ORIGINAL RESEARCH PAPER

Pathology

HISTOPATHOLOGICAL GRADING OF ORAL SQUAMOUS CELL CARCINOMA BY MICRONUCLEI ASSAY, A POTENTIAL BIOMARKER IN ORAL EXFOLIATED CELLS

KEY WORDS: Micronuclei; oral exfoliative cytology; oral squamous cell carcinoma

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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 in exfoliated oral epithelial cells have been shown in some studies to correlate with severity of this genotoxic damage. This severity can be measured in terms of grading of the lesions.

Aim: To correlate frequency of micronuclei (MN) in oral exfoliated cells in clinically diagnosed cases of oral squamous cell carcinoma (OSCC) followed by a histopathological grading.

Materials and Methods : The study subjects consisted of clinically diagnosed cases of OSCC. Healthy subjects without any tobacco consumption habits formed the control group. The cytosmears from both groups were stained with rapid Papanicolaou stain. MN were identified according to the criteria given by Tolbert et al.

Results: The frequency of MN was three to four times higher in patients with OSCC as compared to patients in the control group and the difference was found to be highly significant. In 75% cases, the cytological grade as determined by the frequency of micronuclei correlated with the histopathological grade and this observation was statistically significant.

Conclusions : The MN is potentially an excellent candidate to serve as such a biomarker for prediction of the grade of OSCC.

INTRODUCTION

The micronucleus (MN) assay in exfoliated buccal cells is a useful and minimally invasive method and has generally been used as a biomarker of chromosomal damage, genome instability and cancer risk.[1] Micronuclei are extranuclear cytoplasmic bodies. They are induced in cells by numerous genotoxic agents that damage the chromosomes. The damaged chromosomes, in the form of acentric chromatids or chromosome fragments, lag behind in anaphase when centric elements move towards the spindle poles. After telophase, the undamaged chromosomes, as well as the centric fragments, give rise to regular daughter nuclei. The lagging elements are included in the daughter cells, too, but a considerable proportion is transformed into one or several secondary nuclei, which are, as a rule, much smaller than the principal nucleus and are therefore called micronuclei.[2]

Various studies have shown the correlation of frequency of MN and severity of this genotoxic damage. This severity can be measured in terms of grading of the lesions. This especially holds true for oral squamous cell carcinoma (OSCC). There is lot of controversy surrounding the criteria to be followed for identification and method of counting of MN. To address these uncertainties, the human micronucleus project (HUMN) has initiated a new international validation project for the buccal cell MN assay similar to that previously performed using human lymphocytes. The ultimate goals of the HUMN project are to standardize methodologies, define baseline MN frequency rates, characterize the effects of demographic, genetic, and methodological factors on these frequencies, and to determine whether the MN frequencies in different tissue are predictive of cancer risk.[3]

Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products. Approximately 90 percent of human cancers originate from epithelial cells. Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion.

Recent studies are attempting to show the correlation between frequency of MN in oral exfoliated cells and histopathological grading of OSCC.[4] Present study has made an attempt to validate such correlation, if any. We also put forward a new modified criterion in relation to assessment of size of MN and the method of counting of MN.

AIMS AND OBJECTIVES

The aim of the study was the evaluation of correlation between the frequencies of MN in oral exfoliated cells from patient clinically diagnosed of OSCC. This was followed by histopathological grading of OSCC inthesamesubject. The objective was to test the hypothesis whether MN in oral exfoliated cells can be a parameter for grading of OSCC.

MATERIALS AND METHODS

Subjects included in the study were divided into the following groups:

Group I (study group): comprised 16 subjects diagnosed clinically of OSCC.

Group II (control group): comprised 16 subjects without any oral lesions and any history of habits of tobacco consumption. The age and gender of this group was matched with that of Group I.

A written consent was taken from subjects of both the groups.

Three observers participated in the study for analysis as mentioned below:

Observer 1: Counting of MN in Group I

Observer 2: Counting of MN in Group II.

Observer 3: Histopathological scoring and grading of OSCC.

The slides of each group were exchanged among all the observers, providing each observer a participation in each type of observation. They were not provided with information regarding the study subjects to prevent observer bias. The average score of three observations by the three observers was calculated for each group to resolve the interobserver bias.

Collection of exfoliated cells

To obtain the smear of exfoliated cells from the oral cavity (buccal mucosa in control group), a wooden spatula was used.

