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PAAN WITHOUT TOBACCO: AN INDEPENDENT RISK FACTOR FOR ORAL CANCER

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Oral cancer is the second most common cancer in women and the third most common in men in Pakistan. Tobacco is smoked and chewed extensively in Pakistan. Paan is a quid of piper betel leaf that contains areca nut, lime, condiment, sweeteners, and sometimes tobacco, which is also used extensively. We did this study to clarify the independent association of paan and oral cancer. Between July 1996 and March 1998, we recruited biopsy-proven, primary cases of oral squamous-cell carcinoma, from 3 tertiary teaching centers in Karachi, Pakistan, and controls pair-matched for age, gender, hospital and time of occurrence, excluding persons with a past or present history of any malignancy. There were 79 cases and 149 controls. Approximately 68% of the cases were men, 49 years old on average, the youngest being 22 years old and the eldest 80. People with oral submucous fibrosis were 19.1 times more likely to develop oral cancer than those without it, after adjusting for other risk factors. People using paan without tobacco were 9.9 times, those using paan with tobacco 8.4 times, more likely to develop oral cancer as compared with non-users, after adjustment for other covariates. This study identifies an independent effect of paan without tobacco in the causation of oral cancer. Its findings may be of significance in South Asian communities where paan is used, and among health-care providers who treat persons from South Asia. *Int. J. Cancer* 86:128–131, 2000.

Oral cancer is the second most common cancer in women and the third most common in men in Pakistan (Jafarey and Zaidi, 1987). Tobacco, chewed or smoked, is a well-established cause of oral squamous-cell carcinoma (Gupta *et al.,* 1982; Jayant and Deo, 1986; Brennan *et al.,* 1995). Alcohol, particularly in association with tobacco, increases the risk of oral cancer. Approximately 40% of men in Pakistan over the age of 15 years smoke cigarettes or bidis (locally cured tobacco rolled in a dry leaf) regularly (National Health Survey of Pakistan, 1997). Tobacco is chewed in Pakistan as paan and as naswar. Paan consists of piper betel leaf containing lime, areca nut, condiments, sweeteners and sometimes tobacco. It is chewed and held in the mouth like a quid. Naswar is a mixture of tobacco and lime (Jayant and Deo, 1986). Areca nut without tobacco is suspected of being associated with oral cancer, but epidemiological studies have not clearly demonstrated its independent effect (Dave *et al.,* 1992). A study has shown significantly more chromosomal aberrations, sister chromatid exchanges, and genomic damage among areca-nut users than among non-users, independently of tobacco use (Dave *et al.,* 1992). But areca-nut users, independently of tobacco use, are 100 times more likely to get oral submucous fibrosis, which is a pre-cancerous lesion of the mouth (Maher *et al.,* 1994). A study from South Africa showed increased risk of oral cancer from chewing areca nut, but the estimates were not adjusted for all important risk factors (van Wyk *et al.,* 1993). We did this study to clarify the independent association of paan and oral cancer.

MATERIAL AND METHODS

Between July 1996 and March 1998, we recruited biopsy-proven primary cases of oral squamous-cell carcinoma, from Aga Khan University Hospital (AKUH), Civil Hospital Karachi (CHK) and Abbassi Shaheed Hospital (ASH). All 3 hospitals are tertiary teaching centers located in Karachi, Pakistan. AKUH and CHK have attached undergraduate medical colleges. Patients attend all 3 hospitals from all over the province. Sindh is Pakistan's second largest province, with a population of approximately 30 million. Karachi is the largest city in Sindh, with a multi-ethnic population of 9.8 million according to the 1998 census.

We pair-matched controls from patients who had been admitted to the orthopedic and general surgical wards of the same hospital at the same time as the corresponding case, were of the same sex, and were aged within 5 years of the cases. We excluded as controls persons with a past or present history of any malignancy.

After obtaining verbal consent, a trained interviewer administered a structured, pre-tested, questionnaire in Urdu (the *lingua franca* of Pakistan), examined the mouth, and checked for the presence of oral submucous fibrosis (OSMF) on cases and controls. This was determined clinically by observation of blanching and by palpation for the presence of fibrous bands. The interviewer was clinically trained to check the clinical signs of the disease. In addition to socio-economic and demographic data, we also collected information on the use of cigarettes, bidis, hookah, naswar, paan, areca nut, and alcohol. We defined users of naswar, paan or areca nut as someone who had ever indulged in the habit daily for a month. Smokers were persons who had ever smoked cigarettes, bidis, hookah, cigar, or a pipe daily for at least one month. For each of the substances we asked the date of starting, current use or date of quitting, and average quantity used per day. During anal-

Definitions: Oral submucous fibrosis (OSMF), Progressive fibrosis of the oral mucosa resulting in limited mouth opening. Strongly associated with areca nut use; pre-cancerous. Paan without tobacco, Piper betel leaf containing lime, areca nut, condiments and sweeteners. It is chewed and held in the mouth like a quid. Paan with tobacco, Piper betel leaf containing lime, areca nut, condiments, sweeteners and tobacco. It is chewed and held in the mouth like a quid. Naswar, Tobacco and lime mixture that is chewed and held in the mouth like a quid. Bidi, Locally cured tobacco rolled in a dry leaf and smoked. Hookah, Hubble-bubble. A pipe for smoking tobacco in which the smoke is filtered through water before inhalation.

