Journal of Oral Science, Vol. 51, No. 2, 289-292, 2009

Original

Induction of micronuclei in buccal mucosa on chewing a mixture of betel leaf, areca nut and tobacco

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(Received 25 December 2008 and accepted 25 March 2009)

Abstract: Betel quid containing areca nut and chewing tobacco is used in many parts of India. In this study we evaluated the micronuclei (MN) in buccal mucosa of healthy individuals from southern India, who were regularly chewing a mixture of betel leaf, areca nut and tobacco. A total of 44 subjects were examined. The study population included 15 chewers, 14 chewers with smoking habit and 15 controls with the mean age of 38.57 ± 0.54, 34.50 ± 0.95, and 33.28 ± 0.89 years, respectively. The mean percentage of MN was 1.90 ± 1.03 in chewers, 2.00 ± 1.12 in chewers with smoking habits and 0.81 ± 0.66 in controls. There was no significant difference between the mean percentages of the two experimental groups. It can be concluded that a mixture of betel leaf, areca nut, and tobacco is unsafe for oral health. (J Oral Sci 51, 289-292, 2009)

Keywords: betel quid; areca nut; tobacco chewing; buccal cell micronuclei.

Introduction

Betel quid chewing is an ancient practice common in many Asian countries. Betel quid generally consists of betel leaf, areca nut, and slaked lime, to which tobacco is often added (1). Regular chewing of betel quid has several adverse effects on the oral cavity and upper digestive tract, including inflammation, development of white or gray patches on the tongue and buccal mucosa, and oral cancer. Betel leaf contains large amounts of a carcinogen called safrole, which is readily metabolized and excreted in urine as dihydroxychavicol and eugenol (2). Areca nut and betel quid chewing lead to oral sub-mucous fibrosis, a painful, disabling and potentially precancerous condition of the oral mucosa. Betel quid chewing is a major risk factor for cancer of the buccal mucosa and gingiva. The habit of betel quid and areca nut chewing has been strongly associated with cancers of the mouth, pharyngeal cavity, and upper part of digestive tract (3). Moreover, chewing and smoking habits act synergistically in these cancers (4). Chewing a mixture of betel quid, areca nut and tobacco is a complex behavior and is poorly studied. Betel and areca nut chewing has been extensively studied in populations in many parts of the world (5). However, the habitual chewing of a mixture of betel leaf, areca nut and tobacco has received less attention. The purpose of this study was to evaluate the micronuclei in buccal mucosa of individuals regularly chewing a mixture of betel leaf, areca nut and tobacco.

The buccal cell micronucleus is defined as a microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus (6). Micronuclei originate from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in the daughter nuclei during mitosis. The micronuclei test is the most frequent technique used to detect chromosome breakage or mitotic interference thought to be associated with increased risk for cancer (6).

The frequency of micronucleated cell was measured to

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assess genotoxic damage in betel quid chewers. When compared to other body sites, the mouth offers a unique opportunity to define biomarkers because the mouth permits non-invasive examination in longitudinal studies of smoking and smokeless tobacco-associated acute and chronic diseases. As micronuclei are derived from chromosomal fragments and whole chromosomes lagging behind in anaphase, the micronuclei assay can be used to show both clastogenic and aneugenic effects. Micronuclei formation is undoubtedly an important mechanism associated with chromosome loss (7).

Materials and Methods

The study samples were collected from 44 healthy individuals hailing from rural areas of Coimbatore City, South India. The study population comprised 15 individuals who regularly chewed a mixture of betel leaf, areca nut and tobacco (chewers), 14 individuals who regularly chewed a mixture of betel leaf, areca nut and tobacco and smoked (chewers with smoking habit), and 15 controls (non-chewers/ non-smokers). Before collecting the sample, each subject was interviewed about his lifestyle, food consumption, and health status. Individuals who drank alcohol and more than two cups of tea per day were excluded.

Before sampling, each subject rinsed the mouth thoroughly with tap water. Exfoliated buccal cells were obtained by gently rubbing the inside of both cheeks with an extra soft toothbrush for 1 min each. The participant then rinsed the mouth with 20 ml of 0.9% saline and expectorated into a 50-ml conical-based tube. The toothbrush was then rinsed in the tube and 30 ml saline was added before the cells were pelleted and washed once with Phosphate buffered saline (pH 7.4). Ten microliters of buccal mucosal cell suspension was smeared on a microscopic slide, and the slides were stained by May-Grunwald Giemsa (Sigma, St louis, MO). The micronuclei analysis was done using a light microscope, at ×100 magnification and with a ×10 eye piece. Coded slides were used and 3000 cells from each individual were observed. The results were expressed as frequencies of micronucleated cells in 100 cells. Only cells which were non-fragmented, non-accumulated, and non-overlaid and which had an untouched nucleus were examined.

Results

The average age and distribution of the study population are presented in Table 1. The group under study was analyzed based on age (intervals: 15-30, 31-40, 41-50 years), and no differences were detected among the different age groups.