It was slightly moistened before applying on the mucosa. Patients were also asked to rinse the oral cavity before taking the samples.This was to remove food debris and necrotic slough, if any, which could hamper the quality of the cytosmear. In subjects suspected of OSCC, the most representative site was selected for obtaining the smear, like the margins of the lesion. A light gentle pressure was applied while scraping. The smears were fixed and stained with Papanicolaou (PAP) stain.Whole of the smear was screened for counting of MN. Smear was first screened at X400 for testing the quality of staining, followed by examination at X1000. MN were identified according to the criteria given by Tolbert et al.[5]

Biopsy procedure

Later on an Incisional biopsies were taken from the representative sites taking all aseptic precautions. The tissue specimens were labeled, fixed in 10% formalin for 24 hours, and paraffin embedded. The wax blocks were cut to obtain two tissue sections of 4-μm thickness for each block. The sections were stained by hematoxylin and eosin (H and E) and examined for histopathological grading.

Measurement of micronuclei

Methodological factors that can affect the levels of MN in buccal cells include differences in cell collection (timing and implements used), fixation and staining techniques, selection and number of cells counted. Another important aspect of the MN assay in buccal mucosa cells is the criteria for identifying and scoring the cells.

The suggested criteria for identifying MN by Tolbert et al.are:

(a) Rounded smooth perimeter suggestive of a membrane
(b) Less than a third the diameter of the associated nucleus, but large enough to discern shape and color
(c) Non-refractile and readily distinguishable from artifacts such as staining particles
(d) Staining intensity similar to that of the nucleus;
(e) Texture similar to that of nucleus;
(f) Same focal plane as nucleus; and
(g) Absence of overlap with, or bridge to, the nucleus

Screening of slide was done in a zigzag manner starting from one end of the slide and approaching towards the other end. A simultaneous counting of MN was done for each subject in each group. The average frequency of MN was further tabulated based on following formula:

Average frequency of MN=Total number of MN/ Total number of cells with MN.

Based on the average frequency of MN, a cytological grade was determined [Table 1].

Table 1: Cytological grade of OSCC based on the average frequency of micronuclei

Cytological grading of OSCC	Average frequency of micronuclei
Grade I	**1.0-2**
Grade II	**2.1-3**
Grade III	**3.1-4**

Histopathological grading

All the slides were observed under light microscope so as to see changes in epithelium, basement membrane, and connective tissue. Histopathological grading of squamous cell carcinoma was done according to the malignancy grading system proposed by Anneroth *et al.*[6] The slides were examined for various parameters such as degree of keratinization, nuclear polymorphism, number of mitoses per high-power field, break in the basement membrane, pattern of invasion, depth of invasion, inflammatory infiltrate, etc. Each parameter was scored according to its extent as points 1, 2, 3, or 4. Total malignancy point score for each patient was calculated by adding the points for each parameter and dividing the sum by the number of parameters examined. The final grading was done as follows.

Malignancy point score = Total score/Total number of parameters used.

Total malignancy point score between 1.0 and 2.0 = Grade I SCC; between 2.1 and 3.0 = Grade II SCC; and between 3.1 and 4.0 = Grade III SCC.

Based on malignancy point score, a histopathological grade was assigned to each case of OSCC [Table 2].The obtained cytological grade based on average frequency of MN was correlated with histopathological grade based on malignancy point score. The data obtained was statistically analyzed with the help of Student's *t*-test and Pearson's correlation.

Table 2: Histopathological grade of OSCC based on the malignancy point score

Cytological grade of OSCC	Malignancy point score
Grade I	1.0-2
Grade II	2.1-3
Grade III	3.1-4

RESULTS

The average frequency of MN in Group I and in Group II is summarized in [Table 3]. These observations give an unambiguous implication that the frequency of MN is three to four times higher in patients with OSCC as compared to the patients in control group. These results are also supported by statistical analysis [Table 4]. A highly significant difference was noted in the average frequency of MN in patients with OSCC as compared to subjects in the control group. PAP stained cytosmears revealed cells with MN ranging from one to four, or multiple with dissimilar sizes measuring less than 1/3 the size of the nucleus of the cell [Figure 1] [Figure 2]

Table 3: Frequency of micronuceli in oral squamous cell carcinoma OSCC (group I) and control group (group II)

