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Micronuclei and its application in grading of oral squamous cell carcinoma

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Abstract

Introduction: Presence of micronuclei has been seen in exfoliated oral epithelial cells. This has been correlated with severe genotoxic damage measured in terms of grading of the lesions.

Aim: To correlate frequency of micronuclei (MN) in oral exfoliated cells in cases of oral squamous cell carcinoma (OSCC).

Methods: The study subjects consisted of 20 clinically diagnosed cases of OSCC. 10 healthy subjects without any tobacco consumption habits formed the control group. After informed consent, exfoliated cells were collected from both groups using a wooden spatula and spread on slides, fixed, stained using Papanicolaou stain and subjected to microscopic examination. MN were counted in all cases and calculated.

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. MN frequencies were also found to be raised with increasing histological grades of squamous cell carcinoma.

Conclusions: Micronuclei serve as a biomarker for prediction of the grade of OSCC.

Keywords: Exfoliative cytology, micronuclei, oral squamous cell carcinoma, genotoxic damage.

Introduction

The most common oral malignancy is Oral squamous cell carcinoma (OSCC). It constitutes upto 80-90% of all malignant neoplasms of the oral cavity. Based on the country and gender of the patients, oral cavity is 6th to the 9th most common anatomical location for cancer.[1]The most important fundamental cause of developmental and degenerative disease has been found out to be genomic damage.[2]

Genotoxic risks can be assessed by indicators like deoxyribonucleic acid (DNA) damage and cytogenic markers, chromosomal aberrations, and sister chromatid exchanges. Cells often have errors in chromosome segregation, forming a lagging chromosome that becomes lost during anaphase and is excluded from the reforming nuclei. Micronuclei (MN) is the name given to these microscopically visible, round to oval cytoplasmic chromatin masses.[3]

Micronuclei in oral exfoliated cells can be caused by a variety of genotoxic agents and carcinogenic compound in tobacco, betel nut, and alcohol. The genotoxic and carcinogenic chemicals released from betel nut and tobacco and also the calcium hydroxide content of lime present in the betel quid promote reactive oxygen species which in turn cause damage to the DNA.[4]

The micronucleus test on exfoliated cells has been successfully used to: (1) recognize population groups at an elevated risk for cancer of the oral cavity or urinary bladder; (2) estimate synergistic or additive effects of carcinogen exposure (cigarette smokers plus drinkers of alcoholic beverages); (3) pinpoint the site within an organ from which most carcinomas will develop.[5]

Micronuclei count involves a rapid and economical technique for consistent quantitative examination of the genotoxicity. The buccal cell MN assay can be used as a biomarker of genetic damage.[3]

Materials and Methods

Patient selection

20 patients, between the age range of 24 to 75 years, diagnosed histopathologically as having squamous cell carcinoma (SCC) and who had not received any therapy prior to study were included in the SCC group. Thorough history of each patient, including their oral habits, was recorded. Subjects with no obvious oral lesions or habits of consumption of tobacco were selected as control group. Written informed consents from these patients were taken for the procedures to be carried out on them subsequently. The subjects were grouped as follows:

Group I (SCC): 20 patients having oral squamous cell carcinoma.

Group II (Control): 10 healthy control subjects.

Collection of exfoliated cells

Subjects were asked to rinse their mouth gently with water before the procedure. In subjects of OSCC, the most representative site was selected for obtaining the smear, like the margins of the lesion. Oral cells were collected from buccal mucosa of control group using a wooden spatula. The cells were smeared on microscopic slides, fixed with spray fixative. The slides were numbered and preserved in dust-free boxes until evaluation.

Cytological staining and evaluation

All the cytological smears were stained by papanicolaou technique using a commercially available staining kit RAPIDPAP™. All the slides were observed under light microscope using low magnification (×100) for screening and high magnification (×400) for counting of micronuclei.

Scoring criteria

The zigzag method, most commonly used method was used for screening of slides. One thousand cells with intact nuclei and cell boundaries were counted on each slide.

The criteria for designating an extranuclear body as 'micronucleus' were as follows:

1. Diameter less than one third of the main nucleus.
2. Staining intensity similar to, or slightly weaker than, that of the nucleus.
3. Round-to-oval shape.
4. Texture same as that of the main nucleus.
5. Close proximity but no actual contact with the nucleus.

Only those structures fulfilling the above-mentioned criteria were recorded as micronuclei. Micronucleated cells were counted out of 1000 intact epithelial cells, and they were expressed as percentages.

The average frequency of MN was tabulated using the 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 as shown in Table 1.

Table 1: Cytological grade of OSCC based on the average frequency of micronuclei	
Cytological grade of OSCC	Average frequency of micronuclei
Grade 1	1-2
Grade 2	2-3
Grade 3	3-4

Histopathological grading

Histopathological grading for OSCC for the subjects was also done. Malignancy point score was counted based on grading system given by Anneroth et al. The six parameters used to determine the total score were degree keratinisation, nuclear pleomorphism, number of mitotic figures, pattern of invasion, stage of invasion and plasmalymphocytic infiltration. Each parameter is given score of 1-4. Then, malignancy point score is calculated as follows:

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

Based on malignancy point score, a histopathological grade was assigned to each case of OSCC as shown in Table 2. The obtained cytological grade based on average frequency of MN was correlated with histopathological grade based on malignancy point score.

Table 2: Histopathological grade of OSCC based on the malignancy point score	
Cytological grade of OSCC	Malignancy Point Score
Grade 1	1-2
Grade 2	2-3
Grade 3	3-4

Results

The frequency of MN was three to four times higher in patients with OSCC as compared to the patients in control group. PAP stained cytosmears revealed cells with MN ranging from one to five, with dissimilar sizes ranging from 1/3 to 2/3 the size of the nucleus of the cell as shown in Figure 1.

