, at most, regularly use portion-packed snuff, and they may not be fully comparable to dippers who use ordinary loose snuff, it appears that use of the portion packs may be less harmful (Andersson and Axéll, 1989; Andersson et al., 1989).

Another clinical feature associated with Swedish use of oral snuff is localized gingival recession at the buccal aspect of teeth adjacent to the place of the quid—usually the lateral incisor. Although repeatedly observed, gingival recession has been the object of little study. This condition should be further studied because it is an irreversible sign and probably reflects the accumulated impact on the mucosa. According to Andersson and Axéll (1989) there seems to be a positive correlation between the severity of the lesion and the magnitude of the gingival recession. It might be speculated that gingival recession is an indirect manifestation secondary to contractile actions within the mucosal lesion, rather than a direct reaction of the marginal gingiva. This needs to be substantiated, however.

Table 1
Snuff habits among Swedish men (1988), by age group

	Percentage at Each Level of Use			
	Age 16 to 24 (n=213)	Age 25 to 34 (n=196)	Age 35 to 54 (n=433)	Age 55 to 74 (n=312)
Daily User	29%	20%	13%	14%
Occasional User	6	8	5	1
Non-User	65	72	82	83

Source: Ramström and Tibblin, 1988.

Table 2
Snuff habits among Swedish men (1988), by age and education

	Percentage at Each Level of Use					
	Age 18 to 29		Age 30 to 49		Age 50 to 70	
	Low Education[a] (n=69)	High Education[b] (n=101)	Low Education[a] (n=133)	High Education[b] (n=151)	Low Education[a] (n=260)	High Education[b] (n=63)
Daily User	37%	18%	18%	10%	21%	0%
Occasional User	10	1	6	6	2	0
Non-User	54	81	77	84	77	100

[a] *Low = 9 yr schooling.*
[b] *High = ≥12 yr schooling.*
Source: Ramström and Tibblin, 1988.

The histopathological pattern associated with snuff-induced lesions has been investigated by Axéll et al. (1976) and Hirsch et al. (1982). They found a high incidence of slight dysplasia, but the histomorphologic pattern was not predictable from the clinical state. The occurrence of oral cancer associated with snuff dipping in Swedish populations has been addressed by Axéll et al. (1978) and Sundström et al. (1982), who report a five- or sixfold increase in the risk of developing cancer at the site of snuff placement. Prospective, systematic studies are needed to yield more information about the development and aggravation of snuff-induced lesions and their potential transformation into dysplastic precancerous or cancerous manifestations.

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Periodontal Effects Associated With The Use of Smokeless Tobacco: Results After 1 Year[1]

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ABSTRACT This report of the oral consequences of smokeless tobacco use among 280 professional baseball players examined in both 1988 and 1989 compares findings in 133 non-users with no mucosal changes, 63 users without ST-induced mucosal lesions, and 84 users with mucosal lesions. Subjects completed questionnaires on smokeless tobacco use, rinsed their mouths under supervision, and were cautioned not to discuss their use of tobacco with the dental examiners. They then received an oral examination that included recording of all mucosal abnormalities, missing teeth, decayed and filled surfaces, extrinsic stain, plaque index, gingival index, pocket depth, attachment loss, and gingival recession. In addition, loss of cervical tooth structure was assessed at the 1989 examinations. In both years, about 92 percent of mucosal lesions in users affected the mandibular teeth at sites where the ST quid was placed. These sites exhibited a significantly greater frequency of recession than did the sites not adjacent to lesions in users or similar sites in non-users. By 1989, 36 percent of sites adjacent to lesions in users showed at least 1 mm of recession and 25 percent showed at least 2 mm of recession, compared with 12 and 3 percent, respectively, in non-users. Moreover, an apical shift in the position of the gingival margin of users in sites adjacent to lesions averaged -0.36 mm during the 1-yr period, whereas sites of non-users and users without lesions were unchanged. Loss of tooth structure in areas of recession appeared to result from mechanical abrasion rather than chemical erosion from products of smokeless tobacco, because exposed root surfaces of non-users were affected by cervical depressions as often as sites adjacent to lesions. Compared with sites of non-users, extrinsic stain was more frequent in sites adjacent to lesions in users, but more than 80 percent of these sites did not show stain. Missing teeth, previous caries experience, levels of plaque and gingivitis, pocket depths, and occurrence of severe forms of periodontitis were not related to ST use.

