ORAL MUCOSAL LESIONS ASSOCIATED WITH TOBACCO AND BETEL-CHEWING HABITS : A NEPALESE EXPERIENCE

Prashanta Shrestha DDS, PhD[1]; **Noriaki Ikeda** DDS, PhD[2]; **Hideo Fukano** DDS, PhD[3]; **Yoshiaki Takai** DDS, PhD[1]; **Masahiko Mori**, DDS, PhD[1]

[1]Department of Oral and Maxillofacial Surgery, Asahi University School of Dentistry, Gifu, Japan, [2]Bereau of International Cooperation, International Medical Center of Japan, Ministry of Health and Welfare, Tokyo, Japan and [3]Department of Oral and Maxillofacial Surgery, Aichi Gakuin University School of Dentistry, Nagoya, Japan

Abstract

A pre-existing potentially malignant lesions or conditions, often associated with tobacco and/or areca nut containing quid, may constiute, if not in all cases, pre-malignant stages of oral squamous cell carcinoma, however, their natural history and to what extent these lesions are prevalent and their transformation into a malignant lesion which may vary between different population groups, habit factors and other predisposing or risk factors are difficult to obtain. The present study evaluates the prevalence and characteristics of oral mucosal lesions associated with tobacco and areca nut containing-quid chewing habit in a population based random sample of 1421 individuals in three geographic regions of Nepal, to our knowledge for the first time in the country, and patients presenting with oral mucosal lesions in a hospital setting. Furthermore, to evaluate at least a few aspect of alterations at the molecular level, cellular proliferation and presence of mutant form of p53 oncosupressor gene product, known to affect or contribute in the process of carcinogenesis, in a number of these lesions were assessed by immunohistological methods.

Of a variety of tobacco and areca nut containing quid habit, in the population based survey, the commonest lesions associated with the habit was found to be tobacco pouch associated white or red lesions in 70.4% of tobacco quid users (n=186), betel chewers mucosa in 23.80% of areca nut/ betel quid chewers with or without tobacco (n=143), leukoplakia in 2.4% of smokers (n=495) and submucous fibrosis in 3 subjects who were all areca nut chewers. All these lesions or conditions were significantly correlated with the duration of more than 5 years ($p < 0.05$) and frequency ($p<0.01$, at least once a day vs occasional) of the associated habit factor.

In an analysis of the patients presenting with these lesions in a hospital setting - tobacco pouch lesions in tobacco and lime users (n=35) showed complete remission with the discontinuation of tobacco habit for 1-3 weeks (n=33); betel chewers mucosa (n=12), associated with the area nut/ betel leaves chewing habit, also showed remission with the discontinuation of the habit (n=11) but one patient, a 36-year-old male, was diagnosed as having multicentric squamous cell carcinoma on left and right lateral border of the tongue. All patients presenting with submucous fibrosis (n=12) had areca nut chewing habit of various duration and may suggest a relationship, yet unknown, with the betel chewers mucosa - and one of the patient, a 52-year-old female, was diagnosed as having carcinoma in the buccal mucosa.

Using immunohistological methods, all these tobacco and areca nut quid associated lesions or conditions were found to be hyperproliferative disorder with a statistically significant difference in labeling index of PCNA when compared with clinically healthy mucosa ($p<0.001$). The epithelial proliferation index assessed on the basis of nuclear immunostaining for proliferating cell nuclear antigen PCNA (mean

percentage+/-standard deviation) in the oral mucosa from healthy subjects was 7.2+/-1.96 (n=16) compared with the lesional mucosa of tobacco pouch lesions 21.5+/-1.96 (n=14), submucous fibrosis 28.2+/-4.10 (n=12), betel chewers mucosa 25.4+/-3.20 (n=12), and leukoplakia 18.8+/-4.85 (n=8). Mutant form of p53 was detected in a number of these lesions including nearly 60% of OSF and 25% of clinically healthy oral mucosa restricted to subjects of over 40 years old with some form of tobacco or betel quid habit. The pre-existing oral lesions and SCC, in two patients presenting with betel chewers mucosa and OSF, incidently were found to express the p53 oncoprotein.