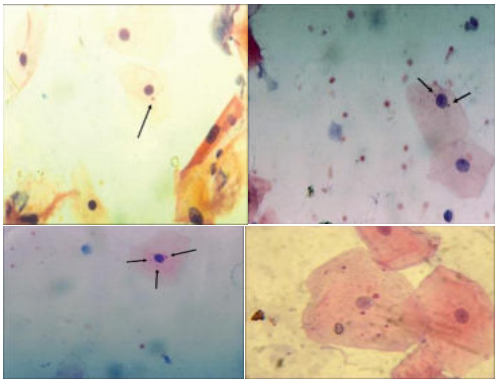
Group I (OSCC)	Group II (Control)
No.of micronuclei /No. of cell with micronuclei	**No. of micronuclei /No.of cell with micronuclei**
244/111	69/43
176/101	64/38
192/111	17/15
506/174	52/23
248/141	40/25
213/131	87/71
76/51	40/25
220/141	62/44
73/51	23/30
172/91	50/21

310/151	51/33
219/145	76/36
203/102	56/47
250/113	56/42
154/97	59/41
430/138	78/51

Table 4: Comparison of frequency of micronuclei between oral squamous cell carcinoma (OSCC) (group I) and control (group II). Statistical analysis is student t test.

Study group	Number of cases	MN range	Mean MN +SD	t -Value	p-Value	Significance
Control	16	17-87	54□18.71	6.194	0.000	Highly significant
OSCC	16	73-506	228.44 □110.69			

Figure 1: PAP stained cytosmears revealing cells with MN (×400): (a) cells with only one micronucleus; (b) cells with two MN; (c) cells with three MN; (d) cells with four MN (x1000)



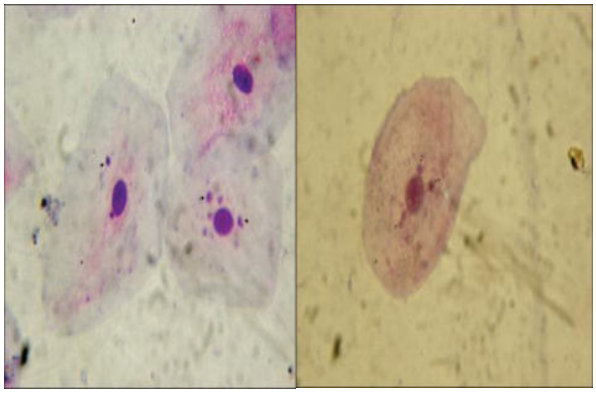


Figure 2: PAP stained cytosmears revealing cells with MN (×1000): (a) cells with multiple micronucleus; (b) cells with three MN;

When cytological grade (based on average frequency of MN) was compared with histopathological grade (based on malignancy point scoring) [Table 5], it was observed that out of 16, cytological grade of 14 subjects (87%) was correlating with the respective histopathological grade. This observation was statistically supported by Pearson's correlation indicating highly significant correlation between the cytological grade and histopathological grade [Table

Table 5: Comparison of cytological grade (based on average frequency of micronuceli) and histological grade (based on malignancy point score)

Average frequency of Micronuclei	Malignancy point score	Cytological Grade	Histological Grade
2.19	2.3	Grade II	Grade II
1.74	1.5	Grade I	Grade I
1.72	1.6	Grade I	Grade I
2.90	2.2	Grade II	Grade II
1.75	1.6	Grade I	Grade I
1.62	2.3	Grade I	Grade II
1.49	2.1	Grade I	Grade II
1.56	1.3	Grade I	Grade I
1.43	1.6	Grade I	Grade I
1.89	1.6	Grade I	Grade i
2.05	2.0	Grade I	Grade I
1.51	2.1	Grade I	Grade I
1.99	1.8	Grade I	Grade I
2.21	2.6	Grade II	Grade II
1.58	1.8	Grade I	Grade I
3.11	3.3	Grade III	Grade III

Table 6: Statistical analysis for the comparison of cytological grade and histological grade (Spearman's correlation)

Grade	Number of cases	r-Value	p-Value	Significance
Cytological Grade	16	0.654	0.006	Highly Significant
Histopathological Grade	16			