INTRODUCTION We previously described the oral consequences of smokeless tobacco use among baseball players examined during the 1988 spring training season in Arizona (Ernster et al., 1990; Grady et al., 1990; Robertson et al., 1990). More than 50 percent of these team members reported using ST, and 39 percent were using ST during the week prior to the examinations. Among these current-week users, 46 percent had oral mucosal lesions, primarily in mandibular sites where the smokeless tobacco quid was placed. Poor oral hygiene and gingivitis were not related to the development of oral lesions. All periodontal measurements on mesial surfaces were similar, and severe forms of periodontal disease were equally rare in non-users, users without lesions, and users with lesions. However, buccal sites adjacent to mucosal lesions in ST users showed significantly greater recession and related attachment loss than in sites not adjacent to lesions in users or comparable sites in non-users.

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The study reported here compared changes in oral and periodontal measurements among smokeless tobacco non-users, ST users without mucosal lesions, and ST users with mucosal lesions, who were examined in both 1988 and 1989.

MATERIALS AND METHODS

The subjects were 280 Major League and minor league baseball team members who were examined during both the 1988 and 1989 spring training seasons in Arizona. The study protocol has been described in detail (Ernster et al., 1990; Robertson et al., 1990). Briefly, each of the subjects completed a comprehensive questionnaire that included a basic demographic profile and dental history and elicited information on the use of smokeless tobacco or other tobacco products. The 1988 examinations included cotinine and thiocyanate determinations to confirm self-reported tobacco use and showed that the self-report was highly accurate in defining use status. Before entering the oral examination area, the subjects were asked not to discuss their use of tobacco with the oral examiners, and all subjects rinsed their mouths repeatedly with water to ensure that no traces of ST remained in the mouth. Mucosal and gingival pathology were recorded, including a complete description of oral lesions. This analysis considers measurements obtained on the buccal surfaces of a subset of 12 teeth: maxillary and mandibular first molars (tooth numbers 3, 14, 19, 30), maxillary and mandibular first premolars (tooth numbers 5, 12, 21, 28), and maxillary and mandibular central incisors (tooth numbers 8, 9, 24, 25). Measurements in both years included the presence or absence of caries, restorations, and extrinsic stain. The plaque index (PI) (Silness and Löe, 1964), gingival index (GI) (Löe and Silness, 1963), pocket depth measured from the gingival margin, and position of the gingival margin measured from the cementoenamel junction were recorded. All probing measurements were recorded to the nearest millimeter. Attachment levels were derived from the pocket depth and gingival margin measurements. Recession was defined as displacement of the gingival margin at least 1 mm and at least 2 mm apical to the cementoenamel junction. In addition, the presence of cervical erosion or abrasion was recorded in 1989 because loss of tooth structure was frequently observed in association with areas of recession during the 1988 examinations. We recorded all buccal sites that showed a loss of tooth structure resulting in smooth, hard depressions adjacent to the cementoenamel junction. No distinction was made between loss of tooth substance by a chemical process (erosion) or by excessive mechanical wear (abrasion).

Prior to both spring seasons, the examiners participated in an extensive training and calibration exercise that included patients with stain, cervical caries, and moderate adult periodontitis with some areas of recession. Differences between examiners were discussed and resolved and the examination process was repeated until at least 90 percent agreement between any two examiners was achieved for all measurements. A final calibration session was conducted with 12 patients in 1988 and 10 patients in 1989. Average percentage of agreement between all examiners for buccal surfaces was essentially equal in both years and ranged from 98 percent (cervical caries) to 93 percent (PI). Average kappa statistics (Fleiss and Chilton, 1983) for any measurement were never lower than 0.78.

