

Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers

Venkatesh Vishwanath Kamath, Praveen Anigol¹, Krishnanand Setlur

Departments of Oral and Maxillofacial Pathology, Dr. Syamala Reddy Dental College, Hospital and Research Centre, Bengaluru, ¹PM Nadevouda Dental College, Bagalkot, Karnataka, India

ABSTRACT

Background: Micronucleus is a microscopically visible round or oval cytoplasmic chromatin mass in the extra nuclear vicinity, originated from aberrant mitosis, which consists of eccentric chromosomes that have failed to reach spindle poles during mitosis and are used as biomarkers for assessment of DNA damage. Micronuclei are characteristically seen in exfoliated cells of the buccal mucosa and urinary bladder wall in precancerous and cancerous conditions. Oral habits of smoking tobacco or chewing areca nut damage the oral tissues. An assessment of the damage is feasible by the detection of micronuclei in exfoliated cells of the oral tissues using smears. **Objectives:** The present study was designed to assess the presence or increase of micronuclei in buccal smears of individuals with tobacco habits against a control group of teetotalers. **Materials and Methods:** Two groups (smokers and non-smokers) of 50 individuals each were examined. Buccal smears of all participants were taken using cytobrush and stained with standard Papanicolaou's (PAP) stain. Presence of micronuclei was assessed under $\times 100$ magnification and a count per 500 cells was determined. The results were analyzed statistically using *t*-test and Mann-Whitney test. **Results:** Smears of individuals with tobacco habits showed a significant increase in the total number of micronuclei per 500 cell counts. There was a definite correlation between the occurrence of micronuclei and the frequency and duration of smoking. A paradoxical age related increase in middle-aged groups was also observed. **Conclusions:** The genotoxic effects of tobacco smoke cause chromosomal damage in the epithelial cells of the oral mucosa and are reflected in the increased micronuclei in smokers. This is present even in the absence of clinically evident changes. This observation is vital in utilization of the micronuclei detection in smears as a prognostic, educational and interventional tool in the management of patients with smoking habits.

Key words: Exfoliated cells, micronuclei, oral cytology, papanicolaou, smear, smoking

INTRODUCTION

Oral cancer affects as many as 274,000 people world-wide annually and the frequency of oral cancer around the world is often indicative of the patterns of use of tobacco products. It has been established that there is a dose-response relationship between the amount of tobacco product used and the development of oral cancer.^[1]

All parts of the oral cavity are susceptible to cancer from tobacco smoking or chewing, including the lip, tongue, palate, gum and cheek. There exists today a need to identify biomarkers of oral cancer and their association with tobacco usage. When compared with other body sites, the mouth offers a unique opportunity for defining biomarkers because the mouth permits noninvasive, repetitive examinations in longitudinal studies of tobacco-associated acute and chronic diseases.^[1]

As a normal process of regeneration superficial cells of the oral mucosa are constantly shed over a specified period (cell turn over time) and these cells are accessible for cytological study through a smear. The local milieu of the oral cavity exerts an influence on the cells of the oral mucosa. These influences are reflected as cellular changes in exfoliated cells.

Access this article online

Quick Response Code:



Website:

www.ccej-online.org

DOI:

10.4103/2278-0513.125794

Address for correspondence: Dr. Venkatesh Vishwanath Kamath, Department of Oral and Maxillofacial Pathology, Dr. Syamala Reddy Dental College, Hospital and Research Institute, Munnekolala, Marathalli, Bengaluru - 560 037, Karnataka, India. E-mail: kamathvv2003@yahoo.com

The association between cigarette use and oral cancer has been firmly established from epidemiologic studies. Tobacco was first introduced to western civilization by the Spanish explorers of America in the early 16th century. Cigarettes were first made in Spain in the mid-17th century and in the 20th century, they became the most popular form of the tobacco habit.^[2]

