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ORIGINAL ARTICLE

Evaluation of Micronuclei in Oral Squamous Cell Carcinoma: A Cytological Study

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ABSTRACT

Introduction: Oral cancer is the leading cause of cancer deaths, and its incidence is still rising particularly in developing countries. Early detection and prevention of oral cancer is the need of the hour to improve the prognosis. Micronucleus test is a noninvasive, cost-effective, and easily acceptable procedure for patients. The aim of the present study was to evaluate the frequency of micronucleated cells in Feulgen- and Papanicolaou-stained smears of oral exfoliated cells in healthy control subjects and oral squamous cell carcinoma (SCC) patients and to compare and correlate the micronucleated cell frequency between both the groups.

Materials and methods: The study comprised 20 cases of histopathologically diagnosed SCC and 10 healthy control subjects without any harmful habits and with absence of any systemic disease. The cytological smears were made from both the groups and stained with Papanicolaou and Feulgen stain. Micronuclei were then counted in both the smears and calculated accordingly.

Results: There was a statistically significant difference between patients with SCC and normal healthy controls as well as between different grades of carcinoma, with $p < 0.001$.

Conclusion: Micronuclei can be used as biomarker or screening test in patients with SCC. Pap stain can be used for routine screening purpose in high-risk populations. The suspected patients with high number of micronuclei found with Pap stain can further be substantiated with Feulgen for confirmation.

Keywords: Cytology, Feulgen, Micronuclei, Oral squamous cell carcinoma, Pap stain.

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INTRODUCTION

Oral cancer is the leading cause of cancer deaths, and its incidence is still rising, particularly in developing countries. The World Health Organization reports that oral cancer is the sixth most common cancer in males and tenth most common cancer in females.[1] Early detection and prevention of oral cancer is the need of the hour to improve the prognosis. Genomic damage is considered as the most fundamental cause of developmental and degenerative disease. This genomic damage can be assessed and evaluated by numerous methods.[2] Among them, one of the most popular and sensitive methods is the micronucleus (MN) test. A number of studies have correlated the frequency of micronuclei and severity of genotoxic damage.[3-7]

Micronuclei were initially discovered by William Henry Howell in red blood cells in 1891, and later by Justin Marie Jolly in 1905 and, thus, named as Howell Jolly bodies.[7,8] Boller, Schmidt, and Heddle were the first to suggest the MN test as a method to detect genotoxic damage. The MN formation indicates genetic damage.[9] These are extranuclear cytoplasmic bodies smaller than the main nucleus. The basic mechanism of its formation is chromosome damage either in the form of small acentric chromatids or chromosome fragments or whole chromosomes that fail to be included in the daughter nuclei at the completion of telophase during mitosis. Sometimes, there is a defect in chromosome segregation machinery, spindle apparatus, leading to micronuclei formation containing whole chromosomes. The MN test is a noninvasive, cost-effective, and easily acceptable procedure by patients.[10-12] Based on this background, this study was undertaken to identify the feasible and economical method that could be used as a screening test in high-risk population for identifying the effects of genomic instabilities.

AIMS AND OBJECTIVES

- To evaluate the frequency of micronucleated cells in Feulgen- and Papanicolaou-stained smears of oral exfoliated cells from healthy control subjects and squamous cell carcinoma (SCC).
- To compare and correlate micronucleated cell frequency between control group and patients with SCC.

MATERIALS AND METHODS

A total of 20 patients with clinically diagnosed oral SCC were taken, and 10 healthy volunteers, age- and sex-matched were selected as controls. All selected individuals were examined by their full medical history and clinical examination to exclude any other systemic or local diseases.

A written informed consent was taken from all the individuals in the study population.

Inclusion Criteria

Group I: 10 healthy volunteers without any harmful habit and with absence of any systemic diseases.
Group II: 20 patients with oral SCC.

Exclusion Criteria

- Medically compromised patients.
- Patients with other systemic illness.
- Patients who have already taken treatment or those who are undergoing any kind of treatment.

