Original Article

Micronucleus investigation in human buccal epithelial cells of gutkha users

Smita Jyoti, Saif Khan[1], Mohammad Afzal, Yasir Hasan Siddique

Department of Zoology, Human Genetics and Toxicology Laboratory, Section of Genetics, [1]Department of Periodontics and Community Dentistry, Dr Z.A. Dental College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Abstract

Background: Gutkha is a cheap and convenient betel quid substitute, which is popular among all age groups. Various studies reveal its carcinogenic nature that leads to oral submucosus fibrosis and increases the chances of oral cancer. The micronucleus (MN) assay in exfoliated mucosal cells is a useful method for observing genetic damage in humans.

Aim: To observe the genotoxic effect of gutkha on human buccal epithelial cells.

Materials and Methods: The MN assay was performed to assess the frequency of MN in human buccal epithelial cells. The study comprises 60 individuals of which 30 individuals were gutkha chewers and another 30 were nonusers (control). The MN frequency was scored to estimate the genotoxic damage.

Results: In gutkha users, the frequency of MN was highly significant (17.4 ± 0.944) as compared with nonusers (control) groups (4.53 ± 0.331) ($P < 0.001$).

Conclusions: The MN assay in human buccal epithelial cells is a useful and minimally invasive method for monitoring genetic damage in humans. A significantly higher frequency of micronucleated cells are found among gutkha users.

Key Words: Buccal, epithelial cells, gutkha, micronuclei

Address for correspondence:
Dr. Yasir Hasan Siddique, Department of Zoology, Human Genetics and Toxicology Laboratory, Section of Genetics, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India. E-mail: yasir_hasansiddique@rediffmail.com
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INTRODUCTION

Gutkha and pan masala are in more demand among all age groups. It is revealed that betel quid chewing with or without tobacco are carcinogenic in humans.[1,2] Gutkha is the mixture of areca nut, catechu, lime, cardamom, spices, unspecified flavouring agents, and tobacco. Gutkha is supposed to be responsible for a number of oral diseases and has addictive effects that leads to the addiction due to the presence of areca nut and tobacco.[3] Areca nut is a main component of gutkha, which is responsible for oral submucous fibrosis (OSMF).[4] OSMF is uncurable disease, and finally leads to oral cancer.[5] After long time of smoking, adverse effects are seen but in case of gutkha users, OSMF develops within a very short span of time.[6] The intake of gutkha and OSMF is very common in young persons.[7] Areca nut increases the chances of formation of precancerous lesion and OSMF. Micronuclei are small chromatin bodies that appear

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in the cytoplasm by the condensation of acrocentric chromosomal fragments or by whole chromosomes, lagging behind during cell division. Thus, it is the only biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells, that are easily detected in interphase cells.[8] MN assay has been used as a biomarker of genetic damage in buccal mucosa cells.[9,10] An elevated micronucleated cell frequency is found in the buccal mucosal epithelium of areca nut chewers.[11] The aqueous extract of N-nitroso compounds related to areca nut, that is, 3-(methylnitrosamino) proprionitrile is highly cytotoxic and genotoxic in cultured human buccal epithelial cells, and enhance the induction of tumors in betel quid chewers.[12] The MN assay in buccal cells can be used to detect cancerous or precancerous lesions and also to monitor the effects of a number of chemopreventive agents.[13,14] In the present study, the effect of gutkha was studied on the micronucleus (MN) frequency in buccal epithelial cells.

Aims and Objectives

The present study showed the frequency variation of MN in the chewers and nonchewers of gutkha by performing MN assay.

MATERIALS AND METHODS

Survey

The study comprised 60 male individuals out of whom 30 individuals were having the habit of chewing gutkha (cases), these were compared with the remaining 30 individuals who were nonusers (control: Those who did not involve in any addiction). A written consent was taken from each individual, and the samples were taken from the Department of Ziauddin Ahmed Dental College and Hospital, A.M.U. Aligarh, U.P. The period of the study was almost 6 months.

Chemicals

Trizma hydrochloride (Tris–HCl), ethylene diamine tetraacetic acid (EDTA) from SRL, India. Giemsa stain, sodium chloride, methanol, and sodium hydroxide pellets from Merck (India). The buffer solution was prepared by dissolving 0.1 M EDTA, 0.001 M Tris–HCl and 0.02 M NaCl in a sterile 1 L distilled water. The pH of the buffer was adjusted to 7.0 with NaOH.