DISCUSSION

DNA damage caused due to the use of tobacco and related products, assessed by MN test is found to be most sensitive when compared with other tests as it neither requires tedious procedures such as cell culture and metaphase preparation nor it requires any specific DNA stains. MN is a microscopically visible round or oval cytoplasmic chromatin mass in the extranuclear vicinity. They originate from mitosis and consist of eccentric chromosomes, chromatid fragments, or whole chromosomes, which failed to reach spindle poles during mitosis. [7] As the focus is to detect early genotoxic damage, the MN test provides a simple, noninvasive, yet reliable screening technique for assessing early genotoxic damage much before any clinical or histological signs of cancer is evident. 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. [8]

The International Collaborative Project on Micronucleus Frequency in Human Populations (HUMN) was organized to collect data on MN frequencies in different human populations and different cell types to determine the extent to which MN frequency is a valid biomarker of ageing and risk for diseases such as cancer.[3] The hypothesis of a direct association between the frequency of MN in target or surrogate tissues and cancer development is supported by the findings like clear increase in the frequency of MN in target tissues as well as in peripheral lymphocytes in cancer patients.[8]

In the present study, making use of exfoliated buccal cells for assaying MN can be arguably explained on two bases. First, since approximately 90% of human cancers originate from epithelial cells, they represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body by way of inhalation and ingestion. The second reason is that the collection of buccal cells is certainly the least invasive method available for measuring the DNA damage in humans. This holds especially true in comparison to obtaining blood samples (for lymphocyte and erythrocyte assays) or obtaining tissue biopsies.[8] Exfoliated buccal mucosa cells can be easily collected using a wooden tongue depressor, a metal spatula, or a cytobrush moistened with water or buffer to swab or gently scrape the mucosa of the inner lining of one or both cheeks. Although cytobrushes appear to be most effective for collecting large numbers of cells, the high expense makes them less feasible for routine purposes. So, we preferred the use of a wooden spatula. Casartelli e*t al.*[9] observed that MN frequencies were higher when cells were collected by vigorous, rather than by light, scraping.

Various methodological factors can affect the levels of MN in buccal cells. The main sources of variability among the laboratories may lie in the scoring criteria and staining procedures used. The effects of these factors on MN assay in the buccal cells have not been properly evaluated or quantified. There are various criteria given by various authors.[5,10,11,12] According to Countryman *et al*.[12] and Sarto *et al.*[10], the criteria for identifying a structure as micronucleus is that it should be of the size less than 1/3 of the diameter of the associated nucleus, but still large enough to discern the shape and color. Whereas according to Belien *et al*.[11] the size should be less than 1/5 of the size of the parent nucleus. These two different views are creating a perplexity. So, in the present study, the criteria regarding assessment of size of MN have been modified.

Sarto *et al.*[10] described that a distinction between MN arising from chromosome breakage and spindle disturbances should be made by restricting the area of a micronucleus to 1/5 of the parent nucleus. Although a number of authors do not explicitly distinguish between the two types of MN, they in fact do distinguish them by their restriction on the diameter or area.[10]

After looking at this diverse origin for MN, it becomes very hard to believe that the size of MN is a constant value. Instead, it should be measured in terms of a range. Bigger MN result from exclusion of whole chromosome, following damage to the spindle apparatus of the cell (aneugenic effect), whereas smaller MN result from structural aberrations causing chromosomal fragments (clastogenic effect). Considering these facts, in the present study, the size of MN has been considered as ranging from 1/3 to 2/3 of the size of the nucleus. This proposition has also been supported by the observations of cells with varied sizes of MN [Figure 3].

Sarto *et al.*[10] have considered the cells from normal mucosa, in order to standardize the size of MN. But this criteria cannot be applied for precancerous and cancerous lesions, as it is very well known that in a cell undergoing atypia secondary to genetic damage, there is always an increase in the size of the nucleus and so also could be the size of micronucleus.[8]

The method of counting of MN also needs to be modified. In many previous studies a method of random screening of 1,000-10,000 cells has been proposed, irrespective of whether they contain MN or not.[4][8] According to these authors, an average frequency of MN was given as:

Average frequency of MN=Total number of MN/1000-10,000 cells.

All the cells in the denominator of above formula (1000-10,000) do not always necessarily contain MN. So, this denominator value does not hold to be significantly convincing and needs to be replaced by the total number of cells which in point of fact do contain MN. Consequently, we intend to put forward a modified formula as follows Average frequency of MN=Total number of MN/Total number of cells with MN.