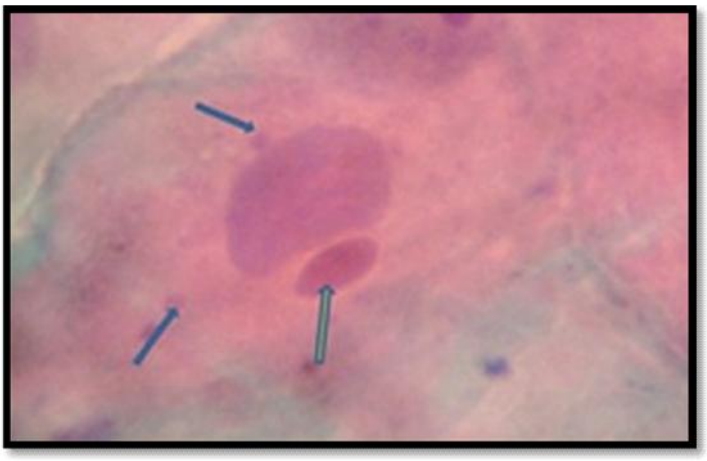

Figure 1: PAP Stained cytosmear showing epithelial cells exhibiting micronuclei of varying sizes (x1000).

When cytological grade which was based on average frequency of MN was compared with histopathological grade, based on malignancy point scoring, it was observed that out of 20, cytological grade of 16 subjects (80%) was correlating with the respective histopathological grade.

Discussion

Micronuclei are extra nuclear 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.[5]

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. According to Tolbert et al., MN are regarded as markers of abnormal mitoses involving chromosomal breakage and mis-segregated chromatin.[5]

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.[6]

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. [6]

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 et al. observed that MN frequencies were higher when cells were collected by vigorous, rather than by light, scraping.[6] Various methodological factors can affect the levels of MN in buccal cells. The main sources of variability 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.[7, 8, 9,10] According to Countryman et al. [7] and Sarto et al.[9], 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.[10] size should be less than 1/5 of the size of the parent nucleus.

Incidence of MN have been analyzed by various studies in normal patients, oral premalignancies and OSCCs.[4] Assessment of MN in exfoliated cells is a promising tool for the study of epithelial carcinogens.[11,12] In our study, the average frequency of MN in patients with OSCC was ranging from 2 to 3.5 micronuclei per cell. In the study conducted by Palve and Tupkari,[4] the overall level of MN in the OSCC group was observed to be in the range of 1.1-3.0. The levels in the present study were slightly lower than those reported by Kumar V et al.[13] where the values ranged between 1.4% - 9.15%.

In studies done by Palve and Tupkari[4], the frequency of MN increased significantly from grade I to grade III, in squamous cell carcinoma group. Similar results were obtained for increasing grades of OSCC in our study.

Frequency of MN in oral mucosal cells of patients with OSCC was 3-4 fold higher as compared with the control group. A 80% 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.

A significant correlation of MN frequency with histopathological grading was observed in this study. Routine histopathological grading is subjective and it depends upon the individual experience and assessment of microscopic observations. Since the histopathological grading of SCC is correlated with the MN frequency in the present study, the reliability of the results decreases if the grading itself is not accurate.

Conclusion

From the present study,

1. It is evident that the percentage of micronuclei is uniformly elevated in all histologic grades of oral squamous cell carcinoma, suggesting a strong cytogenetic damage of the oral epithelium.

2. The mean micronucleus frequency in oral exfoliated cells was significantly increased in oral squamous cell carcinoma (SCC) group as compared to the control group.

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 used for grading of OSCC.

References

1. Pires FR, Ramos AB, Oliveira JB, Tavares AS, Luz PS, Santos TC. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single oral pathology service during an 8-year period. J Appl Oral Sci. 2013; 21(5):460–467. doi:10.1590/1679-775720130 317.
2. Shashikala R, Indira AP, Manjunath GS, Rao KA, Akshatha BK. Role of micronucleus in oral exfoliative cytology. J Pharm Bioallied Sci. 2015; 7(Suppl 2):S409–S413. doi:10.4103/0975-7406.163472.
3. Kiran K, Agarwal P, Kumar S, Jain K. Micronuclei as a predictor for oral carcinogenesis. J Cytol. 2018; 35:233-6.
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. Ankit Agarwal, Rinky Ahuja, Manisha Tijare, Sonalika Ghate, Anand Tegginamani, Sanyog Pathak. Micronuclei: A Prognostic Tool. Journal of Evolution of Medical and Dental Sciences. 2014; 3 (49): 11762-11765.
6. Jadhav K, Gupta N, Ahmed MB. Micronuclei: An essential biomarker in oral exfoliated cells for grading of oral squamous cell carcinoma. J Cytol. 2011;28(1):7–12. doi:10.4103/0970-9371.76941
7. Countryman IP, Heddle JA. The production of micronucleus from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Research.1976; 41:321-32.
8. Tolbert PE, Shy CM, Allen JW. Micronuclei and other anomalies in buccal smears: method development. Mutat Research.1992; 271:69-77.
9. 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.
10. 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.
11. Kumar S, Vezhavendhan N, Priya S. Role of exfoliative cytology in oral leukoplakia and squamous cell carcinoma. Int J Clin Dent Sci 2011; 2:93-7.
12. Casartelli G, Bonatti S, De Ferrari M, Scala M, Mereu P, Margarino 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.

13. Kumar V, Rao NN, Nair NS. Micronuclei in oral squamous cell carcinoma: A marker of genotoxic damage. Indian J Dent Res. 2000;11:101-6.

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