Procedure

The detailed history of each patient was obtained. The subjects were clinically examined and relevant data were entered. The purpose of the study was explained and a written informed consent was obtained from all the participants.

A provisional diagnosis of oral SCC was made based on clinical examination. Patients were asked to rinse the mouth thoroughly before taking the scrapings in order to remove the dirt particles or any debris from the oral cavity. Lesions were cleaned with a sterile gauge piece. Area was anesthetized with topical lignocaine spray. The scrapings were taken from the most representative site of the lesion using moist wooden spatula.

The scraped material was smeared on a precleared, precoded microscopic glass slide. Two smears were made. The obtained smears were placed in Coplin jars containing 95% ethanol, which is used as fixative. One smear was stained with Pap and the other was stained with Feulgen reaction. Incisional or excisional biopsy of the lesion was obtained under local anesthesia. The specimens were preserved in 10% formalin for further laboratory procedures. The biopsy specimens were subjected to routine histopathological procedures and stained with hematoxylin and eosin for histopathological diagnosis.

RESULTS

The present study comprised 20 cases of histopathologically diagnosed SCC and 10 healthy control subjects without any harmful habit and with absence of any systemic disease.

The SCC cases were categorized into three subgroups or grades based on histopathological diagnosis according to Broder's classification – well differentiated (WDSCC), moderately differentiated (MDSCC), and poorly differentiated (PDSCC). The micronuclei were counted according to criteria given by Tolbert et al.[12]

The micronucleated cell frequency was calculated and the data obtained were subjected to statistical analysis. Only 1 out of 10 cases showed positivity with both Feulgen and Pap stain in control group, and in SCC, all cases showed positivity for micronuclei (Table 1).

All the histological grades of SCC showed positivity in both the stains for micronuclei (Table 2 and Figs 1 to 6).

The mean micronucleated cells detected in SCC were higher with both the stains. The p value was significant (Tables 3 to 6). The mean and standard deviation (SD) was calculated in different grades of SCC with both the stains, and p value was found significant (Tables 7 to 10).

Table 1: Number of cases showing positivity for micronuclei with both Feulgen and Pap in groups

Groups	*Feulgen*	*Papanicolaou*
Normal (10) group 1	1	1
SCC (20) group 2	20	20

Table 2: Number of cases showing positivity for micronuclei with Feulgen and Pap in subgroups in SCC