Although a number of factors such as the ethnic variation, geography and lifestyles, nutritional, immune-modulating, and many other factors may affect the ultimate outcome of tobacco and areca nut -quid related lesions, the results presented in the present study reasonably allowed us to conclude that due to a very high incidence of tobacco pouch lesions in tobacco quid users, in order to clarify its nature and potential for malignant transformation, the lesion may be evaluated as a separate lesion from leukoplakia as defined by the WHO criteria. The tobacco and areca nut-quid associated lesions or conditions are indeed hyperproliferative disorders and even clinically healthy oral mucosa in elderly individuals with tobacco or areca nut/betel quid habit, on the basis of detection of mutant form of p53, may be considered as a high risk mucosa.

Introduction

Oral mucosal lesions with a potential of malignant transformation and oral cancer in South and South-East Asia, reported to be associated with tobacco and areca nut containing quid chewing habits, still present an unresolved dilemma on their prevalence, natural history, association with the habit factors which are indeed too numerous and to what extent thay have a potential or risk factors for malignant transformation which are likely to vary with various predisposing or risk factors as well as between ethnic origins, geography and lifestyles.

Nepal, lying on the lap of the highest mountain ranges of Himalayas, the highest peak of which is known throughout the world as Mount Everest, displays a variety of geographical setting and lifestyles. A lack of population based cancer registry or central source of such information, and, in addition to, inaccessibility of many regions of this mountainous country, are difficulties in the assessment of epidemiological status of oral cancer in Nepal. A hospital based study in Kathmandu, on the basis of malignant tumors diagnosed by the biopsy service at one of the tertiary care center of the country, has shown that oral cancer accounts for 14 and 7.3 percent of all cancers in males and females, respectively (1).

The strategies of prevention or early detection of potentially malignant and malignant oral lesions should be based on their prevalence and, additionally, the habit factors and other associated predisposing or risk factors for malignant progression should be clearly identified.The present study aims to investigate the prevalence and characteristics of oral mucosal lesions related with tobacco and areca nut containingquid habit in a sample of Nepalese poulation and further attempts are made to identify markers of risk, known to contribute or affect neoplastic changes in various neoplasia including oral neoplasms, from biopsy specimens using immunohostological methods (2). We present our findings on proliferating fraction of cells detected by using immunoreactivity of proliferating cell nuclear antigen (PCNA) and mutant form of p53 oncoprotein, resulting from mutation in the 53 oncosuppressor gene, in these tobacco and areca nut containing quid associated lesions.

Materials and Methods

Target population and methodology: A random sample of inhabitants from three geographic locations with different social and economic backgrounds, firstly, a village in Dolkha in the mountainous region; secondly, Butwal and surrounding areas in the plains near the southern border and lastly, the city of Kathmandu, were selected for the study. An adult population of more than 20 years were interviewed, examined and the information recorded according to Criteria for Diagnosis of Oral Mucosal Lesion prepared by Ikeda et al with slight modifications (3).

The interview and examination of each individual was carried by one examiner (PS) in the month of June and July 1996. Oral examination was performed in natural light with an additional torch light using two mouth mirrors.

Statistical analysis: The test of non-parametric comparison in the three locations, Dolkha, Butwal and Kathmandu, were used. For comparison of several parameters the Kruskal-Wallis-test and the chi-square tests were used. The critical value for statistical significance was considered as $p<0.05$.

Immunohistological evaluation: Tissue specimens of tobacco pouch lesions (n=14), betel chewers mucosa (n=12), submucous fibrosis (n=12), leukoplakia (n=8) and clinically healthy oral mucosa (n=16) were evaluated for expression of proliferating cell nuclear antigen (PCNA) and mutant form of p53 using monoclonal antibodies in a three stage streptavidin-biotin immunoperoxidase method, the details of which have been described elsewhere (4).

Results

The sample population was 1421 adults (760 male and 661 female) where age ranged from 20-85 years (mean age 42.6). Smoking was the commonest tobacco habit in both male and female subjects followed by use of mucosal pouch tobacco with lime (Fig 1) mostly among males and areca nuts/ betel leaves chewing with or without tobacco. Except for the tobacco smoking habit among males, there was a statistically significant difference in the prevalence of various habit factor in both the sexes in three geographical regions (Table 1).