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ysis, we combined persons reporting only areca nut with those consuming paan without tobacco, after evaluating them separately and finding them similar.

We calculated the sample size to detect a difference in cases and controls, assuming that users of paan without tobacco were 4 times as likely to get oral cancer as those who did not chew paan, and that the prevalence of paan chewing in this population was 13%. With Epi-Info (Dean *et al.*, 1994), we used the Bonferroni method, to be able to simultaneously evaluate risk factors. We took the power to be 90% and the alpha error to be 1%. With a ratio of 2 controls to 1 case, the sample size turned out to be 72 cases and 144 controls.

The data were double-entered for verification in Epi-info. We did conditional logistic regression analysis (SAS, 1996) in which the outcome variable was presence of oral cancer. The predictor variables included education, presence of oral submucous fibrosis, ever-use of naswar, of paan with tobacco, of paan without tobacco, ever-smoking of tobacco, ever-use of alcohol. We calculated matched odds ratios in the univariate analysis and considered variables that were significant at alpha = 0.15 in this analysis for further evaluation in the multivariate analysis.

We then added potentially confounding variables to the model and observed changes in the beta coefficients of the variables in the core model. Potentially confounding variables, which made at least 10% change in the beta-coefficient estimate of one of the variables in the core model, were considered as confounders and were added in the final model. To assess collinearity between variables, 2 variables were added together into the model to see whether or not the standard errors became inflated. After obtaining the main effects model, we proceeded to check for the effect modification between the co-variates. A total of 9 interaction variables were created, to test whether OSMF and smoking have synergistic effects with other co-variates. We assessed paan with and without tobacco for evidence of a dose-response relationship, by dividing cases and controls into tertiles of paan-years. Paan-years were calculated by multiplying the average number of paan consumed per day into the average number of years of use.

RESULTS

In our matched case-control analysis, there were 79 cases and 149 controls from 3 different hospitals in Karachi, Pakistan (Table I). This gave us 99% power to detect a 4-fold difference in cases and controls with respect to exposure. Cases were 49 years old on average, the youngest being 22 years old and the eldest 80. Controls were 48 years old on average, ranging from 18 to 78 years. Men made up 68% and 63% of the subjects in the case and the control groups, respectively. Cases and controls were not different with respect to education level, and ethnicity (Table I), but did differ significantly by religion. This difference did not persist in the multivariate analysis, possibly because of few subjects in the sub-groups. The most common site of cancer was the buccal mucosa (Table II). The prevalence of bidi smoking in cases was 7/79 *vs.* 8/149 for controls ($p = 0.31$), of hookah smoking was 2/79 among cases and 1/149 among controls ($p = 0.24$), and of cigarette smoking was 31/79 in cases and 40/149 in controls ($p = 0.06$). Since there were few bidi and hookah users, we combine all smokers in one category. In the univariate analysis, cases were more likely than controls to have oral submucous fibrosis ($p < 0.001$), to report ever using alcohol ($p = 0.036$), naswar ($p = 0.011$), paan with tobacco ($p < 0.001$) and paan without tobacco ($p < 0.001$) (Table III).

Oral submucous fibrosis, naswar, paan with and without tobacco were statistically significant in the multivariate model. Ever-use of alcohol and of tobacco in any form markedly changed the coefficient estimates of all these co-variates by 13 to 25%. Thus, both variables were considered as strong confounders and included in all subsequent analyses. No clinically important interaction term was statistically significant, found to have clinical importance.

