Lack of Correlation of Betel Nut Chewing, Tobacco Smoking, and Alcohol Consumption with Telomerase Activity and the Severity of Oral Cancer

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Background: Oral cancer is one of the most frequent cancers. A strong association has been found between oral cancer incidence and the use of betel nut, alcohol, and tobacco. Telomerase activity (TA) has also been shown to play a role in carcinogenesis. We therefore surveyed the consumption habits of betel nut chewing, alcohol drinking, and tobacco smoking in oral cancer patients and evaluated the association of these habits with TA level and clinical stage.

Methods: In total, 154 oral cancer patients were recruited. TA was measured in paired (grossly normal and cancerous) tissues using a polymerase chain reaction-based enzyme immunoassay. Associations of these factors with clinical stage and TA level were analyzed using the Pearson chi-square test.

Results: In these patients, 86.4%, 61.0%, and 83.3% used betel nut, alcohol, and tobacco, respectively. For all tissue assayed, 80.5% of cancerous and 3.2% of grossly normal mucosae were positive for TA. However, neither clinical stage nor TA level in cancerous tissues had a statistical association with the use of individual substances (betel nut, alcohol, and tobacco) or their combined use.

Conclusion: Even though the habitual uses of these substances have been previously reported to be associated with oral cancer incidence, we found a lack of correlation with TA level or with disease severity in our study. These results imply that these consumption habits might only be associated with the early stages of oral cancer development, while the later stages of cancer progression may be more associated with other external factors or dependent on host cellular factors, all of which require confirmation through further investigations.

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Key words: **oral cancer, telomerase activity, betel nut, cancer stage.**

Oral squamous cell carcinoma is one of the most frequent cancers in the world.[1] This disease occurs much more frequently in males.[1] Epidemiologic studies of oral cancer show strong associations with the consumption of betel nut, alcohol, tobacco, and cigarette smoking.[2-6] For example,

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betel nut chewers have a 10-30-times greater risk of developing oral carcinoma than non-chewers.[6] Alcohol potentiates tobacco-induced carcinogenesis and is also an independent risk factor.[4] In Taiwan, approximately 85% of oral cancer patients habitually use betel nut.[5] The incidence of oral cancer in Taiwan is one of the highest in the world, comprising 4%-5% of all malignancies.[7,8] The incidence of oral cavity cancer is approximately 20 per million in our male population.[7,8] The majority of oral cancers in Taiwan occur in the buccal mucosa (ICD 145), which is relatively less common in Western populations.[1,8] Such geographical differences in the incidences and oral cancer sites may result from exposure to different carcinogens and/or possible genetic predispositions.

The overall 5-year survival rate for patients with oral cancer is among the lowest of the major cancers and has not changed during the past 2 decades.[9] The standard treatment is surgery, radiation, or multidisciplinary therapy.[9] Although standard care is frequently initially successful in the treatment of patients with early-stage oral cancer (stage I or II), disease relapses still occur in about 35%, with a risk of local tumor recurrence or lymph node metastasis.[9,10] Apparently, clinical stages represent the severity of oral cancer and determine its prognosis.

Telomerase is a specialized reverse transcriptase that directs the synthesis of telomeric DNA onto chromosomal ends using a segment of its integral RNA component as a template.[11] In most human somatic cells, telomerase activity (TA) is undetectable, and the telomeric length progressively shortens during cell proliferation. Cell senescence is thought to occur when the telomeric length is shortened to a critical point.[11] Conversely, immortalized human cells exhibit stabilized telomeric lengths and are positive for TA.[11] Accumulating evidence indicates that TA is frequently detectable in primary tumor specimens from malignancies of diverse tissue origins, less frequently in premalignant and benign proliferative tissues, and rarely or not at all in grossly normal somatic tissues.[12] Apparently, activation of telomerase plays an important role during carcinogenesis.

Although the use of betel nut, alcohol, and tobacco smoking have been reported to be associated with oral cancer, the significance of the impact of these substances on cancer severity, used either alone or in combination, remains unclear. We designed this study to survey the consumption habits of betel nut, alcohol, and tobacco smoking in oral cancer patients, and to examine TA levels in paired grossly normal and cancerous tissues from these patients. Our aims were to look for a correlation between the use of betel nut, alcohol, and tobacco with clinical stage and levels of TA, in order to evaluate possible links between these substances and molecular changes involved in carcinogenesis and cancer progression.