A cigarette contains numerous cytotoxic substances, such as polycyclic aromatics hydrocarbons, aromatics amines, nitrosamines, heavy metals, poisonous gases and pesticide residues.^[3] It is well-established that these elements in the cigarette smoke exert a genotoxic effect on the oral tissues. The major addictive substance in cigarette tobacco is nicotine, which may exist in an ionized or a non-ionized form determined by the pH of the smoke. Alkaline smoke produced by cigars and pipe tobacco, nicotine, being predominantly non-ionized, is mainly orally absorbed.^[4]

Globally, about 82,000-99,000 young individuals are initiated to the habit of smoking a cigarette each day. The addiction component in cigarette smoking is nicotine. On an average an American cigarette contains 8-9 mg of nicotine whereas average Indian cigarette contains 15 mg. The increased level of this nicotine concentration causes individuals to get addicted.^[5]

The free radicals released as the combustion of cigarette have an adverse effect on oral and general health of an individual. It has been detected that cigarette smoke contains 1×10^{17} radicals/g of tar or 4×10^4 per puff using Electron Spin Resonance spectroscopy.^[6] Tar is a composite term for the particulate matter that can be condensed from tobacco smoke and is the most damaging component of tobacco smoke.^[4] Interestingly, most Indian cigarettes do not display the tar content despite it being a requirement by law.

Micronucleus (MN), a microscopically visible round or oval cytoplasmic chromatin mass in the extra nuclear vicinity, originates from aberrant mitosis and consists of eccentric chromosomes, chromatid fragments or whole chromosomes, which have failed to reach spindle poles during mitosis. MN has been used consistently as a biomarker for assessment of DNA damage.^[7]

Since 1937 micronuclei have been regarded as indicators for genotoxic exposure. Clinical studies show that the determination of the MN rates in different cytological preparations can be reproducible. The loss of chromatin in the main nucleus due to a mutagenic exposition, contributes to the formation of micronuclei.^[8]

Epithelial tissues are in immediate contact with inhaled and ingested genotoxic agents^[9] of the cigarette smoke

and are accessible for sample collection. More than 90% of cancers arise in epithelial tissues and these cells can be easily collected from the mouth^[9] without causing discomfort to patients. The standard laboratory procedure is feasible, cheap and accurate; final results can be obtained in less time.^[9]

Dr. George N. Papanicolaou was the first to introduce PAP smear in 1928 in cervical tissues and since then this technique has helped reduce cervical cancer incidence and mortality rates by 75%.^[10] This is an easy technique and can be replicated in the oral cavity for analysis of the changes caused by smoking.

The present study was designed to identify and correlate the occurrence of MN in exfoliated oral squamous cells in smokers without clinically evident lesions.

MATERIALS AND METHODS

Two groups, smokers ($n = 50$) and nonsmokers ($n = 50$), aged above 18 years with sound medical history were randomly selected. Criteria for inclusion were pursuance of the smoking habit for more than six months in the smoker group and absence of any clinically evident changes in the oral cavity related to the habit. Alcohol consumption in any form was an exclusion factor for both groups. No attempts were made to balance sex bias as all smokers were male. Consent forms of all participants were taken and approval from the Ethical Committee was obtained. After a thorough medical history and oral examination participants were asked to rinse the oral cavity. Using a sterile cyto-brush, buccal smears were taken and fixed in alcohol (Bio-fix). The smears were stained using standard Papanicolaou (PAP) staining protocol. The smears were observed under 100 magnifications. An eyepiece grid was used and 500 cells per slide were counted for micronuclei. MN identification and scoring was performed following the criteria established by Tolbert *et al.*^[11]

RESULTS

There was a definitive expression of micronuclei in cells cultivated from the smears [Figure 1]. Micronuclei were single or multiple in many smear cells and more prominent in the smoker group [Figure 2] than in the non-smokers [Figure 3]. None of the smear cells showed any dysplastic changes [Figure 4].

The smoker group presented higher MN count compared with that of the non-smoker group. Results were statistically significant using *t*-test ($P < 0.01$) [Table 1].