TABLE I – COMPARISON OF THE DEMOGRAPHIC AND SOCIO-ECONOMIC CHARACTERISTICS OF THE STUDY SUBJECTS, KARACHI, 1996–1998

Characteristics	Case (n = 79)		Control (n = 149)	
	Number	%	Number	%
Hospital				
AKU	32	40.5	44	29.5
Civil	38	48.1	89	59.7
Abbasi	9	11.4	16	10.7
Gender				
Male	54	68.4	94	63.1
Female	25	31.6	55	36.9
Education				
Never attended school	40	51.9	72	48.3
Primary	11	13.9	20	13.4
Middle	7	8.9	11	7.4
Secondary	10	12.7	18	12.1
Higher	10	18.8	28	18.8
Ethnicity				
Urdu speaking	40	50.6	70	47.0
Sindhi	13	16.5	28	18.8
Punjabi	4	5.1	16	10.7
Balochi	9	11.4	15	10.1
Pathan	4	5.1	9	6.0
Gujarati	7	8.9	4	2.7
Other	1	1.3	3	2.0
No response	1	1.3	4	2.7
Religion				
Islam	68	86.1	143	96.0
Christianity	1	1.3	3	2.0
Hinduism	9	11.4	2	1.3
No response	1	1.3	1	0.7
Age in years				
Mean ± sd	48.8 ± 11.6		47.6 ± 12.6	
Range	22–80		18–78	

AKU, Aga Khan University; sd, standard deviation.

TABLE II – DISTRIBUTION OF CANCER BY LOCATION

Location of cancer	Number	Percentage[1]
Buccal	56	49.1
Gingiva	18	16.1
Floor of mouth	9	8.0
Tongue	14	12.5
Palate	7	6.3
Fauces	4	3.6
Others	5	4.5

[1]Some of the cancers were present on more than one site, consequently the total number of locations exceeds the number of cases.

People with oral submucous fibrosis were 19.1 times more likely to develop oral cancer than those without OSMF after adjusting for smoking, and for use of alcohol, naswar, and paan with or without tobacco (Table IV). People using paan without tobacco were 9.9 times more likely to develop oral cancer than non-users, after adjustment for other co-variates. The risk of getting oral cancer increased with higher intake of paan with tobacco (Fig. 1) and without tobacco (Fig. 2), after adjusting for other co-variates in the model.

DISCUSSION

In this study we found an association between use of paan without tobacco and oral squamous-cell carcinoma, after adjusting for oral submucous fibrosis, smoking, use of alcohol and tobacco chewing. Paan contains areca nut, which contains arecoline and other alkaloids that are converted into *N*-nitroso compounds, and are carcinogenic (Dave *et al.*, 1992). The demonstration of a direct association of paan without tobacco and oral squamous-cell carcinoma has been elusive, mainly because most people who use paan also use tobacco, and tobacco is a well-established risk factor for oral cancer (Daftary *et al.*, 1993). We were able to observe this

TABLE III – UNIVARIATE ANALYSIS OF RISK FACTORS FOR ORAL CANCERS

Risk factors	Prevalence of risk factor in cases (%) (n = 79)	Prevalence of risk factor in controls (%) (n = 149)	Odds ratio (OR)	95% confidence interval (CI)	*p* value
Never attended school[1]	40 (51.9)	72 (48.3)	1.64	0.84, 3.21	0.14
Presence of oral submucous fibrosis	46 (58.2)	10 (7.0)[2]	23.46	7.25, 75.91	<.001
Ever smoked tobacco	38 (48.1)	48 (32.2)	1.83	0.89, 3.77	0.103
Ever used alcohol	21 (26.6)	20 (13.4)	2.70	1.07, 6.84	0.036
Ever used naswar	13 (16.5)	10 (6.7)	3.42	1.33, 8.80	0.011
Ever used paan with tobacco	41 (51.9)	15 (10.1)	12.45	4.86, 31.93	<.001
Ever used paan without tobacco	26 (32.9)	16 (10.7)	5.17	2.04, 13.11	<.001

[1]Effect of educational level as a trend variable; OR 0.7, 95% CI 0.59–0.96. $p = 0.02$.—[2]n = 143.

TABLE IV – MULTIVARIATE ANALYSIS OF RISK FACTORS FOR ORAL CANCERS[1]

Risk factors	Adjusted OR	95% CI	*p* value
Prevalence of oral submucous fibrosis	19.07	4.15, 87.66	<0.001
Ever used naswar	9.53	1.73, 52.53	0.010
Ever used paan with tobacco	8.42	2.31, 30.64	0.001
Ever used paan without tobacco	9.90	1.76, 55.62	0.009

[1]Adjusted for cigarette smoking and alcohol use.

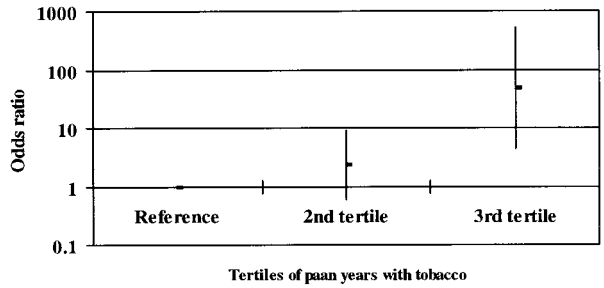

FIGURE 1 – Odds ratios and 95% confidence intervals for textiles of paan-years (with tobacco) after adjustment for smoking, oral submucous fibrosis and use of alcohol, naswar, and paan without tobacco. *p* value for trend, 0.004. Note: y axis, logarithmic scale.