METHODS

Patients, tissues, and cells

Biopsies of malignant and grossly normal mucosal tissues from 154 patients newly diagnosed with oral cancer at the Otorhinolaryngology Head and Neck Surgery Clinics at Chang Gung Memorial Hospital (Taoyuan, Taiwan) were obtained. Written informed consent to use a patient's tissue samples for molecular analysis was obtained from each patient participating in the study. A questionnaire was filled out by each patient before the first clinical visit to determine the habitual consumption patterns of betel nets (daily chewer), cigarettes (daily smoker), and alcohol (daily drinker). Clinical stages and tumor anatomic sites were classified according to the AJCC system, based on pathological staging.[13] For each patient, the grossly normal mucosal tissue sample was obtained from the opposite site of the oral cavity from the location of the cancerous tissue. The grossly normal tissue sample was stored in liquid nitrogen until being assayed for TA. A portion of the grossly diseased tissue was examined histopathologically, and the rest was stored in liquid nitrogen until being assayed for TA. During these processes, all samples were treated equivalently, and all data were carefully controlled during acquisition. An oral cancer cell line OC2[14] was used as a positive control. OC2 cells were grown at 37˚C and 5% CO2 in RPMI medium containing 10% fetal bovine serum and antibiotics (100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 μg/ml amphotericin B).

Extraction of cellular proteins

The investigators assaying TA were blinded as to the source and type of tissue being assayed. Tissue samples (~50 mg) were homogenized in 300

μl of lysis buffer (10 mM Tris-hydrogen chloride (HCl), pH 7.5, 1 mM $MgCl_2$, 1 mM ethylene glycol-bis-aminoethylether tetra-acetic acid (EGTA), 0.5% CHAPS, 10% glycerol, 5 mM mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride) in Kontes tubes with matching pestles rotating at 450 rpm. For protein extraction from OC2 control cells, cells were counted in a hemocytometer and then resuspended at 3000 cells per μl of lysis buffer. After 30 min at 4˚C, the lysate was centrifuged at 15,000 rpm for 30 min at 4˚C. The supernatants of tissue or OC2 cell extracts were transferred and prepared for the TA assay. The supernatant from OC2 cells was diluted serially to obtain 400 and 10 cells/μl. The protein concentration of each tissue sample was determined using the Coomassie protein assay reagent (Pierce).

Telomerase activity assay

TA was assayed using the polymerase chain reaction-enzyme immunoassay (PCR-EIA) method we developed previously.[15] In principle, in a properly buffered condition, telomeric repeats are synthesized in the presence of biotinylated TS (TS-B) primer, the sample, and a control cellular protein extract. Telomere products are subsequently amplified by PCR in the presence of reverse digoxigeninated CX (CX-D) primer. The PCR products are then detected using an enzyme immunoassay. Both TS-B and CX-D oligonucleotides were purchased from Genasia Scientifics, Taipei, Taiwan. Briefly, 0.3 μg of protein extract was added to a 30-μl reaction mixture containing 0.1 μg TS-B and CX-D primer, 2 units of Taq DNA polymerase, 20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM potassium chloride (KCl), 0.005% Tween-20, 1 mM EGTA, 50 μM dNTPs, and 0.1 mg/ml bovine serum albumin. The reaction mixture was incubated at 24˚C for 15 min, followed by PCR amplification of 30 cycles of 94˚C for 30s, 55˚C for 30s, and 72˚C for 1 min in a DNA thermal cycler. After PCR, 5 μl of the PCR products was dispensed into streptavidin-coated wells and incubated with 100 μl of anti-digoxigenin antibody conjugated with horseradish peroxidase at 30˚C for 60 min in an EIA reaction buffer. After washing 4 times with 200 μl of washing solution, enzyme reactions were initiated by the addition of 100 μl of a tetramethylbenzidine substrate solution (Sigma) to each well. Ten minutes later, the reactions were stopped by the addition of 100 μl of 2N HCl to each well. Colorimetric signals were determined by measuring the absorbance at 450 nm using an automatic microwell reader. A mock control was performed using all of the reaction components except the cell extract. The absorbance value of each sample was reported after subtraction of that of the mock control. Relative levels of TA in tissue samples were measured by comparing them with levels in different dilutions of the OC2 cell line (10 and 400 cells). Tissues with a TA equivalent of less than 10 OC2 cells were scored as "undetectable" or "negative", those with activity equivalent to between 10 and 400 cells as a "low" level, and those with TA greater than or equal to 400 OC2 cells were scored as a "high" level.