Belien et al.[11] have concluded that at least 10,000 cells should be screened to monitor a significant reduction of 50% in the number of MN. The present study focused at screening of the whole smear for cells with MN in order to obtain a large sample size which directs us to a more reliable method of counting of MN.

The MN assay has been reported to correlate well with the histological grading of OSCC and leukoplakia. Incidence of MN has been analyzed by various studies in normal patients, oral premalignancies and OSCCs.[1] We detected that the average frequency of MN in patients with OSCC was ranging from 1.05 to 3.1 MN per cell. whereas it ranged from 1.4% to 9.15% in the SCC group as obtained by Kumar V *et al.* [13] Thus the levels in the present study were slightly lower than those reported by Kumar V *et al.* In the present study, exfoliated cells were utilized to make the smears for micronucleus analysis. Exfoliative cytology does not include the cells from the basal layer, which actually has the cells in the dividing stage that are more prone to genotoxic damage. These cells that mature and reach to the most superficial layer can then become available for screening when exfoliative cytology is executed. Therefore, the smears made by exfoliative cytology may have less chance of including micronucleated cells. This may be the reason for slightly lower levels of mean micronucleus frequency in the present study. In the study by Kumar V *et al.,* the cells from SCC lesions were obtained by mincing the biopsy tissue and preparing single cell suspensions from it. Since after mincing and making cell suspensions the smears include cells from all the epithelial layers (basal layer to superficial), the smears have more chance of including micronucleated cells. The other reason for higher levels in the study by Kumar V *et al.* may be that they had followed a fluorescent-acridine orange staining method and the analysis was done under fluorescence microscope, increasing the specificity to identify DNA-containing structures. This technique is a time consuming method, and it requires costlier chemicals and equipment. Therefore, in the present study, rapid papanicolaou technique was used in place of fluorescent dyes for staining purpose since it is very simple to use, less time consuming, and economical. Although the method of counting of MN was modified as compared to previous studies, these results are correlating with the results of the studies conducted by Palve and Tupkari, [4] where the overall level of MN in the OSCC group was observed to be in the range of 1.1-3.0.

There was a significant correlation between the cytological grade (based on average frequency of MN) and the histological grade (based on malignancy point score) with coefficient of correlation (r value) being 0.654 and level of significance of 0.006 (P<0.05). This observation was similar to those reported by Kumar *et al.* [13] and Palve and Tupkari, [4]

where the frequency of MN increased significantly from grade I to grade II to grade III, respectively, in squamous cell carcinoma group. Comparable results were obtained for increasing grades of OSCC in the present study.

Palve and Tupkari [4] have raised a question regarding subjectivity of histopathological grading system and its dependence on individual experience and assessment of microscopic observation. The reliability of correlation of frequency of MN and histopathological grading decreases if grading itself is not correct. So, they have suggested the new malignancy grading system given by Anneroth et al.[6] instead of other grading systems. As each observer was given the opportunity for counting (Group I and Group II), scoring and grading (in subjects diagnosed of OSCC) of all slides, without any exchange of information regarding case details, we strived to prevent the interobserver bias.

One more aspect regarding MN is the accurate identification of MN because many similes would create confusion and misguide the observer leading to defective count. These are condensed chromatin, fragmented nuclei (karyorrhectic cells), pyknotic nuclei, karyolitic or ghost cells and broken eggs cell. An easy way to exclude these is by alteration of the fine adjustment in microscope whereby these structures are lost.This indicates that these structures are not in one plane, and hence not to be considered as MN.MN is non-refractive, round to oval shaped body and the shape, color, and texture of MN are similar to that of the main nucleus. Many of such organizations should be differentiated from MN in order to attain a more valid count.

To summarize the results, MN can be seen in normal mucosal cells. Frequency of MN in oral mucosal cells of patients with OSCC was threefold to fourfold higher as compared with the control group. A 87% correlation was found between frequency of micronuclei MN and histopathological grade. Hence, it can be put forward that the frequency of MN in oral exfoliated cells of clinically suspected OSCC can be a candidate for histopathological grading of OSCC in the same subject.

CONCLUSIONS

There is a significant increase in the frequency of MN as compared to normal counterparts, indicating strong cytogenetic damage secondary to genotoxic and carcinogenic agents released by tobacco and areca products. The present study has revealed that there is a correlation of frequency of MN and histopathological grading in OSCC. These results should always be compared with a control. Thus, MN in oral exfoliated cells can be a candidate for grading of OSCC.