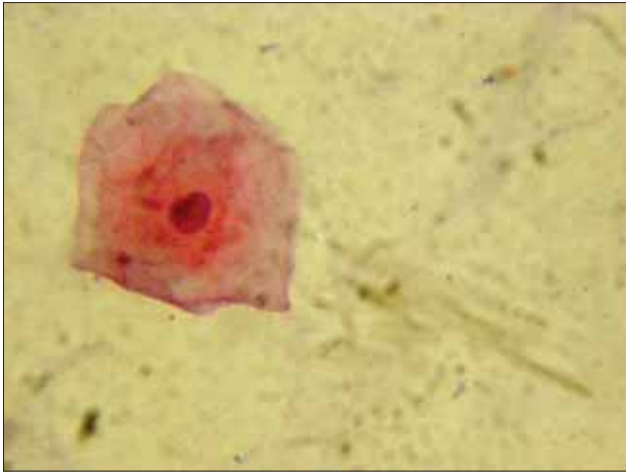


Figure 1: Smear from non-smoker showing morphology of a squamous cell without micronuclei (PAP, ×100)



Figure 2: Single squamous cell in a smoker showing multiple micronuclei (arrows). Note the fusion of micronuclei in one end (PAP, ×100)

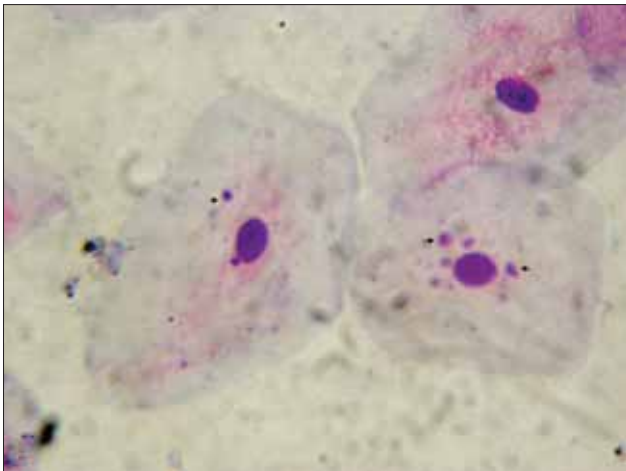


Figure 3: Smear from non-smoker showing clump of squamous cells with one cell exhibiting micronuclei (PAP, ×100)

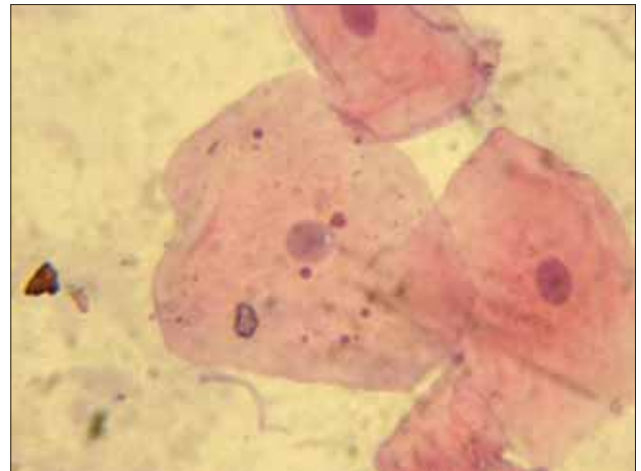


Figure 4: Smear from smoker showing multiple micronuclei and granular cytoplasmic changes in one cell (PAP, ×100)

On comparing the MN count in relation to the age of the samples, the age group between > 30 years showed more MN count than the age group between 18 and 30. However, statistically the *P* value was not significant [Table 2].

Duration of smoking was correlated with MN count. Groups smoking 5-10 years showed more MN count than the groups smoking <5 years and >10 years [Table 3].

When frequency of smoking was related to the MN count, the group smoking >10 cigarettes/day revealed high MN count than the groups smoking 5-10 cigarettes/day and >10 cigarettes/day [Table 4].