Statistical analysis

To examine how clinical stage and TA varied with different factors, the Pearson chi-square test was used. A chi-square test of trend was used to examine whether a linear association existed among different consumption habits (betel nut, alcohol, and tobacco smoking) of individual substances and the combined use of any of the 3. McNemar's test was used to compare TA between cancerous and grossly normal tissues. All p values reported were 2-sided; $p \leq 0.05$ was considered statistically significant.

RESULTS

Patient characteristics and the distribution of telomerase activity.

Patient characteristics are summarized in Table 1. Their mean age was 51.7 (range, 30-82) years; 144 (93.5%) were male and 10 (6.5%) were female. A total of 133 (86.4%) of the patients chewed betel nut, 94 (61.0%) consumed alcohol, and 129 (83.8%) smoked tobacco. Cancers included 64 (41.6%) in the buccal mucosa, 46 (29.9%) in the tongue, 15 (9.73%) in an alveolus, 10 (6.48%) in the retromolar trigone, 8 (5.19%) in the hard palate, 6 (3.89%) in the floor of the mouth, and 5 (3.25%) in the lip. TA in oral tissues is shown in Table 2. Of the grossly normal mucosal samples, 149 (96.8%) were negative for TA, with the 5 (3.2%) positive samples all having a low level of activity except for 1 with a high level. Of the malignant tissue samples, 124 (80.5%) were positive for TA, with 101 (65.6%) having a low level and 23 (14.9%) a high level. TA levels differed sig-

nificantly between grossly normal and malignant tissues ($p<0.001$), but not with anatomic sites of the tumors (data not shown).

Association of the use of betel nut, alcohol, and tobacco smoking with telomerase activity.

As shown in Table 3, TA was statistically associated with none of the habits individually. When analyzing for the combined use of these substances (Table 4), no statistical association was found between the TA level and the combined use of betel nut and alcohol ($p=0.733$), betel nut and tobacco ($p=0.670$), alcohol and tobacco ($p=0.450$), or all 3 substances together ($p=0.785$).

Association of the use of betel nut, alcohol, and tobacco smoking with cancer severity.

As shown in Table 5, neither T stage, N stage, or

Table 1. Characteristics of 154 Oral Cancer Patients

Item	Number	Percentage (%)
Gender		
Female	10	6.5
Male	144	93.5
Age (years)		
30-40	24	15.6
40-50	56	36.3
50-60	42	27.3
60-70	20	13.0
> 70	12	7.8
Habit		
Betel nut chewing	133	86.4
Alcohol drinking	94	61.0
Tobacco smoking	129	83.8
Cancer site		
Buccal mucosa	64	41.6
Tongue	46	29.9
Alveolus	15	9.73
Retromolar trigone	10	6.48
Hard palate	8	5.19
Mouth floor	6	3.89
Lip	5	3.25
Total	154	100

Table 2. Levels of Telomerase Activity in Grossly Normal and Malignant Tissues of Oral Cancer Patients

Telomerase activity	Number	Percentage (%)
Grossly normal mucosa		
Negative	149	96.8
Positive	5	3.2
Low level	4	2.6
High level	1	0.6
Malignant tissue		
Negative	30	19.5
Positive	124	80.5
Low level	101	65.6
High level	23	14.9
Total	154	100

Table 3. Association of Telomerase Activity with Exposure to Tobacco, Alcohol, and Betel Nut

		Telomerase Activity			
Habit	N	Negative (%)	Low (%)	High (%)	*p*
Betel Nut					
No	21	6 (28.6)	12 (57.1)	3 (14.3)	0.413
Yes	133	24 (18.0)	89 (66.9)	20 (15.0)	
Alcohol					
No	60	12 (20.0)	35 (58.3)	13 (21.7)	0.294
Yes	94	18 (19.1)	66 (70.2)	10 (10.6)	
Tobacco					
No	25	7 (28.0)	12 (48.0)	6 (24.0)	0.959
Yes	129	23 (17.8)	89 (69.0)	17 (13.2)	
Total	154	30 (19.5)	101 (65.6)	23 (14.9)	

Pearson's chi-square test of trend was used.