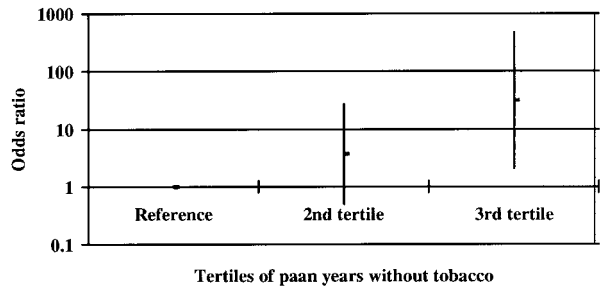

FIGURE 2 – Odds ratios and 95% confidence intervals for textiles of paan-years (without tobacco) after adjustment for smoking, oral submucous fibrosis and use of alcohol, naswar, and paan with tobacco. *p* value for trend, 0.0008. Note: y axis, logarithmic scale.

effect because there were persons in this study who used paan, but did not use tobacco. Oral submucous fibrosis is recognized as a pre-cancerous condition (Daftary *et al.*, 1993*a*) (Pillai *et al.*, 1992). One study from India reported that 40% of the cases of oral cancer also had submucous fibrosis (Pillai *et al.*, 1992). Another study reported that 7.6% of oral submucous-fibrosis cases became malignant over a 17-year period (Daftary *et al.*, 1993*b*). In our study, oral submucous fibrosis was the strongest independent risk factor for oral squamous-cell carcinoma (adjusted odds ratio, 19.1, $p < 0.001$) (Table IV). This further underscores the role of paan in carcinogenesis, since the main ingredient of paan is areca nut, and areca nut is the single strongest risk factor for oral submucous fibrosis, as shown in our study and in other studies (Maher *et al.*, 1994). But this study also demonstrates that paan without tobacco can be associated with oral cancer independent of its association with oral submucous fibrosis. Moreover, we observed higher risk of oral cancer with increasing paan years, whether it was used with or without tobacco. Even though the confidence intervals around the point estimates of the third tertiles in particular were wide, owing to small sample size, they did not include the null value, and the trend was statistically significant (Figs. 1, 2).

Smoking was not significantly associated with oral squamous-cell carcinoma in association with alcohol use, tobacco chewing, paan, and oral submucous fibrosis. It should not be construed or implied from this finding that smoking is safe. Several studies show unequivocally that cigarettes are associated with oral squamous-cell carcinoma (Choi and Kahyo, 1991; Negri *et al.*, 1993). Cigarettes contain carcinogens that not only stimulate genetic damage, resulting in the production of atypical cells, mutations and eventually cancer, they also impair the function of the *p53* gene which, when functioning normally, prevents mutations from developing into cancer (Langdon and Partridge, 1992). There are at least 3 explanations for not observing smoking as a risk factor in this study. First, it could be because among persons aged 25 to 44 years in Pakistan, approximately 40% men and less than 4% women smoke (National Health Survey of Pakistan, 1997). This was consistent with the prevalence of smoking in controls in this study, which was 50% and 2% for men and women respectively. As a result of this distribution, the sample size may not have been large enough to capture the effect of smoking.

Second, there could be more important factors than smoking, such as tobacco chewing, areca-nut use and oral submucous fibrosis, associated with the disease. In this study, the oral squamous-cell-carcinoma lesion was present on the buccal mucosa and tongue about 50% and 13% of the time respectively. The lesion on the buccal mucosa is rare in communities where chewing tobacco or areca nut is not common (Fahmy *et al.*, 1983). One study reports that carcinoma of the inside of the cheek occurs 7 times more frequently in chewers, but only 1.5 times more in smokers (Jayant and Deo, 1986). Finally, controls in our study came from hospitals, and hospital-based controls have been reported to have a higher prevalence of smoking than the general population. If this were so in our study, then the association between smoking and oral cancer may have been under-estimated.

Since few people reported that they drank, we observed an association with alcohol in the univariate analysis (Table III), but not in the multivariate model, when alcohol was evaluated with other factors. Alcohol use may be under-reported because it is a restricted substance in Pakistan.

As in any case-control study, there was a chance of recall bias: the cases may have recalled exposure better than the controls. To allow for this, we used pre-tested, structured questions and trained the interviewers. It is possible that the interviewers, who were aware of the hypothesis, probed the cases more than the controls, creating a bias away from the null.

This study identifies an independent effect of paan without tobacco in the causation of oral cancer. Its findings are of significance for South Asian communities, where paan is used and sold freely, and for health-care providers who treat persons from South Asia.

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