Oral Mucosa Cell Collection and Processing

Oral mucosa cells were collected from each subject using a soft toothbrush gently from the oral mucosa of cheeks.[15] The brush was then swirled into a centrifuge tube containing a buffer solution of pH 7.0, thereby creating a cell suspension. The cells were washed three times by centrifugation at 1500 rpm for 10 min in the buffer solution.[15] About 15 mL of buffer in a 30 mL conical tube was used in every washing step. About 50–100 μL of the cell suspension was laid and spread on clean, preheated (37°C) glass slide and allowed to air dry for 5–10 min. The slides were fixed in methanol, stained with 5% Giemsa and observed under microscope.[16] A total of 2000 oral mucosal cells were scored per individual.

Statistical Analysis

Statistical analysis was carried out by Student's *t* test using commercial software Statistica Soft Inc.

RESULTS

MN frequency among individuals having chewing habit was found to be 4 times higher (21.3 ± 1.788) as compared with the control (4.56 ± 0.331) [Table 1]. The number of micronucleated cells in the controls and cases were 4.53 ± 0.331 and 17.4 ± 0.944, respectively [Table 1]. The distribution of micronucleated cells is given in [Table 2] and Figure 1. The age distribution of cases and controls is given in Table 3. Among users, the youngest was of 12 years and the oldest one was of 65 years of age. Figures 2–5 shows the MN in buccal epithelial cells of the users.

DISCUSSION

MN has been used since 1937 as an indicator of genotoxicity.[17] Studies on MN frequencies support that MN as a product of early events in human carcinogenic processes, particularly in oral regions.[14,18,19] MN test is especially used for the identification of preclinical steps of the cancer.[20] Various studies from 1985 till date have shown significant increase in micronucleated frequency in betel quid chewers as compared with healthy individuals.[21] The present study shows the higher frequency of MN

Table 1: Total micronucleus frequency per 2000 cells per individual in the buccal region of 30 cases and 30 controls

Group	Number of individuals	Age range	Age (Mean±SE)	MC (Mean±SE)	TM (Mean±SE)
Control	30	16–85	31.4 ± 2.518	4.53 ± 0.331	4.56 ± 0.331
Cases	30	12–65	29.7 ± 2.46	17.4 ± 0.944[a]	21.3 ± 1.788[a]

MC, micronucleated cell; TM, total number of micronucleus. [a]Significant at $P<0.001$ compared with control.

Table 2: Micronucleus distribution from combined data

Number of micronucleus	Number of controls	Number of cases
1	29	6
2	1	14
3	0	7
4	0	3
Total number of individuals	30	30