Table 4. Associtaion of Telomerase Activity

		Telomerase Activity			
Habits	N	Negative (%)	Low (%)	High (%)	*p*
Betel nut and alcohol					
Neither	16	6 (37.5)	7 (43.8)	3 (18.8)	0.733
One	49	6 (12.2)	33 (67.3)	10 (20.4)	
Both	89	18 (20.2)	61 (68.5)	10 (11.2)	
Betel nut and tobacco					
Neither	13	5 (38.5)	5 (38.5)	3 (23.1)	0.670
One	20	3 (15.0)	14 (70.0)	3 (15.0)	
Both	121	22 (18.2)	82 (67.8)	17 (14.0)	
Alcohol and tobacco					
Neither	19	6 (31.6)	7 (36.8)	6 (31.6)	0.450
One	47	7 (14.9)	33 (70.2)	7 (14.9)	
Both	88	17 (19.3)	61 (69.3)	10 (11.4)	
Betel nut, alcohol, and tobacco					
None	13	5 (38.5)	5 (38.5)	3 (23.1)	0.785
One	9	2 (22.2)	4 (44.4)	3 (33.3)	
Two	49	6 (12.2)	36 (73.5)	7 (14.3)	
Three	83	17 (20.5)	56 (67.5)	10 (12.0)	
Total	154	30 (19.5)	101 (65.6)	23 (14.9)	

Pearson's chi-square test of trend was used.

Table 5. Association of Clinical Stage with Exposure to Tobacco, Alcohol, and Betel Nut

		T stage (%)			N stage (%)			Overall stage (%)		
Habits	N	T1-T2	T3-T4	*p*	N = 0	N > 0	*p*	I-II	III-IV	*p*
Betel nut										
No	21	12 (57.1)	9 (42.9)	0.481	11 (52.4)	10 (47.6)	0.279	9 (42.9)	12 (57.1)	0.645
Yes	133	65 (48.9)	68 (51.1)		86 (64.7)	47 (35.3)		50 (37.6)	83 (62.4)	
Alcohol										
No	60	31 (51.7)	29 (48.3)	0.741	39 (65.0)	21 (35.0)	0.679	24 (40.0)	36 (60.0)	0.731
Yes	94	46 (48.9)	48 (51.1)		58 (61.7)	36 (38.3)		35 (37.2)	59 (62.8)	
Tobacco										
No	25	15 (60.0)	10 (40.0)	0.275	14 (56.0)	11 (44.0)	0.429	11 (44.0)	14 (56.0)	0.523
Yes	129	62 (48.1)	67 (51.9)		83 (64.3)	46 (35.7)		48 (37.2)	81 (62.8)	
Total	154	77 (50.0)	77 (50.0)		97 (63.0)	57 (37.0)		59 (38.3)	95 (61.7)	

Pearson's chi-square test was used.

Table 6. Association of Clinical Stage with Combined Exposure to Tobacco, Alcohol, and Betel Nut

		T stage (%)			N stage (%)			Overall stage (%)		
Habits	N	T1-T2	T3-T4	*p*	N = 0	N > 0	*p*	I-II	III-IV	*p*
Betel nut and alcohol										
Neither	16	10 (62.5)	6 (37.5)	0.553	9 (56.3)	7 (43.8)	0.802	7 (43.8)	9 (56.3)	0.631
One	49	23 (46.9)	26 (53.1)		32 (65.3)	17 (34.7)		19 (38.8)	30 (61.2)	
Both	89	44 (49.4)	45 (50.6)		56 (62.9)	33 (37.1)		33 (37.1)	56 (62.9)	
Betel nut and tobacco										
Neither	13	8 (61.5)	5 (38.5)	0.296	8 (61.5)	5 (38.5)	0.282	6 (46.2)	7 (53.8)	0.523
One	20	11 (55.0)	9 (45.0)		9 (45.0)	11 (55.0)		8 (40.0)	12 (60.0)	
Both	121	58 (47.9)	63 (52.1)		80 (66.1)	41 (33.9)		45 (37.2)	76 (62.8)	
Alcohol and tobacco										
Neither	19	10 (52.6)	9 (47.4)	0.424	11 (57.9)	8 (42.1)	0.899	8 (42.1)	11 (57.9)	0.731
One	47	26 (55.3)	21 (44.7)		31 (66.0)	16 (34.0)		19 (40.0)	28 (59.6)	
Both	88	41 (46.6)	47 (53.4)		55 (62.5)	33 (37.5)		32 (36.4)	56 (63.6)	
Betel nut, alcohol, and tobacco										
None	13	8 (61.5)	5 (38.5)	0.380	8 (61.5)	5 (38.5)	0.615	6 (46.2)	7 (53.8)	0.541
One	9	4 (44.4)	5 (55.6)		4 (44.4)	5 (55.6)		3 (33.3)	6 (66.7)	
Two	49	26 (53.1)	23 (46.9)		32 (65.3)	17 (34.7)		20 (40.8)	29 (59.2)	
Three	83	39 (47.0)	44 (53.0)		53 (63.9)	30 (36.1)		30 (36.1)	53 (63.9)	
Total	154	77 (50.0)	77 (50.0)		97 (63.0)	57 (37.0)		59 (38.3)	95 (61.7)	