TABLE 1: Prevalence of tobacco and areca nut containing quid habit factor in three geographic regions of Nepal

Habit factors	Dolkha		Butwal		Kathmandu	
	Male (n=278)	Female (n=240)	Male (n=188)	Female (n=202)	Male (n=290)	Female (n=219)
Smoking 130	78*	100	34*	144	9*	
Mucosal pouch tobacco	62**	2#	84**	8#	28**	2#
Areca nut quid chewing	54***	6##	58***	15##	53***	20##

* $p<0.001$,
** $p<0.001$; # $p<0.05$,
*** $p<0.01$; ## $p<0.01$

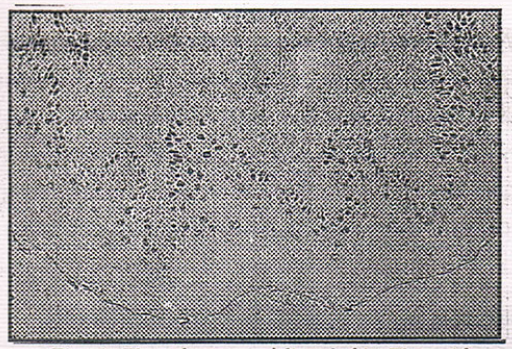

Fig. 1. The tobacco and lime habit. The quid remain on the surface of the oral mucosa.

The commonest oral mucosal lesion associated with tobacco and areca nut containing quid chewing habit was tobacco pouch associated lesions followed by betel chewers mucosa, leukoplakia and submucous fibrosis (Table 2).

TABLE 2 : Prevalence of oral mucosal lesion (in both sexes)

Lesion	Dolkha	Butwal	Kathmandu
Suspicious of oral cancer	-	2	1
Tobacco pouch lesion	37	77	16
Leukoplakia	2	8	4
Submucous fibrosis	-	2	1
Betel chewer's mucosa	8	12	14
Nicotinic stomatitis	2	5	6
Lichen planus	1	3	3
Others	2	3	3

(Others - candidiasis, migrating glossitis, aphthous ulcers and mucosal pigmentation)

Fig. 2. Whilte and red lesions on the oral mucosa in contact with tobacco and lime quid.

Tobacco pouch lesions featuring hom ogeneous and/or non-homogeneous leukoplakia (Fig 2) was observed at sites, the lower labial and gingival or alveolar mucosa, where the chewing tobacco quid is placed most frequently ($p<0.001$) than any other site. The lesion typically was velvety with fissured or rippled surface often showing white or red patches with a frequent destrution of the periodontium resulting in gingival recession and exposure and yellow to brown staining of the root surface of the affected teeth. The appearance of tobacco pouch lesion was associated with the duration of the habit of more than 5 years ($p<0.05$), frequency and duration of quid in the oral cavity ($p<0.01$).

Betel chewer's mucosa, seen as a condition where the direct action of the quid or traumatic effects of chewing or both results in a tendency to desquamation or peeling off of the oral epithelium and loose or detached tags of epithelium may be seen or felt, was associated with the areca nut /betel-leaves chewing habit with or without tobacco and/or slaked lime. Betel chewers mucosa was most significantly associated with the duration and

frequency of the habit ($p<0.001$). Not infrequently yellow or brow encrustations were found on the oral mucosal surface and this effect was more prominent among betel quid chewers.

The prevalence of leukoplakia was nearly 1%, the lesion was most frequent in men where buccal mucosa, lateral border of the tongue and floor of the mouth were most commonly afected and was associated with smoking habit.

Submucous fibrosis (fig 3) was associated with the history of areca nut chewing ($p<0.001$) with a prevalence rate of nearly 0.2% in the population.

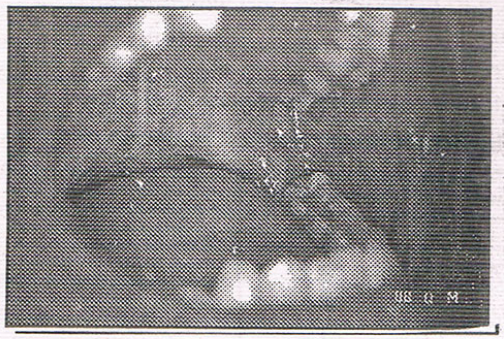

Fig. 3. Oral submucous fibrosis in a patient with areca nut chewing habit. The oral mucosa show persistent blanching and marlbe-like appearance.

Fig. 4. Oral squamous cell cardinoma arising at the site where tobacc lime quid used to be kept. The patient had this habit for last 15 years.

Among the subjects suspicious of oral cancer (two of them were proven as squamous cell carcinoma by biopsy), one had history of tobacco and areca nut containing quid chewing for 15 years (Fig 4) and the other had submucous fibrosis and habit of areca nut chewing and tobacco quid for 12 years.