Table 3: Age distribution of cases and controls

Age group	Control	Cases
≤ 24	12	14
25–40	13	10
>40	05	06
Total	30	30

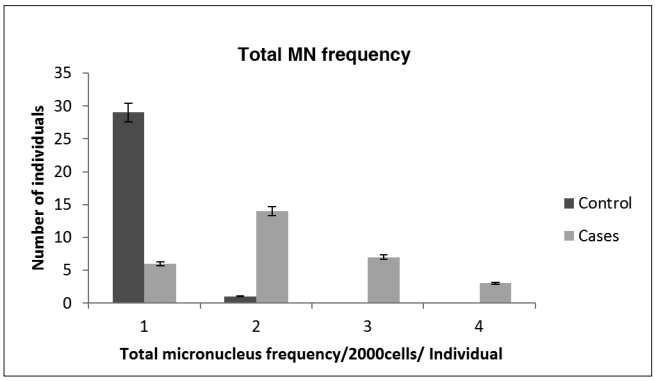

Figure 1: Total micronucleus frequency in 2000 cells

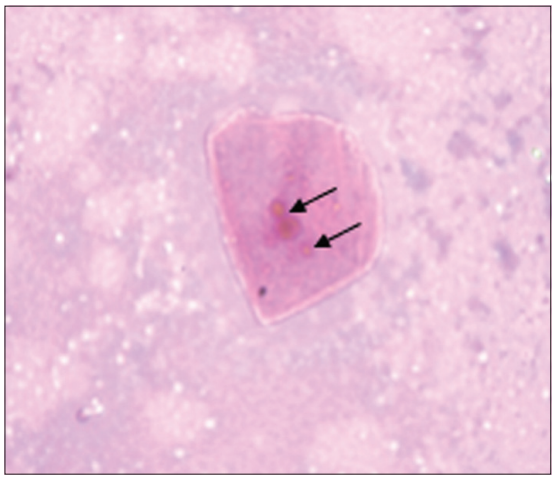

Figure 3: Buccal epithelial cell with two micronuclei

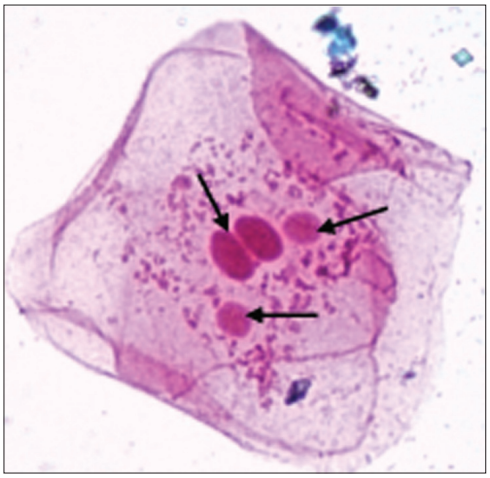

Figure 5: Buccal epithelial with Binucleated cell and two micronuclei

among the users of gutkha, this was proved by previous studies.[22,23] The main carcinogens in gutkha is derived from their ingredients areca nut, catechu, and tobacco. Tobacco-specific nitrosamines are formed due to chewing of gutkha.[24] That leads to exposure of buccal cells to volatile nitrosamines derived from areca nut alkaloids.[25] A high level of nitrite and nitrate reductase activity have been reported in the saliva of gutkha chewers.

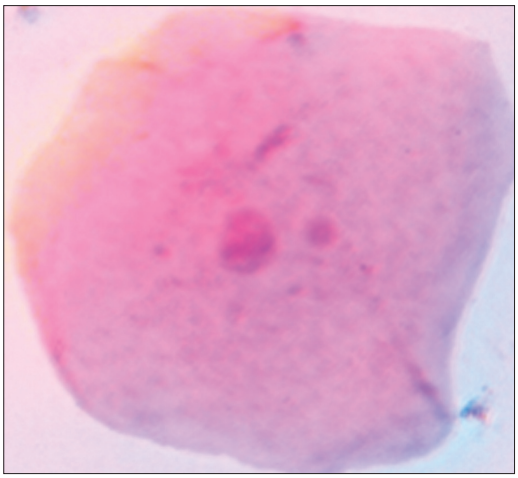

Figure 2: Buccal epithelial cell with one micronuclei

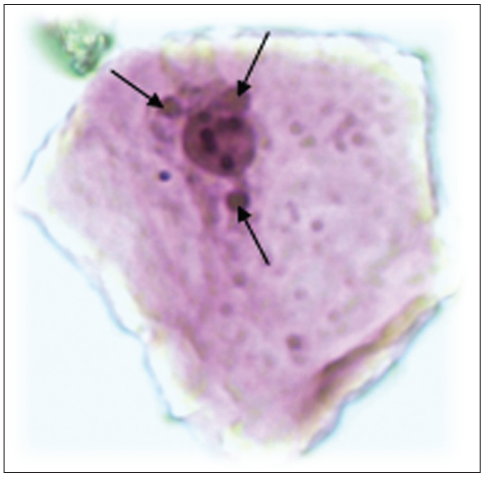

Figure 4: Buccal epithelial cell with three micronuclei

[26] Swallowing of the quid leads to the nitrosation of secondary and tertiary amines due to the acidic pH of stomach. Urinary levels of N-nitrosoproline were 4- to 6.5-fold higher in gutkha chewers.[27,28] Aqueous extracts of areca nut and catechu responsible for the generation of reactive oxygen species that cause the genotoxic damage in buccal epithelial cells.[29] Variations in the number of micronucleated cells may be affected by the ingredients in the quid, the number of quids per day and different lifestyles, gender, age, and food habits.[30] We observed the difference in the frequencies of micronucleated cells in the control group, which may be due to the different food habits of the population groups. Individuals ingest various types of chemicals in their daily diet, which was the reason for the variable levels of micronucleated cells.[31] The duration of addiction of the chewing habit in the present study of 30 individuals was in average of 1–20 years and their frequency was 2–18 pouches/day. A majority of degenerative and developmental diseases are caused by genomic damage, which is produced by environmental exposure of radiation, chemicals, micronutrient deficiency, and

lifestyle factors, for example, alcohol, smoking, drugs, gutkha, pan masala and stress. So it is important to biomonitoring, identifying, and treatment of diseases caused by, or associated with genetic damage. The MN assay in buccal cells serves as an excellent biomarker.[32] A supplement of vitamins and beta-carotene found to be an effective measure used for reduction in the number of micronucleated cell frequency in healthy chewers as well as precancerous lesions.[33]

CONCLUSIONS

This study reveals that gutkha is highly genotoxic and responsible for oral cancer in near future, so it is important to increase the awareness programs to inform and educate the public regarding the adverse health consequences and possible cancer risk associated with gutkha.

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