Pearson's chi-square test of trend was used.

overall stage was statistically associated with any of these habits individually. When analyzing combined exposures to these substances (Table 6), no statistically significant association was found between clinical stage and the combined use of betel nut and alcohol, betel nut and tobacco, alcohol and tobacco, or the 3 substances together.

DISCUSSION

The presence of TA in tissues of head and neck cancer patients has been investigated in a number of studies.[16-21] In malignant tissues, the presence of TA has been reported in 80% to 100%, while in grossly normal tissues it has ranged from 0% to 74%. In our study, we found TA in 80.5% of malignant tissue and

3.2% of grossly normal mucosal samples (Table 2). Differences in TA levels found in different groups can be explained in several ways. Compared to other studies, we used a much higher number of tissue samples (154 matched samples compared with 16 to 30 malignant samples and 17 to 40 grossly normal samples in other reports).[16-21] Second, the type of carcinogen exposure plus possible genetic predispositions may result in different oral cancer carcinogenic pathways, theoretically resulting in differences in TA levels in different populations. For example, tobacco exposure and alcohol consumption are the habits most commonly associated with oral cancer in Western countries[3,4] while betel nut chewing is a common habit in Southeast Asia.[5,22-24] In India, oral cancer patients usually combine tobacco with betel nut chewing, whereas in Taiwan, oral cancer patients often have all 3 habits: betel nut chewing, alcohol drinking, and tobacco smoking.[2,5,22]

An interesting coincidence has been found between TA in grossly normal tissues and the incident rate of oral cancer in different geographical regions. For example, positive TA in adjacent grossly normal tissues was found in 74% of Indian patients[16] and 38% of Thai patients,[20] but not in US or Japanese patients,[17,18,21] the latter being closer to the 4.6% we found in Taiwanese patients. Oral cancer is highly prevalent in India, comprising 35%-40% of all malignancies, but only 10% in Taiwan and 2%-4% in Western countries.[7,8] With the coincidence of higher percentages of betel nut chewing in regional oral cancer groups (Southeast Asia), higher frequencies of positive telomerase activity in adjacent grossly normal mucosa, and higher incidence rates of oral cancer in the region, it is suspected that betel nut chewing may be associated with the reactivation of telomerase in oral mucosa, and such a molecular change may potentiate the process of oral carcinogenesis. However, in our present study, although we found that most cancer tissues (80.5%) but only a few grossly normal mucosa samples (3.2%) were positive for TA, in general agreement with other reports and which suggests that telomerase reactivation plays a role in the carcinogenesis of oral cancer, we found no correlations between TA and any single habit of betel nut, alcohol, and tobacco consumption (Table 3), or with their combined exposure (Table 4). These results suggest no direct association of telomerase reactivation with any of these factors. The exact role of potential carcinogen exposure with regard to telomerase reactivation and molecular changes leading to the development of oral cancer thus requires further investigation.