In patients presenting with tobacco associated lesions in a hospital setting - tobacco pouch lesions in tobacco users (n=35) showed complete remission with the discontinuation of tobacco habit for 1-3 weeks (n=33). The hypperkeratosis remain unchanged (n=1) and gradually became thickened after cessation of the habit (n=1) during a follow up of 3 months in the present study. Betel chewers mucosa (n=12), as described above, also showed remission with the discontinuation of the habit (n=11) but one patient, a 36-year -old male, had multicentric squamous cell carcinoma on left and right lateral border of the tongue at the time of presentation. The commonest site involved in betel chewers mucosa (n=48) was the buccal mucosa in 60% of the cases, which was followed by lateral border of the tongue (21%), floor of the mouth (6%), gingivae (4%), palate and dorsum of the tongue (3%).

Immunohistological findings: The epithelial proliferation index assessed on the basis of nuclear immunostaining for proliferating cell nuclear antigen PCNA (mean percentage+/-standard deviation) in all the tobacco and areca nut containing quid associated lesions and oral mucosa from healthy subjects was stastically significant ($p<0.001$). A number of these lesions and clinically normal oral mucosa restricted to subjects of over 40 years old with some form of tobacco or areca nut containing quid habit had expression of mutant form of p53 particularly in the basal cell layer of the oral epithelium (Fig 5 and Table 3). No

significant corelation was observed between the growth fraction of cells and p53 status in any of these lesions. However, cases of squamous cell carcinoma in betel-chewers mucosa (n=1) and preexisting submucous fibrosis (n=1) had positive immunoreactivity of p53 in carcinoma cells.

Table 3: Growth fraction and expression of p53 oncoprotein in tobacco and betel-quid associated lesions

	No. of cases	Growth fraction PCNA index mean =/- SD	p53 oncoprotein expression number of cases
Clinically healthy mucosa	16	7.2 +/- 1.96	4
Tobacco pouch lesion	14	21.5 +/- 5.40	4
Betel chewers mucosa	12	25.4 +/- 3.22	3
Submucous fibrosis	12	28.2 +/- 4.10	7
Leukoplakia	8	18.8 +/- 4.85	2

The difference between growth fraction of cells in clinically healthy mucosa and tobacco and areca nut-quid associated lesions are statistically significant ($p<0.001$).

Discussion

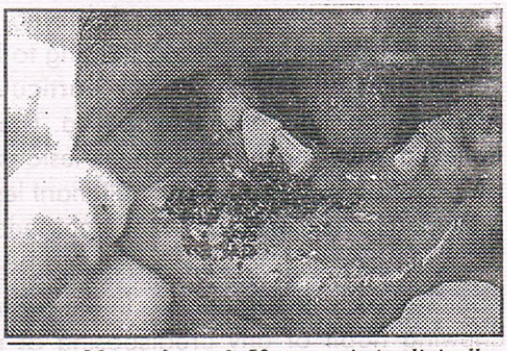

Fig. 5. Mutant form of p53 expressin in clinically healthy mucosa of a smoker.

The present study describing prevalence of tobacco and areca nut containing quid associated oral lesions or conditions, to our knowledge for the first time from Nepal, in three different geographic locations with a diferent social and economic background, showed as might be expected and reported in numerous other studies from different regions of the world, a difference in the prevalence of tobacco and areca nut containing quid chewing with a significant correlation between tobacco or areca quid habit and incidence of oral mucosal lesions, a significant number of which, if not all, were potentially malignant lesions or conditions in the oral cavity. However, oral squamous cell carcinoma arising in a pre-existing potentially malignant lesions or conditions and the predisposing or risk fators involved or those arising without the patients or clinicians being aware of such lesions or conditions are yet to be quantified.

The prevalence of tobacco and areca quid habit and associated oral lesions or conditions in male, in the present study, was remarkably higher than in the female, although smoking habit was highly prevalent among rural women (data not shown). The trends in the habit groups differ from countries, such as India, Thailand and Philippines, in south and South East Asia, where either female subjects are found to have more oral lesions than the males as more women are indulged in betel-quid chewing habit or where habits are equally frequent in both sexes (5)

The overall incidence of leukoplakia, combined with white lesions associated with the mucosal pouch tobacco, in the present study was 10.2% in our sample population. When

tobacco pouch lesions were excluded, the incidence of leukoplakia, standing at 1.2%, significantly associated with the smoking habits, is comparable with results obtained in many other studies, such as those in Japan and Cambodia, where smoking and alcohol has been found to be the most frequently associated habit in the genesis of the lesion (6,7). In our sample, nearly 70% of the mucosal pouch tobacco users had leukoplakia at sites where the tobacco quids remain in contact with the oral mucosa compared with less than 2% of the tobacco smokers. The prevalence of leukoplakia may vary between 0.7% to 24% in the same population depending upon the clinical criteria for the diagnosis of white lesions (8) and we have excluded frictional white lesions and nicotinic stomatitis in the diagnosis of leukoplakia.