Some precautions and recommendations put forward are the following. The method of obtaining the sample should be standardized and repeatable. Complete smear needs to be screened for counting the frequency of MN for more valid results. The clarification on the size of the MN, as to whether to consider a constant value or a range, demands further studies.

REFERENCES

1. Sivasankari NP, Kaur S, Reddy KS, Vivekanandam S, Rao KR. Micronuclei index: An early diagnosis in oral carcinoma. J Anat Soc india 2008;57:8-13
2. Schmid W. The micronucleus test. Mutat Res 1975;31;9-15
3. M. Fenech, N. Holland, W.P. Chang, E. Zeiger, S. Bonassi. The Human Micronucleus Project-an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, Mutat. Res. 1999; 428:271-283
4. Palve DH, Tupkari JV. Clinico-pathological correlation of micronuclei in oral squamous cell carcinoma by exfoliative cytology. J Oral Maxillofac Pathol 2008; 12:2-7.
5. Tolbert PE, Shy CM, Allen JW. Micronuclei and other anomalies in buccal smears: method development. Mutat Research 1992; 271:69-77.
6. Anneroth G, Batsakis J, Luna M. Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. Scand J Dent Res 1987; 95:229-49.
7. Agarwal M, Sunitha JD, Dawar G, Rallan NS. Micronuclei assay of exfoliated oral mucosal cells: A review. Ann Dent Spec 2014;2:47-50.
8. 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.
9. Casartelli G, Bonatti S, De Ferrari M, Scala M, Mereu P, Marquarino G *et al*. Micronucleus frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. Anal Quant Cytol Histol 2000; 22:486-92.
10. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Toraanin R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal mucosa. Mutagenesis 1987; 2:11-7.
11. Belien JA, Copper MP, Braakhuis BJ, Snow GB, Baak JP. Standardization of counting micronuclei: Definition of a protocol to measure genotoxic damage in human exfoliated cells. Carcinogenesis 1995; 16:2395-400.
12. Countryman IP, Heddle JA. The production of micronucleus from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Research 1976; 41:321-32.
13. Kumar V, Rao NN, Nair NS. Micronuclei in oral squamous cell carcinoma: a marker of genotoxic damage. Indian J Dent Rese 2000; 11:101-6.

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1. Evaluation of cytogenic damage in the form of micronuclei in oral exfoliated buccal cells in tobacco users
   Gopal K.S., Padma M. Indian J Dent Res 2018; 29:773-80
2. Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers
   Kamath VV, Anigol P, Setlur K Clin Cancer Investig J 2014; 3:49-54
3. Clinico-pathological correlation of micronuclei in oral squamous cell carcinoma by exfoliative cytology
   Palve DH, Tupkari JV, J Oral Maxillofac Pathol 2008; 12:2-7
4. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps
   Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, et al. Mutat Research 2008; 659:93-108.
5. Micronucleus as Potential Biomarker of Oral Carcinogenesis
   Harshvardhan S. Jois , Alka D. Kale , Mohan Kumar K.P. Indian J Dent. Adv.2010; 2:197-201
6. Evaluation of Micronucleus Assay in Oral Exfoliated Buccal Cells in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma
   Kumar N, Indira AP, David MP, Kumar TS, Int J Prev Clin Dent Res 2016;3(3):173-177
7. Oral cancers – Micronuclei as biomarker of genotoxicity a population-based study to establish usable dynamic cut off limits in tobacco users
   Amin S, Patel N, Chattoo B, Int. J of Mol. & Immu. Oncology 2019; 4:9-12
8. Micronuclei as a Predictor for Oral Carcinogenesis
   Kiran K, Agarwal P, Kumar S, Jain K, J Cytol 2018; 35:233-6
9. Role of fine needle aspiration, imprint and scrape cytology in the evaluation of intraoral lesions
   Khan N, Afroz N, Haider A, Hassan MJ, Hashmi SH, Hasan SA, Journal of Cytology 2013; 30(4):263-269
10. Significance of micronucleus in cervical intraepithelial lesions and carcinoma
    Gayathri BN, Kalyani R, Hemalatha A, and Vasavi B, Journal of Cytology 2012; 29(4):230-35