Measurements were expressed as the percentage of total sites that showed caries, stain, cervical depressions, visible plaque (PI > 1), and gingival bleeding (GI > 1), and the mean and standard deviation for age, missing teeth, percentage of decayed and filled teeth, pocket depth, attachment loss, and position of the gingival margin. Where players were used as the unit of analysis (non-users, users without lesions, and users with lesions), differences between categories were evaluated by χ^2, and differences between mean scores were evaluated by Wilcoxon tests.

To assess the effects of ST on the tooth and adjacent tissue where the tobacco quid was held, the data were also analyzed by tooth site in four categories: sites in the mouths of subjects who were not using ST and did not have any oral mucosal lesions in either 1988 or 1989 (non-user, no lesion); sites in the mouths of subjects who used ST in 1988 and 1989 but did not have any oral mucosal lesions in 1989 (user, no lesion); sites in the mouths of subjects who used ST in 1988 and 1989 and had a mucosal lesion in 1989 not adjacent to the site (user, nonadjacent lesion); and sites in the mouths of subjects who used ST in 1988 and 1989 and had a lesion on the mucosa in 1989 that was immediately adjacent to the site (user, adjacent lesion). The analyses of differences between sites were evaluated by χ^2, which was corrected for the interclass correlation that resulted from use of multiple tooth sites in the same player (Donner and Donald, 1988).

RESULTS Of 280 team members who were seen in 1988 and 1989, 133 subjects were non-users of ST and showed no mucosal lesions, 63 subjects were users but did not show a mucosal lesion in 1989, and 84 subjects were users and did show a mucosal lesion in 1989. Of the 63 users without lesions in 1989, 46 had also been without a lesion in 1988, and 54 of the 84 users with lesions in 1989 also had shown lesions in 1988. For 1989, the age, race, average number of missing teeth, and percentage of all buccal surfaces that were decayed or filled are shown in Table 1. There were no differences in age among the three subject groups. A higher proportion of users than non-users—with and without lesions—were white. Values for missing teeth and previous caries experience were very low and reflected the excellent general dental health and regular dental care exhibited by these team members. About 65 percent of all subjects showed no missing teeth, and most of the tooth loss in remaining subjects was associated with premolar extraction prior to orthodontic therapy.

A total of 104 oral lesions were identified in the 84 ST users with lesions in 1989, 67 of whom had 1 lesion, and 17 of whom had 2 or more separate lesions. These lesions were characterized by white, slightly raised, and irregularly corrugated changes in the mucobuccal fold, typical of ST use (Grady et al., 1990; Greer and Poulson, 1983; Greer et al., 1986; Pindborg and Renstrup, 1963). Although most lesions terminated coronally at the mucogingival junction, the color of the adjacent gingiva was usually more pale than surrounding areas. On average, each lesion involved 4.6 ±1.9 teeth. Lesion patterns among teeth of ST users with lesions in 1988 and 1989 are shown in Table 2. The majority of lesions in both years showed a bilateral pattern involving the mandibular central incisors. The second most common site for lesions was in the mandibular left quadrant, followed

Table 1
Age, race, missing teeth, and decayed and filled teeth in 1989 among team members examined in 1988 and 1989

	n	Age[a]	Race			Missing Teeth[a]	Decayed and Filled Teeth[a]
			White	Black	Latino		
Non-Users	133	25.7 ± 5.8	57%	26%	16%	3.0 ± 3.5	2.4 ± 3.8%
Users Without Lesions	63	25.5 ± 3.9	83	8	10	2.8 ± 3.6	2.9 ± 3.9
Users With Lesions	84	25.3 ± 5.5	82	14	4	3.0 ± 3.8	3.6 ± 4.6