Table 2 represents the average number of micronucleated cells in the subjects who chewed a mixture of betel leaf, areca nut and tobacco and the chewers with smoking habit. There was no significant difference between the mean percentages of micronucleted cells for these two groups (1.90 ± 1.03 in chewers, 2.00 ± 1.12 in chewers with smoking habit).When compared to controls (0.81 ± 0.66), the result was statistically significant (Table 3).

Discussion

In the present study, we observed a low number of MN cells in the oral mucosa of the control groups (non-chewers). However, the mean number of MN in chewers with smoking habit was higher than that in the chewers who did not smoke, which was similar to findings reported previously in oral cancer samples (3). This result suggests that both groups practiced oral hygiene.

The micronucleus test has been receiving increasing attention as a simple and sensitive short-term assay for detection of environmental genotoxicants (8). By applying this test, an elevated incidence of micronuclei has been recorded in the buccal mucosa cells of smokeless tobacco chewers.

Sufficient and compelling evidence shows that the constituents of betel leaf, areca nut and tobacco have cytogenic, genotoxic and mutagenic effects on mammals. For example, these products enhance chromatid breakage and exchange in the range of 12-37% in human cells *in vitro* and DNA strand breakage in mouse kidney cells or human epithelial cells (9,10). The strong and intriguing relation between the use of betel quid and tobacco chewing was found to be a public health hazard. A similar work was conducted by Kayal et al. 1993 in North Indian subjects, and it was reported that the chewing of areca nut, alone or in combination with betel leaf and lime, caused damage to the oral mucosa. The habit of chewing a mixture of betel leaf, areca nut and tobacco is widespread in south India and it should be considered in terms of its carcinogenic effect and other known risks. The use of betel leaf, areca nut and tobacco in any form is unsafe for oral health.

The present study demonstrated that the percentage of micronuclei was significantly higher in smokers chewing a mixture of betel leaf, areca nut and tobacco than in non-smokers/non-chewers. The control subjects, who were healthy subjects with no apparent nutritional deficiencies, showed a minimal number of micronuclei when compared to experimental subjects. Finally, the present study showed that the buccal cell MN assay is a cost-effective and accurate procedure, which can be easily carried out for population-based studies.

Table 1 Characterization of participants

Groups	*n* = 44 (%)	Mean age
1. Control	15 (34.09%)	38.57 ± 0.54
2. Chewers with smoking habit	14 (31.80%)	34.50 ± 0.95
3. Chewers	15 (34.09%)	33.28 ± 0.89

Chewers: Person with Mixture of betel leaf, areca nut and tobacco chewing habit.
Control: Non smokers/Non chewers.

Table 2 Frequency distribution of micronuclei in buccal mucosa of betel leaf, areca nut and tobacco chewers

Subjects (Number)	Tobacco (Year)	Period of use (Year)	Cells with micronuclei (mean ± SD)
Chewers without smoking habit			
U01	–	27	3.80
U02	–	25	3.00
U03	–	20	2.33
U04	–	18	2.89
U05	–	15	0.30
U06	–	13	2.81
U07	–	9	0.80
U08	–	8	1.00
U09	–	7	0.40
U010	–	5	1.70
U11	–	4	1.20
U12	–	10	2.60
U13	–	16	1.00
U14	–	20	2.44
U15	–	12	2.30
		13.93 ± 6.80	1.90 ± 1.029
Chewers with smoking habit			
UA01	24	–	2.40
UA02	20	–	3.00
UA03	18	–	3.54
UA04	12	–	2.20
UA05	15	–	2.45
UA06	10	–	1.64
UA07	19	–	3.48
UA08	17	–	3.30
UA09	7	–	2.32
UA10	5	–	1.91
UA11	4	–	0.15
UA12	11	–	0.20
UA13	13	–	0.31
UA14	10	–	1.60
	13.21 ± 5.69		2.00 ± 1.119

Chewers-Mixture of betel leaf with areca nut and tobacco chewing.

Table 3 Frequency of distribution of micronuclei in buccal mucosa of controls

Subjects (Number)	Tobacco (Year)	Period of use (Year)	Cells with micronuclei (mean ± SD)
Non smoker/Non chewers			
C001	–	–	0.06
C002	–	–	0.33
C003	–	–	1.25
C004	–	–	2.52
C005	–	–	1.03
C006	–	–	0.95
C007	–	–	1.57
C008	–	–	0.20
C009	–	–	0.90
C010	–	–	1.68
C011	–	–	0.66
C012	–	–	0.18
C013	–	–	0.32
C014	–	–	0.45
C015	–	–	1.00
			0.81 ± 0.659

Acknowledgments

The authors are grateful to the authorities of Karpagam University, Coimbatore, Tamil Nadu, India for granting permission to use their facilities and for their encouragement, and also to the subjects for their cooperation.

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