The findings in tobacco pouch lesions, homogeneous and non-homogeneous and occasionally with areas of erythroplakia at sites where the tobacco quid are usually placed and associated mucosal changes in the surface texture and destruction of periodontium with discolouration of the exposed root surface of the tooth, as the most common lesion among tobacco quid users, was associated with the duration of practice of habit, frequency per day and duration of the quid in contact on the mucosa. This altered mucosa , with a very high incidence among mucoasl pouch tobacco users only at sites where the tobacco most frequently remain into contact, however, may suggest a differential behavior of these lesions from leukoplakia classified according to the WHO criteria (9) and therefore we believe it is reasonable to segregate this particular habit associated lesion from other tobacco associated or idiopathic leukoplakia. The relative risk of malignant potential of these lesion, believed to be much less than the lesions described as leukoplakia but adequate enough to be classified as a potentially malignant lesion, may however never be known as many lesions disappear after cessation of the habit and many will be treated before it is too advanced.

There was a clear association between submucous fibrosis and areca nut chewing habit with or without tobacco in the present study. The individual response to the areca nut chewing habit or any predisposing of risk factors in the genesis of OSF are, however, incompletely understood. Betel chewer's mucosa, on the other hand, may be a reversible condition, and if not always, be a precursor of submucous fibrosis and the relationship, if any, is yet known. Our finding of a single patient presenting with betel chewers mucosa and a multicentric squamous cell carcinoma, although rare, on both the lateral border of the tongue in a young male patient necessiate further evaluation of this condition as having a malignant potential. The chemical and mechanical trauma inflicted on oral mucosa resulting in the Betel chewer's mucosa may substantiate this condition as a condition potentiating the effect of carcinogenesis as in animal models infliction of trauma during carcinogen application results in potentiating the effects of carcinogens and the detection of viral genome in oral mucosa (10). In addition, an increased prevalence of human papilloma virus, a common virus associated with carcinoma in the cervix but the role of which in oral carcinogenesis yet not clear (11,12), in oral mucosa have been reported from Cambodian subjects (13).

The prevalence of oral lichen planus was comparable to the results obtained in European and Asian countries - 1.9% in sweeden (14), 0.08% in Hungary (15) 0.2-1,6% in India (16), 0.5% in Japan (6) and 1.8 in Cambodia (7). However, we fail to substantiate relationship,

due to a limited number of cases in our sample, with the tobacco-betel habit or snuff as suggested by Pindborg et al (16) and Axell and Rendquist (14).

An accumulation of a number aberrations at the cellular and molecular level contribute in the genesis of malignant lesions. Studies on such alterations in potentially malignant lesions and conditions and those leading to increasing risk of malignant transformation, however, do not demonstrate predictive value of an individual marker of combination of markers. Our finding of all our tobacco and areca nut containing quid associated lesions or conditions, histologically and immunohistologically, as hyperproliferative disorders with a statistically significant difference in labeling index of PCNA compared with healthy oral mucosa and detection of mutant form of p53 oncoprotein may have an implication in further understanding of these lesions at the molecular level. The role of mutant form of p53 in the transformation of a potentially malignant lesions into a malignant one (17), although suggested, needs further evaluation during oral carcinogenesis. An unexpected finding of p53 in healthy oral mucosa in our patients of above 40 and with some form of tobacco and areca nut containg quid habit may warrant the oral mucosa in these subjects as a high risk mucosa. It may , therefore, be reasonable to substantiate the potential of screening for potentially malignant oral lesions in elderly individuals as suggested by Ikeda et al (18).

A number of factors such as the ethnic variation, geography and lifestyles, nutritional, immune-modulating and many other factors may affect the ultimate outcome of tobacco and areca-quid related lesions, the results presented in the present study reasonably allowed us to conclude that due to a very high incidence of tobacco pouch lesions in tobacco quid users, in order to clarify its nature and potential for malignant transformation, the lesion should be evaluated as a separate lesion from leukoplakia as defined recently. The tobacco and areca nut containing quid associated lesions or conditions are indeed hyperproliferative disorders and even clinically healthy oral mucosa in elderly individuals with tobacco or areca nut containing quid habit, on the basis of detection of mutant form of p53, may be considered as a high risk mucosa.