[a] *Mean ± SD.*

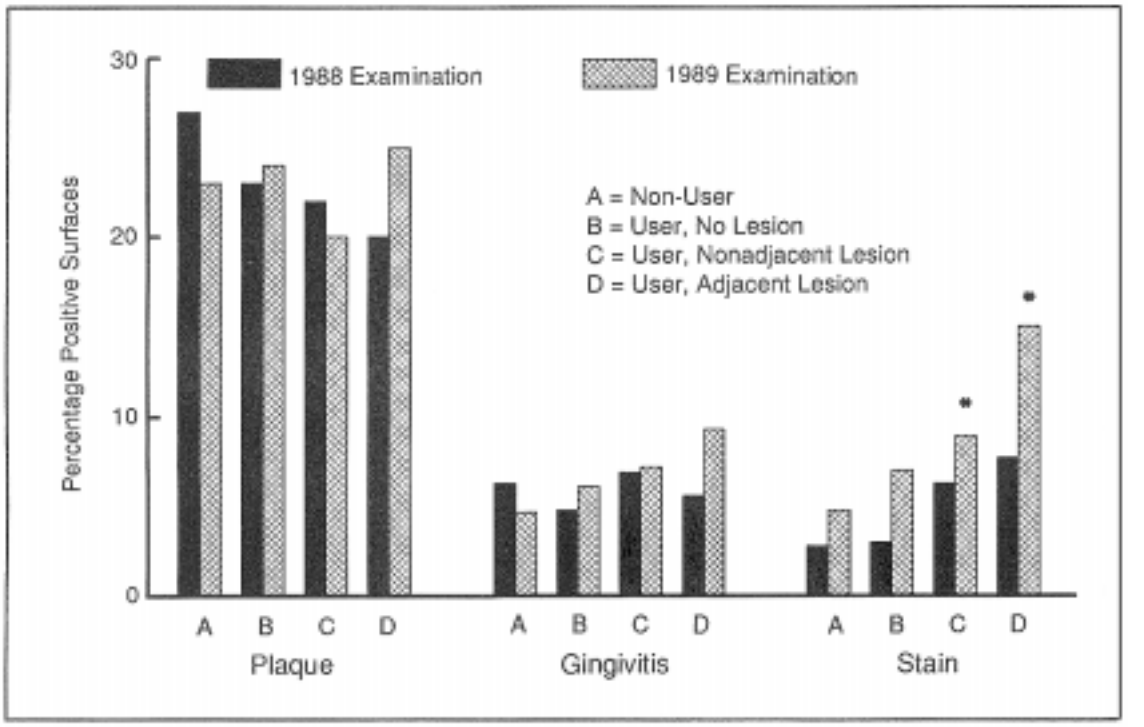
Table 2
Lesion patterns among teeth of smokeless tobacco users with lesions in 1988 and 1989

	1988	1989
Bilateral Mandibular Anterior	46%	45%
Bilateral Mandibular Posterior	2	9
Unilateral Mandibular Right	16	18
Unilateral Mandibular Left	32	20
Maxillary	4	8

by the mandibular right quadrant. The maxilla was not a preferred site to hold the tobacco quid, and maxillary lesions were infrequent. Despite comments from users that they tried to move the quid around the mouth in anticipation of the 1989 examinations, the general patterns of lesions in both years remained fairly constant, although in 1989 there was a slight shift of lesions to the maxilla.

The remaining dental and periodontal results are given only for the mandibular teeth where more than 90 percent of mucosal lesions were located. The percentage of mandibular surfaces that showed visible plaque, gingival bleeding, and extrinsic stain for each site group in 1988 and 1989 is shown in Figure 1. About 20 to 28 percent of all surfaces showed visible plaque, and there were no large or statistically significant differences between the groups in either year, nor were there significant changes from 1988 to 1989 in any group. A similar lack of significant differences was observed in gingival bleeding, which occurred in < 10 percent of

Figure 1
The percentage of mandibular buccal surfaces in 1988 and 1989 that showed visible plaque (PI > 1), gingival bleeding (GI > 1), and extrinsic stain for sites in non-users with no ST-induced mucosal lesions in 1989 (A), sites in users with no lesion (B), sites in users with a lesion elsewhere but not adjacent to the site (C), and sites in users with a lesion immediately adjacent to the site (D).