DISCUSSION

Cancer is one of the most life-threatening diseases afflicting mankind. The word “cancer,” in itself, generates fear

Table 1: Statistical analysis of micronuclei in smokers and non-smokers

Group	Mean	Standard deviation	Mean difference	<i>t</i>	<i>P</i> value
Smokers	57.96	32.46	23.04	2.847	0.006
Non-smokers	34.92	24.17			

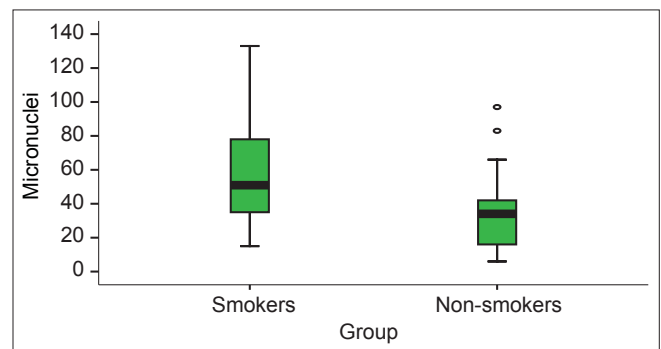


Table 2: Micronuclei count in relation to age in smokers

Age group (years)	N=Total number of members	Mean	Standard deviation	Mean difference	t	P value
18-30	34	54.65	34.91	-10.353	-0.737	0.469
>30	16	65.00	27.26			

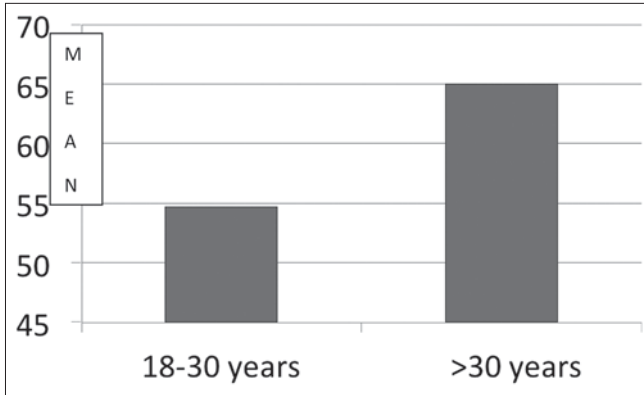
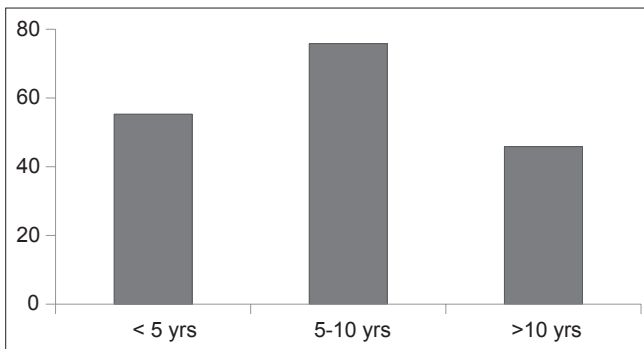


Table 3: Micronuclei count correlated with duration of smoking

Duration of smoking (years)	N=Total members in the group	Mean	Standard deviation	F	P value
<5	26	55.31	32.03	1.420	0.263
5-10	12	75.83	37.78		
>10	12	45.83	24.29		

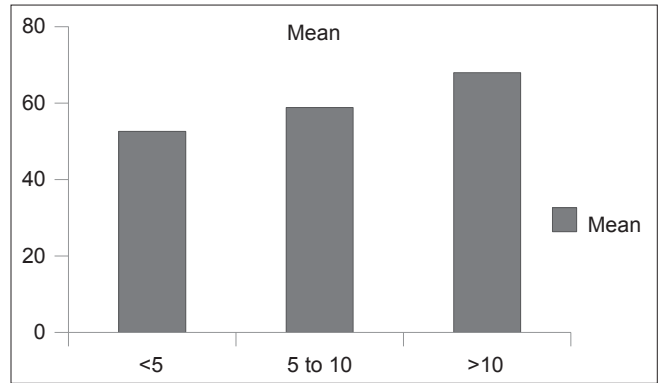


amongst all human beings, to whichever strata of the society they may belong. It is often diagnosed at an advanced stage because of the lack of early diagnostic markers and therefore, the survival rate is markedly reduced despite the best available treatment options. Oral cancer mostly occurs as a result of malignant transformation of a preexisting lesion. It has been associated with smoking, tobacco chewing and alcohol consumption.^[12]