References

1. Shrestha P: Incidence and pattern of cancer with special reference to oral cancer at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Oral and Macillofacial Surgery - Proceedings of the 16th IAMFS, Oita, Japan, 197-200, 1991.
2. Coltrera MD, Zarbo RJ, Sakr WA, Gown AM. Markers for dysplasia of the aerodigestive tract. Suprabasal expression of PCNA, p53 and CK19 in alcohol fixed, embedded tissue. Am J Pathol 141: 817-825, 1992.
3. Ikeda N, Zain R, Handa Y, Axell T. Criteria for diagnosis of oral mucosal lesion - an aid for examiners of epidemiological surveys in South East Asia. 1995.
4. Lan H A, Zain RB, Shrestha P et al. Proliferating cell nuclear antigen and p53 in epithlial dysplasia and squamous cell carcinoma of the oral cavity - a marker of risk? Anticancer Res (in press, 1996).
5. Hirayama T. An epidemiology study of oral and pharyngeal cancer in Central and Southeast Asia. Bull WHO 34: 442-69, 1966.
6. Ikeda N, Ishii T, Iida S, Kawai T. Epidemiological study of oral leukoplakia based on mass screening for oral mucosal diseases in a selected Japanese population. Community Dent Oral Epidemiol 19: 160-163, 1991.
7. Ikeda N, Handa Y, Khim SP et al. Prevalence study of oral mucosal lesions in a selected Cambodian population. Community Dent Oral Epidemiol 23: 49-54, 1996.
8. Axell T. Occurrence of leukoplakia and some other white lesions among 20333 adult Sweedish people. Community Dent Oral Epidemiol 15: 46-51, 1987.

9. Kramer JR, Pindborg JJ, Brezrukov V, Infirri JS. Guide to epidemiology and diagnosis of oral mucosal diseases and conditions. Community Dent Oral Epidemiol 8: 1-26, 1980.
10. Iwasaki T, Maeda H, Tanaka H, Konishi S, Kurata T, Kameyama Y. Identification of a hamster oral papilloma virus in the dysplastic lesion of hamster lingual mucosa induced by applicationof 9,10-dimethly-1,2-benzanthracene and excisional wounding (Abstract). 4th International Congress on Oral Cancer, Ogaki, Japan, 1995.
11. Cruz IBF, Snijders PJF, Steenbergen RDM et al. Age-dependence of human papilloma virus DNA presence in oral squamous cell carcinomas. Oral Oncol, Eur J Cancer 32B: 55-62, 1996.
12. Greer RO, Eversole LR, Crosby LK. Detection of human papilloma virus -genomic DNA in oral epithelial dysplasias, oral smokeless tobacco-associated leukoplakias, and epithelial malignancies. J Oral Maxillofac Surg 48: 1201-1205, 1990.
13. Maeda H, Okeda N, Zain R et al. Detection of human papilloma viruses in betel chewer's mucosa and leukoplakias of Cambodian patients (Abstract). Second Asia Pacific Workshop on Oral Mucosal Lesions. Nov 1995 Chiang Mai, Thailand.
14. Axell T, Rundquist L. Oral lichen planus - a demographic study. Community Dent Oral Epidemiol 15: 52-56, 1987.
15. Banoczy J, Rigo O. Prevalence of oral precancerous lesions within a complex screenin system in Hungary. Community Dent Oral Epidemiol 19: 265-267, 1991.
16. Pindborg JJ, Mehta FS, Daftary DK, Gupta PC, Bhonsle RB. Prevalence of oral lichen planus among 7639 Indian villagers in Kerala, South India. Acta Dermatoveneral (Stockholm) 52: 216-220, 1972.
17. Ness M, Homann N, discher H et al. Expression of mutated p53 occurs in tumor-distant epithelia of head-and-neck cancer patient; a possible molecular basis of the development of multiple tumors. Cancer Res 53: 4189-4196, 1993.
18. Ikeda N, Downer MC, Ishii T, fukano H, Nagao T, Inoue K. Annual screening for oral cancer and precancer by invitation to 60-year-old residents of a city in Japan. Community Dent Health 12: 133-137, 1995.