** Significant difference from non-user with no lesion sites.*

surfaces in all groups. Severe forms of gingivitis were observed infrequently and were equally distributed among subject groups. However, sites in users with lesions had a significantly greater frequency of extrinsic stain than sites in non-users at both the 1988 (p=0.04) and 1989 (p=0.03) examinations. Extrinsic stain was seen most frequently in sites with an adjacent lesion, and the number of these sites that exhibited stain increased significantly (p=0.01) from the 1988 to 1989 examination.

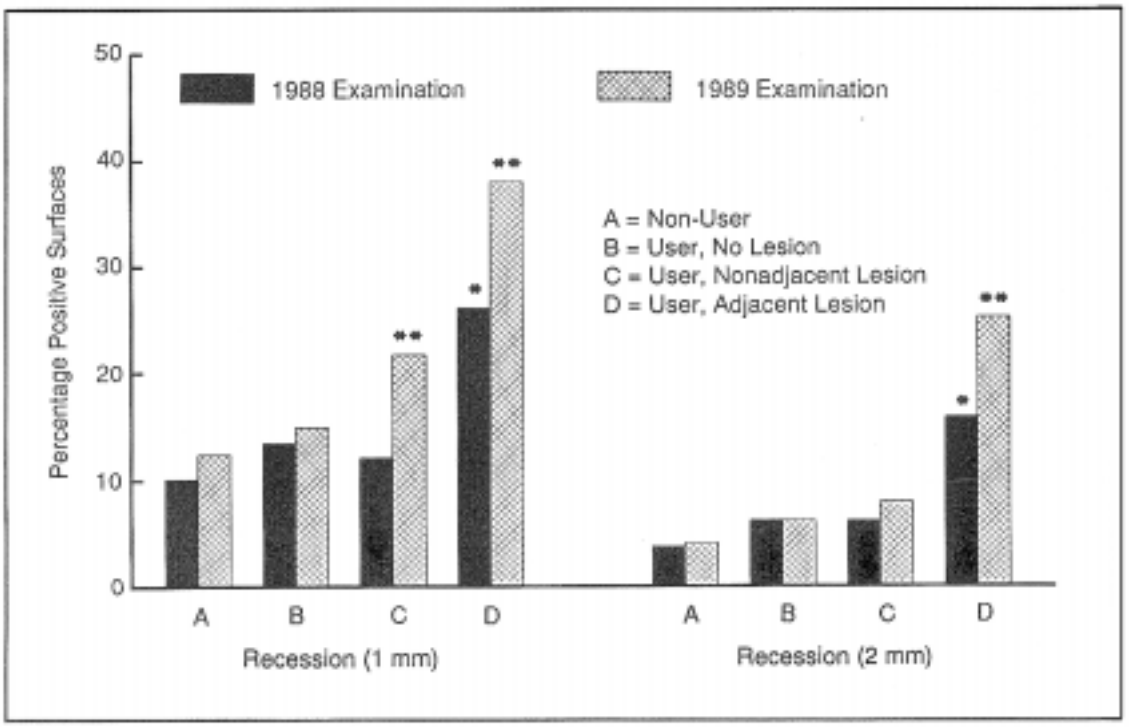
Because probing measurements were made to the nearest millimeter, we were concerned that, in cases where the gingival margin was positioned near the cementoenamel junction, very slight apical or coronal movement might distort the change in number of sites defined as showing recession. Thus, in Figure 2 we have expressed recession as the percentage of all mandibular surfaces with at least 1 mm, and at least 2 mm of displacement of the gingival margin apical to the cementoenamel junction for each site group at both examinations. Using the 1-mm threshold, we observed a significant increase (p=0.04) relative to non-users in the percentage of sites with recession in sites of users with nonadjacent lesions in 1989 and sites of users with