Subgroups	*Feulgen*	*Papanicolaou*
WDSCC (6)	6	6
MDSCC (10)	10	10
PDSCC (4)	4	4

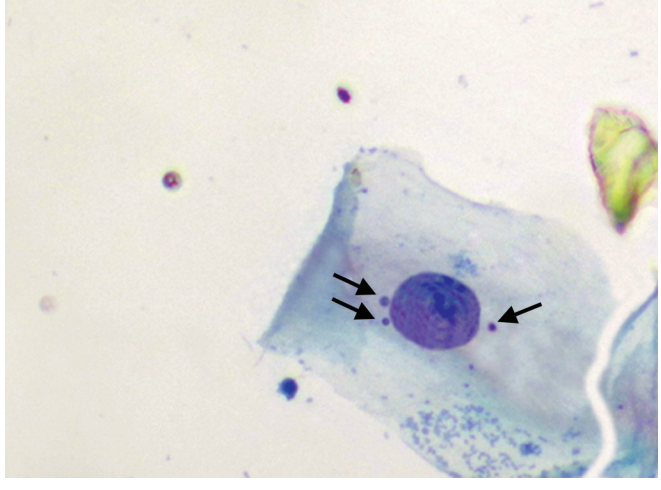

Fig. 1: Epithelial cell representing three micronuclei with Pap stain in WDSCC

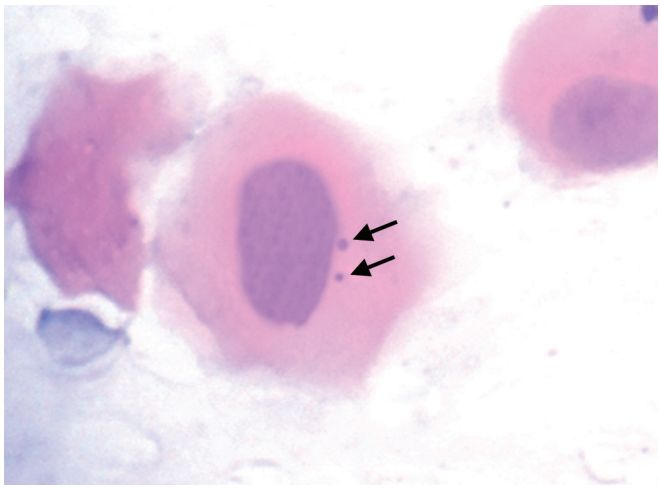

Fig. 2: Epithelial cell representing two micronuclei with Pap stain in MDSCC

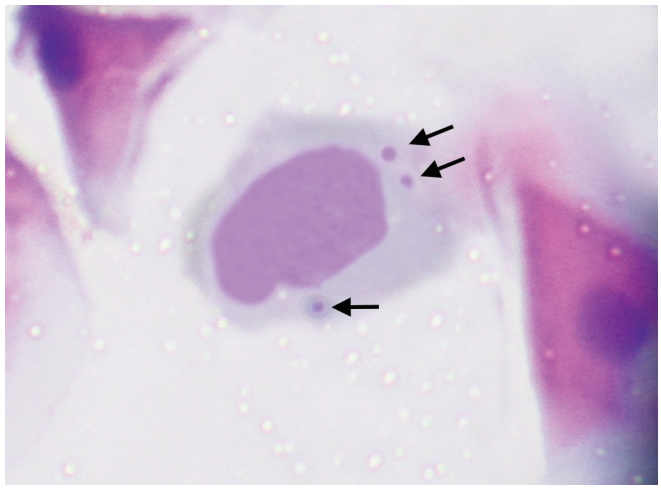

Fig. 3: Epithelial cell representing three micronuclei with Pap stain in PDSCC

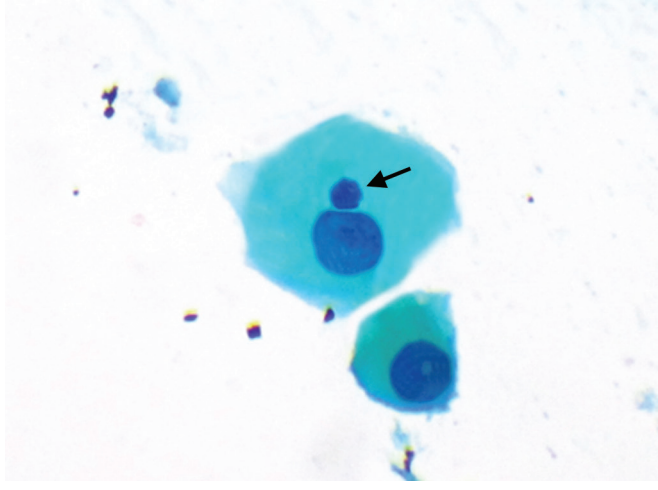

Fig. 4: Epithelial cell representing one micronuclei with Feulgen stain in WDSCC

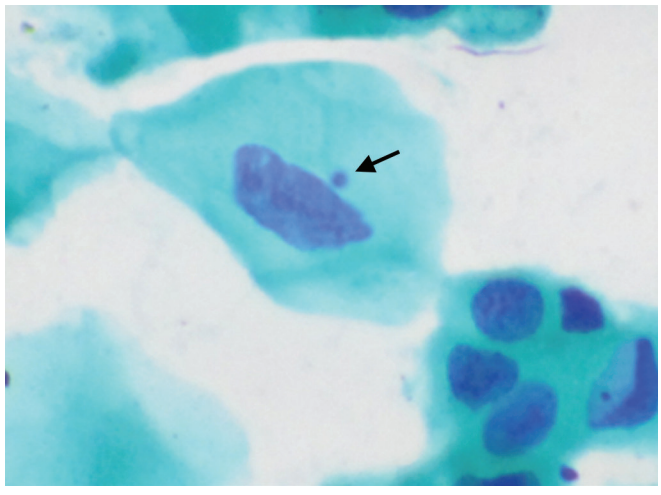

Fig. 5: Epithelial cell representing one micronuclei with Feulgen stain in MDSCC

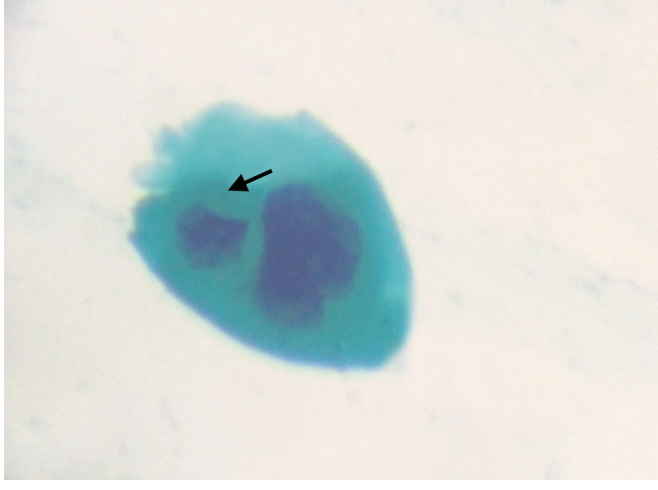

Fig. 6: Epithelial cell representing one micronuclei with Feulgen stain in PDSCC

Table 3: Mean and SD of micronucleated cells groupwise with Feulgen stain

				ANOVA test	
Group	*No. of subjects*	*Mean*	*SD*	*F-value*	*p-value*
Normal	10	0.10	0.316	56.72	<0.001
SCC	20	5.05	2.188		

Table 4: Bonferroni comparison test result for the micronucleated cells groupwise with Feulgen stain

Bonferroni comparison test result		
Groups	*Mean difference*	*p-value*
Normal and SCC	4.950	<0.001