Biomarkers are instruments of individual tumor prevention and help to detect high-risk patients. They are divided into three groups: First to define the exposure to carcinogenic agents, the second to show biological effects on the target

Table 4: Micronuclei in relation to frequency of smoking

Frequency of smoking (cigarettes/day)	N=Total members in the group	Mean	Standard deviation	F	P value
<5	16	52.63	29.35	0.241	0.788
5-10	28	58.86	32.81		
>10	6	68.00	48.54		



tissue and the third to give information about the individual susceptibility.^[13] The MN assay in exfoliated buccal cells is a useful and minimally invasive method for monitoring genetic damage in humans.^[14]

The use of the MN test on exfoliated cells as an approach to identify genotoxic damage in human tissues that are targets for organ specific carcinogens and from, which carcinoma will develop is well-established. Chromosomal damage by carcinogens in dividing basal cells of the epithelium results in the production of micronuclei in the daughter cells that migrate up through the epithelium and are exfoliated.^[15]

In the present study, MN count was assessed in the smoker group and compared with non-smokers to recognize the population group at an elevated risk of cancer. A significant increase in the MN in smokers was found compared with non-smokers indicating the genotoxic effect of tobacco smoke. Sarto *et al.*^[16] showed that MN count was double in the smoker group as compared with the non-smokers with high statistical significance, which is in accordance with our results. We also support the suggestion given by Sellappa *et al.*^[17] who stated that oral cavity offers a unique opportunity to define biomarkers as it permits non-invasive examination in longitudinal studies of smoking.

There was increase in MN frequency in the group aged above 30 years that is in accordance with previous studies.^[18] We suggest that this effect could be due to compounded genotoxic effect of tobacco on mucosal cells

over a period of time. It is well-known effects of genotoxic agents such as radiation and tobacco are cumulative and DNA damage is passed on to the daughter cells. The effects of these damages reflected by MN may lead to the development of pre-cancer and cancer. Thus, MN assessment and a high index would presuppose predisposition of the individual to the development of these changes. This knowledge can be used to monitor, prognosticate and educate the individuals into cessation of the habits and thus prove to be an educational and screening tool. Longitudinal studies need to be done to quantify and support this contention.

Interestingly, higher mean MN was found in people with 5-10 years of smoking duration followed by those with >10 years and 5-10 years of duration. This paradoxical observation could probably be explained by the fact that development of MN is a cytological feature under regulation of the nucleus. Increased MN count was found in people who smoke >10 cigarettes/day followed by those who smoke between <5 cigarettes/day and 5-10 cigarettes/day. This is due to the increased genotoxic effect of cigarette smoke on the oral mucosa and is in accordance with the previous study.^[19]

The frequency of occurrence of MN is a measure of chromosome breakage in early cell divisions and the number of micronuclei is known to increase with carcinogenic stimuli, long before the development of clinical symptoms. The advantage of MN assay lies in its simplicity, as the scoring of micronuclei is rapid and does not require much expertise. Thus, MN in a cell represents an “internal dosimeter” to estimate exposure to genotoxic and carcinogenic agents.^[12]

SUMMARY AND CONCLUSIONS

The present study highlights the use of oral exfoliative cytology as an effective tool in non-invasive screening of population under the risk of oral cancer. In our study, there was a direct correlation with increased MN in smokers and increased correlation of presence of MN with age, duration and frequency of smoking. Interestingly, peak MN detection was seen in patients in the 3rd decade and above, when effects of smoking would be considered the most. It was also found that direct correlation in the expression of MN in patients with increased frequency of smoking (>10 cigarettes/day).

We strongly feel and conclude that assessment of pre-dysplastic changes using noninvasive, easy procedure like smears can be advocated in all routine field screening and MN would be better indicators for such changes and

patients with high risk can be counseled by delivering the ill effects of tobacco accordingly.