an adjacent lesion in 1988 and 1989 (p=0.02). In addition, nonadjacent and adjacent sites showed significant increases (p=0.01) in the frequency of recession from 1988 to 1989. Using the 2-mm threshold, we found sites adjacent to lesions at both examinations showed a significant increase (p=0.03) in the frequency of recession compared with non-users. The frequency of recession in these sites also increased significantly (p=0.01) from 1988 to 1989. In general, incisor and premolar teeth showed more recession than molar teeth in all site categories. However, patterns of recession for incisor, premolar, and molar teeth were similar to those shown in Figure 2, at both the 1-mm and 2-mm thresholds. All tooth types adjacent to ST-induced lesions showed a significantly greater frequency of recession than did the same tooth types in non-users, users without lesions, and users with a lesion not adjacent to the tooth.

The average position of the gingival margin relative to the cemento-enamel junction in 1988 was 0.5±0.8 mm for sites of non-users, 0.4±0.8 mm for sites of users without lesions, 0.4±0.9 mm for sites not adjacent to lesions in users, and 0.0±0.8 mm for sites adjacent to lesions in users. In 1989, no changes were observed in the average position of the gingival margin in the same sites of non-users or users without lesions. Sites in users with lesions that were not adjacent to the lesion showed a slight apical displacement (-0.11±0.5 mm) during the 1-yr period. However, a significant (p=0.02) apical shift in the gingival margin, which averaged -0.36±0.6 mm during the 1-yr period, was observed in sites adjacent to lesions in users. By 1989, 36 percent of sites adjacent to lesions in users showed at least 1 mm of recession and 25 percent showed 2 mm of recession compared with 12 and 3 percent, respectively, of sites with recession in non-users. About 98 percent of sites in non-users and all sites in users with 2 mm of recession in 1988 also showed recession in 1989.

Loss of cervical tooth structure recorded at the 1989 examination occurred primarily on the root surface in areas of recession. The majority showed hard, smooth, and often highly polished depressions in the root surface that were clinically consistent with mechanical abrasion. The percentage of all mandibular buccal surfaces with loss of cervical tooth substance and the percentage of mandibular buccal surfaces with recession that also showed loss of tooth substance is given in Figure 3. Cervical depressions were more frequent in sites of users with an adjacent lesion than all other sites, although differences were not statistically significant. The finding was consistent with the higher frequency of recession in sites adjacent to lesions. However, sites with recession that also showed loss of cervical tooth substance occurred at least as often in non-users as in users with and without lesions, and there appeared to be no relationship between ST use and cervical depressions in sites with existing recession. Because these cervical depressions were common on exposed root surfaces of all subjects and were seen as often in sites that were not adjacent to ST-induced mucosal lesions as in lesion-adjacent sites, we have used the term *abrasion* in Figure 3 to suggest that this loss of tooth substance is not related to chemical decalcification from products of smokeless tobacco.

Figure 2
The percentage of mandibular buccal surfaces in 1988 and 1989 that showed recession of 1 mm or greater and recession of 2 mm or greater for the categories of tooth sites described in Figure 1.