Cancer development is recognized as a micro-evolutionary process that requires the cumulative actions of multiple events. These events can be simplified into a 3-stage model: the induction of DNA mutation in a somatic cell (initiation), the stimulation of tumorigenic expansion of the cell clone (promotion), and the malignant conversion of the tumor into cancer (progression). As indicated before, uses of betel nut, alcohol, and tobacco smoking have been reported to be related to oral cancer development.[2-6] In addition, many reports have indicated the presence of synergetic effects of combined exposures to these factors.[2,4,24] For example, it was reported that the addition of tobacco to betel nuts increased the risk of carcinoma from 4 to 29 times.[24] The risk of oral cancer is increased by the use of betel nut, alcohol, and tobacco by 18-, 10-, and 28-fold, respectively, while the combined use of these substances increases the risk by 123-fold.[2] Since those studies focused on the oral cancer incidence, comparing differences in consumption habits between oral cancer patients and healthy controls, their results may also suggest that these factors play a role in early cancer development, such as at the initiation and/or promotion steps. In the present study, we examined whether these factors were associated with disease severity, that is, associated with further cancer progression. We used newly diagnosed oral cancer patients who were unlikely to have changed their consumption habits at the first doctor visit, providing an opportunity to examine the association of substance use with disease severity. Our results showed no association between the use of these substances with clinical stage (Tables 5, 6), suggesting that these factors are not involved in later cancer progression. In this regard, other risk factors, such as diet, dental radiation, or viral infections,[25-29] as well as factors that are intrinsic to the host may play more-significant roles in the later stages of oral cancer development.

In conclusion, although the frequencies of betel nut chewing, alcohol consumption, and cigarette smoking are very high in oral cancer patients, there is no association of these factors with telomerase activity or with the severity of oral cancer. These results imply that the consumption habits of these

substances might only be associated with the early stages of oral cancer development, while the later stages of cancer progression may be more closely associated with other external factors or be dependent on host cellular factors, confirmation of which requires further investigation.

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REFERENCES

1. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. N Eng J Med 1993;328:184 -94.
2. Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel nut chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J Oral Pathol Med 1995;24:450-3.
3. Harris EL. Association of oral cancers with alcohol consumption: exploring mechanisms. J Natl Cancer Inst 1997;89:1656-7.
4. Dave BJ, Trivedi AH, Adhvaryu SG. Role of areca nut consumption in the cause of oral cancers. Cancer 1992; 70:1017-23.
5. Chong KM. Betel nut chewing and mouth cancer in Taiwan. Observation of the oral mucosa in the betel nut chewers. J Formosan Med Assoc 1996;65:79-85.
6. Jayant K, Balakrishnan V, Sanghvi LD, Jussawala DJ. Quantification of the role of smoking and chewing habits in oral, pharynx and oesophagus cancer. Br J Cancer 1997;35:232-5.
7. Daftary DK, Murti PR, Bhonsle RR, Gupta PC, Mehta FS, Pindborg JJ. Risk factors and risk markers for oral cancers in high risk areas of the world. In "Oral Cancer: detection of patients and lesions at risk," ed. N. W. Johnson. Cambridge, Cambridge University Press, 1991; 29-63.
8. Chen CH. An epidemiological study of oral squamous cell carcinoma in southern Taiwan. J Formosan Dent Assoc 1987;10:268-74.
9. Clayman GL, Lippman SM, Laramore GE, Hong WK. Head and neck cancer. In "Cancer Medicine," ed. Holland JF, Frei E, Bast RC, Kufe DW, Morton DL, Weichselbaum R. Philadelphia, Williams and Wilkins, 1996;1645-709.
10. Vikram B. Changing patterns of failure in advanced head and neck cancer. Arch Otolaryngol Head Neck Surg 1994;110:564-5.
11. Morin GB. The implications of telomerase biochemistry for human disease. Eur J Cancer 1997;33:750-60.
12. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997;33:787-91.
13. Fleming ID, Cooper JS, Henson DE, Hutter RVP, Kennedy BJ, Murphy GP, O'Sullivan B, Sobin L, Yarbro JW. In "AJCC Cancer Staging Manual (5th ed)". Philadelphia, Lippincott Williams Wilkins, 1998;21-58.
14. Wong DYK, Chang KW, Chen CF, Chang RCS. Characterization of two new cell lines derived from oral cavity human squamous cell carcinomas - OC1 and OC2. J Oral Maxilofac Surg 1990;48:385-90.
15. Cheng AJ, Tang R, Wang JY, Chang JT, Wang TC. Polymerase chain reaction-based enzyme immunoassay for quantitation of telomerase activity: application to colorectal cancers. Jpn J Cancer Res 1999;90:280-5.
16. Patel MM, Patel DD, Parekh LJ, Raval GN, Rawal RM, Bhatavdekar JM, Patel BP, Patel PS. Evaluation of telomerase activation in head and neck cancer. Oral Oncol 1999;35:510-5.
17. Miyoshi Y, Tsukinoki K, Imaizumi T, Yamada Y, Ishizaki T, Watanabe Y, Sasakura Y, Lin y, Hosaka M, Kubota Y. Telomerase activity in oral cancer. Oral Oncol 1999;35: 283-9.
18. Sumida T, Sogawa K, Hamakawa H, Sugita A, Tanioka H, Ueda N. Detection of telomerase activity in oral lesions. J Oral Pathol Med 1998;27:111-5.
19. Curran AJ, St Denis K, Irish J, Gullane P, Mac Millan C, Kamel-Reid S. Telomerase activity in oral squamous cell carcinoma. Arch Otolaryngol. Head Neck Surg 1998;124: 784-8.
20. Mutirangura A, Supiyaphun P, Trirekapan S, Sriuranpong V, Sakuntabhai A, Yenrudi S, Voravud N. Telomerase activity in oral leukoplakia and head and neck squamous cell carcinoma. Cancer Res 1996;56:3530-3.
21. Mao L, El-Naggar AK, Fan YH, Lee JS, Lippman SM, Kayser S, Lotan R, Hong WK. Telomerase activity in head and neck squamous cell carcinoma and adjacent tissues. Cancer Res 1996;56:5600-4.
22. Singn AD, Von Essen CF. Buccal mucosal cancer in South India. Am J Roentgenol 1966; 96:6-14.
23. Marsden ATH. Betel cancer in Malaya. Med J Malaysia 1960;14:162-5.
24. Awang MN. Betel nut and oral carcinogenesis. Sign Med J 1988;29:589-93.
25. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, Monnier P. Food groups and risk of oral and pharyngeal cancer. Int J Cancer 1998;77:705-9.
26. Franceschi S, Munoz N, Bosch XF, Snijders PJ, Walboomers JM. Human papillomavirus and cancers of the upper aerodigestive tract: a review of epidemiological and experimental evidence. Cancer Epidemiol Biomarkers Prev 1996;5:567-75.
27. Das C, Schantz SP, Shillitoe EJ. Antibody to a mutagenic peptide of herpes simplex virus in young adult patients with cancer of the head and neck. Oral Surg Oral Med