REFERENCES

1. Proia NK, Paszkiewicz GM, Nasca MA, Franke GE, Pauly JL. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer – A review. *Cancer Epidemiol Biomarkers Prev* 2006;15:1061-77.
2. Silverman Jr S. *Oral Cancer*. American Cancer Society. 4th ed. London: B.C. Decker Inc.; 1998.
3. Crispino CC, Fernandes KG, Kamogawa MY, Nóbrega JA, Nogueira AR, Ferreira MM. Multivariate classification of cigarettes according to their elemental content determined by inductively coupled plasma optical emission spectrometry. *Anal Sci* 2007;23:435-8.
4. Blakely T, Bates M. Nicotine and tar in cigarette tobacco: A literature review to inform policy development. Prepared as a Ministry of Health Contract for Scientific Services, 1998. Available from: [http://www.moh.govt.nz/moh.nsf/pagescm/1006/\\$file/nicotinetartobacco.pdf](http://www.moh.govt.nz/moh.nsf/pagescm/1006/$file/nicotinetartobacco.pdf).
5. Oral Cancer. Report of global adult tobacco survey India 2010. Available from: <http://www.oralcancerawareness.blogspot.com/2010/10/report-of-global-adult-tobacco-survey.html>.
6. Wigand JS. Additives, cigarette design and tobacco product regulation. A report to: World Health Organization tobacco free initiative tobacco product regulation group. Available from: <http://www.jeffreywigand.com/whofinal.pdf>.
7. Sivasankari NP, Kaur S, Reddy KS, Vivekanandam S, Rao KR. Micronucleus index: An early diagnosis in oral carcinoma. *J Anat Soc India* 2008;57:8-13.
8. Bloching M, Reich W, Schubert J, Grummt T, Sandner A. Micronucleus rate of buccal mucosal epithelial cells in relation to oral hygiene and dental factors. *Oral Oncol* 2008;44:220-6.
9. Alexandrescu I, Havârneanu D, Popa D. New approaches in biomonitoring human populations exposed to genotoxic agents: Epithelial cell micronucleus assay. *J Prev Med* 2006;14:57-65.
10. Mehta V, Vasanth V, Balachandran C. Pap smear. *Indian J Dermatol Venereol Leprol* 2009;75:214-6.
11. Palaskara S, Jindal C. Evaluation of micronuclei using Papanicolaou and May Grunwald Giemsa stain in individuals with different tobacco habits – A comparative study. *J Clin Diagn Res* 2010;4:3607-13.
12. Kamboj M, Mahajan S. Micronucleus – An upcoming marker of genotoxic damage. *Clin Oral Investig* 2007;11:121-6.
13. Bloching M, Hofmann A, Lautenschläger C, Berghaus A, Grummt T. Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral Oncol* 2000;36:550-5.
14. Halder A, Chakraborty T, Mandal K, Gure PK, Das S, Raychowdhury R, *et al.* Comparative study of exfoliated oral mucosal cell micronuclei frequency in normal, precancerous and malignant epithelium. *Int J Hum Genet* 2004;4:257-60.
15. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, *et al.* The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. *Mutat Res* 2008;659:93-108.
16. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis* 1987;2:11-7.

17. Sellappa S, Balakrishnan M, Raman S, Palanisamy S. Induction of micronuclei in buccal mucosa on chewing a mixture of betel leaf, areca nut and tobacco. *J Oral Sci* 2009;51:289-92.
18. Di Giorgio M, Nasazzi NB, Heredia MI, Fernandez JA. Influence of age, sex and life style factors on the spontaneous and radiation induced micronuclei frequencies. Presentado en el Ninth International Congress of Radiation Protection (IRPA9), 1996. Available from: <http://www.arn.gov.ar/menoriat/mt95-97/pi4-96.pdf>.
19. Bonassi S, Neri M, Lando C, Ceppi M, Lin YP, Chang WP, *et al.*

Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the Human MicroNucleus project. *Mutat Res* 2003;543:155-66.

Cite this article as: Kamath VV, Anigol P, Setlur K. Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers. *Clin Cancer Investig J* 2014;3:49-54.

Source of Support: Nil, **Conflict of Interest:** None declared.