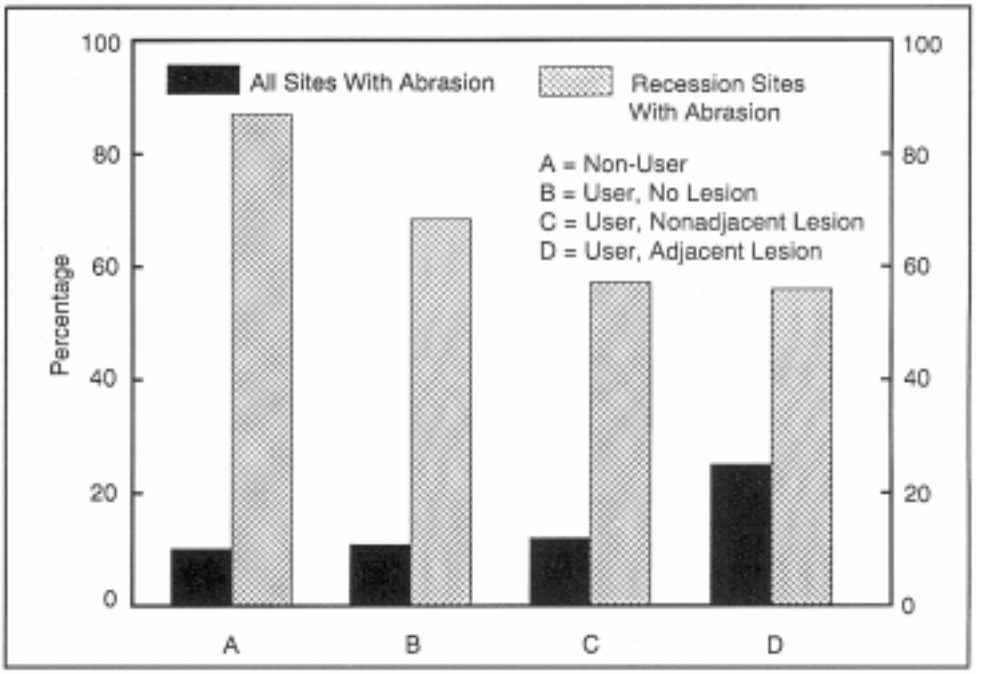


** Significant difference from non-user with no lesion sites.*
*** Significant difference from non-user with no lesion sites as well as from 1988 to 1989.*

No significant differences in pocket depth were found among the four groups of sites, and the average pocket depth for all subjects was 1.7±0.6 mm. Fewer than 1 percent of all buccal sites showed pocket depths > 6 mm, which were distributed proportionally among sites in non-users, and in users with and without lesions. About 95 percent of attachment loss observed in the subjects was a function of recession, and no severe forms of early onset or rapidly progressive periodontal disease were observed in any of the team members.

DISCUSSION The primary oral consequences of using ST for at least 1 yr are increased risk of mucosal lesions among mandibular teeth where the quid is most often placed and increased frequency of gingival recession affecting buccal tooth surfaces adjacent to ST-induced lesions. Compared with sites of non-users, extrinsic stain was also more frequent in ST users, adjacent to lesions, but more than 80 percent of these sites did not show stain. Missing teeth, previous caries experience, levels of plaque and gingivitis, pocket depths, and occurrence of severe forms of periodontitis were not related to the use of smokeless tobacco.

Figure 3
Percentage of mandibular buccal sites that showed abrasion in 1989 and the percentage of mandibular buccal sites with recession ≥ 1 mm that also showed abrasion in 1989 for the tooth sites in Figure 1.



Recession associated with ST use is permanent and progressive, and it affects about one-quarter to one-third of sites with adjacent mucosal lesions. In the 1-yr period, few new areas of recession were observed in non-users, whereas about 20 percent of sites adjacent to lesions in ST users showed recession in 1989 but not 1988. The recession presumably results from localized damage to gingival tissue by products of the ST quid, particularly in areas with a thin or absent alveolar housing (Löst, 1984; O'Leary et al., 1971; Robertson et al., 1990). In addition to esthetic problems, these areas of recession may develop thermal sensitivity and root caries (Gorman, 1967).

Exposed root surfaces are also at risk for abrasion or erosion. Abrasion is the loss of tooth substance by abnormal mechanical wear, primarily as a result of toothbrushing or use of highly abrasive dentifrices. Erosion is the loss of tooth substance by chemical processes that do not involve bacterial action. We had hypothesized that products of the ST quid, held against the surfaces of the teeth for considerable periods of time, might result in loss of some tooth structure by erosion. This seems not to be the case, however, as loss of enamel was rare in all groups, and exposed root surfaces of non-users and users without lesions were affected by cervical depressions at least as often as sites adjacent to lesions. Thus, this loss of root structure appears to be caused by abrasion.

We conclude that, although ST use in young subjects with good oral hygiene and regular professional care does not result in severe forms of periodontal disease, such use is associated with a significantly increased risk for permanent and progressive recession in areas adjacent to ST-induced mucosal lesions.

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