Table 5: Mean and SD of micronucleated cells groupwise with PAP stain

				ANOVA test	
Group	*No. of subjects*	*Mean*	*SD*	*F-value*	*p-value*
Normal	10	0.10	0.316	76.18	<0.001
SCC	20	9.85	3.514		

Table 6: Bonferroni comparison test result for the micronucleated cells groupwise with PAP stain

Groups	*Mean difference*	*p-value*
Normal and SCC	9.75	<0.001

Table 8: Multiple comparison tests for Kruskal–Wallis test result

Groups	*Mean difference*	*Significant level at 5%*
WDSCC and MDSCC	3.17	S
WDSCC and PDSCC	5.67	S
MDSCC and PDSCC	2.5	S

Table 10: Multiple comparison tests for Kruskal–Wallis test result

Groups	*Mean difference*	*Significant level at 5%*
WDSCC and MDSCC	5.83	S
WDSCC and PDSCC	8.83	S
MDSCC and PDSCC	3.00	S

Table 7: Mean and SD of micronucleated cells in different grades of SCC with Feulgen stain

				Kruskal–Wallis test result	
Category	*No of subjects*	*Mean*	*SD*	*Test value*	*p-value*
WDSCC	6	2.33	0.516	16.205	<0.001
MDSCC	10	5.50	0.850		
PDSCC	4	8.00	0.816		

Table 9: Mean and SD of micronucleated cells in different grades of SCC with PAP stain

				Kruskal–Wallis test result	
Category	*No of subjects*	*Mean*	*SD*	*Test value*	*p-value*
WDSCC	6	5.17	0.753	15.824	<0.001
MDSCC	10	11.00	0.133		
PDSCC	4	14.00	8.16		