Oral Path 1993;75:610-4.
28. Chang KW, Chang CS, Lai KS, Chou MJ, Choo KB. High prevalence of human papillomavirus infection and possible association with betel quid chewing and smoking in oral epidermoid carcinomas in Taiwan. J Med Viral 1989; 28:57-61.
29. Underhill TE, Kimura K, Chilvarquer I, McDavid WD, Langlais RP, Preece JW, Barnwell G.. Radiobiologic risk estimation from dental radiology. Part II. Cancer incidence and fatality. Oral Surg Oral Med Oral Pathol 1988; 66:261-7.

口腔癌症病患之檳榔、煙、酒的食用習性與端粒脢活性及與臨床癌期缺乏相關性

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背 景： 口腔癌是台灣十大癌症之一，流行病學的研究顯示，口腔癌的發生率與檳榔、煙、酒的食用習性有關。近年來，研究又顯示端粒脢在癌化過程中扮演一個重要角色，因此在本研究中，我們探討在口腔癌症病患中，檳榔、煙、酒的食用習性與臨床癌期及與端粒脢是否具相關性。

方 法： 本研究中共有154位口腔癌症病患收錄參與，每位病患均取一小塊的正常口腔黏膜與癌化組織檢體來研究。端粒脢活性的檢測是以PCR-EIA的方法，而以Pearson chi-square的統計學方法來分析檳榔、煙、酒與臨床癌期或與端粒脢之相關性。

結 果： 病患中分別有86.4%、61.0%和83.3%有嚼食檳榔、喝酒及吸煙的習慣。在所有檢測的檢體中，80.5%的癌組織及3.2%的正常黏膜細胞呈現陽性的端粒脢活性，但是統計學的研究顯示，臨床癌症或端粒脢活性的高低均與檳榔、煙、酒的食用習性 (不論個別或結合習用) 無關。

結 論： 過去的許多報告顯示，檳榔、煙及酒與口腔癌的發生率有密切相關，但本研究中卻顯示這些食用習性，與癌化程度或與端粒脢活性無統計相關，這些結果引申隱喻，這些食用習性也許只在口腔癌的癌化初期扮演某些角色，然而癌組織形成後期的繼續惡化，也許與環境因素較無相關，反之，更端賴於細胞分子本身。

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關鍵字： 口腔癌，端粒脢，檳榔，癌症分期。

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