DISCUSSION

Oral SCC is the major cause of morbidity and mortality in both developing and developed countries nowadays.[1] Chromosomal damage is positively associated with cancer risk and can be used as a biomarker of cancer susceptibility.[13,14] Although there are numerous methods to assess this genetic damage, micronuclei have been considered as upcoming, reliable, and economic markers of genomic damage. Human MN was an international collaborative project launched in 1997 to determine the effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei, and it aimed to provide the information on the extent of variation of micronuclei frequency ranges for different laboratories and protocol factors. Countryman and Heddle in 1976 were the first to use micronuclei as a measure of chromosome damage in peripheral blood lymphocyte.[15-18] Though the histopathology is considered as gold standard for diagnosis, exfoliative cytology has been used as a promising diagnostic tool due to its rapid and noninvasive nature.[19] Thus, this study aimed to evaluate the micronucleated cell frequency in Feulgen- and Papanicolaou-stained smears of oral exfoliated cells from healthy controls and oral SCC and compare the MN frequencies in controls and cases with SCC. The SCC cases were divided into three subgroups or grades according to Broder's classification as WDSCC, MDSCC, and PDSCC.

To find out the statistical difference between different grades of SCC in both Feulgen and Papanicolaou stains, the mean MN count was calculated, and it was found that mean values increased with grade of differentiation, with higher mean values (8.00 ± 0.816) in grade III (PDSCC). There was statistically significant difference between grades I and II, II and III, and III and I in Feulgen stain (Tables 7 and 8).

Similarly, in Papanicolaou stain, the mean values were calculated and compared between different grades of SCC to find statistical status. Mean values increased with increasing grades of carcinoma, and highly statistically significant difference was found between grades I and II, II and III, and I and III (Tables 9 and 10).

The increase in mean MN count with increasing grades of carcinoma correlates with progression of cancer.

Our results are also consistent with the studies done by Palve and Tupkari,[13] which showed the statistically significant correlation of mean MN frequency with histological grades of SCC. Among the different histological grades, the mean MN frequency statistically significantly increased from grades I, II and III. Dorea et al[20] also showed higher frequency of chromosomal damage in the form of MN in carcinoma patients and found statistically significant difference as compared with control.

Pap staining could lead to misinterpretation of artifacts, as it may not distinguish true MN from keratohyalin granules, bacterial clumps, or even stain deposit. The comparison indicates that micronuclei formation in epithelial cells may be overestimated with Pap stain.[11]

However, the Pap stain is still being used. The reasons to use Papanicolaou stain in this study were simple to use, less time consuming, and economical.

Feulgen reaction is the gold standard for MN, but still there are certain limitations that were observed during the procedure, such as time-consuming and technique-sensitive procedure.

The smear contains dividing, differentiated, as well as degenerated cells, so the scoring ratio of basal, differentiated,

and degenerated cells also affects the frequency of micronuclei. Therefore, the time at which smear was taken also affects MN. Moreover, micronuclei can be expressed only in cells that have completed one nuclear division following any genotoxic damage. So, the proportion of those cells in the overall population affects MN frequency.

CONCLUSION

The following results were drawn from the present study:

- No significant number of micronuclei were found in normal healthy controls.
- There was statistically significant difference between normal controls and SCC in both Feulgen and Papanicolaou stain.
- There was highly statistical significant difference between various histopathological grades of SCC in both stains.
- There was moderate level of agreement between both the stains.

From the present study, we conclude that MN can be used as biomarker or screening test in malignant lesions.

Feulgen reaction, which stains MN specifically, has definite advantages over Papanicolaou stain.

Pap stain can be used for routine screening purpose in high-risk populations. The suspicious patients with high number of micronuclei found with Pap stain can further be substantiated with Feulgen for confirmation.

Further studies on a larger scale with larger sample size and inclusion of large number of cells to be counted for micronuclei are warranted.

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