Micronuclei frequency as an early diagnostic tool for detection of Oral Cancer: A comparative study

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Abstract

Aim & Objectives: To assess and compare the levels of micronuclei in oral exfoliative cytology of healthy controls and potentially malignant and malignant oral disorders subjects and also to compare according to different ages and gender.

Material & Method: A total of 50 patients with oral lesions and 50 healthy controls were randomly selected for the study. The subjects were divided into control group and study group. The study group was further subdivided into premalignant and malignant oral disorders. Oral mucosal cells were scraped from buccal mucosa of control group and from lesional tissues of study group using a premoistened wooden spatula. The slides were prepared which were then stained with 10% Giemsa solution and the frequency of micronuclei in epithelial cells were evaluated by scoring 1000 cells on each slide.

Results: Micronuclei frequency was significantly higher in malignant oral disorder group as compared to potentially malignant disorder group and control group. Also, it was significantly higher in potentially malignant disorder group as compared to control group (P value < 0.001).

Conclusion: Micronuclei frequency increases in buccal exfoliated cells of patients with increased risk of cancer; hence, it can be used as an early diagnostic tool for oral precancer and cancer detection.

Key words: Micronuclei frequency, Potentially malignant disorders, Malignant oral disorders, Exfoliative cytology

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2395-499X.2016.00014.9

Introduction

Cancer is the end product of an unregulated proliferation of cells resulting from the accumulation of sequential genetic alterations (mutations) in a precursor cell.[1] Carcinogenesis is a multistep process characterized by genetic, epigenetic and phenotypic changes. Such changes involve genetic damage, mutation in critical genes related to the control of cell division, cell death, metastatic potential and activation of signalling or metabolic pathways that give the cells favourable growth and survival characteristics. Many chemical, physical and biological environmental agents are able to interact with DNA to induce mutations. When the normal function of DNA is lost as a consequence of mutation, the risk of cancer development increases.[2]

The assessment of molecular changes may become the primary means of diagnosis and may guide management.[3] To evaluate genetic instability, there are biomarkers that predict if a premalignant lesion or condition is likely to develop into an aggressive metastasizing tumor.[2] In 1978, working group of WHO classified 'precancer' into 'lesions' and 'conditions' with following definitions: A precancerous lesion is 'a morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart'. A precancerous condition is 'a generalized state associated with a significantly increased risk of cancer'.[4]

The current Working Group of WHO (2005) did not favour subdividing precancer to lesions and conditions and the consensus view was to refer to all clinical presentations that carry a risk of cancer under the term 'potentially malignant disorders' to reflect their widespread anatomical distribution.[4]

Various methods such as routine histopathology (H and E-stained sections), exfoliative cytology and immunochemistry are available today for diagnosis of premalignant and malignant oral disorders. Out of these, exfoliative cytology is particularly valuable for mass screening purposes.[5] Exfoliative cytology is the microscopic examination of shed or desquamated cells from the epithelial surface usually the mucous membrane. It also includes the study of those cells that have been collected by scraping the tissue surface or collected from body fluids such as sputum, saliva, etc.[6] It is the nucleus that expresses the genotypic alterations caused in the process of malignancy; and exfoliative cytology is a method that gives better insight of the nuclear changes in the individual cells.[5]

Micronuclei (MN) is a small extranuclear DNA particle formed when chromosome fragment or acentric chromosomes lag behind and fails to be included in the main nuclei of daughter cells.[7] They remain in the cytoplasm of interphase cells, where they can be observed as structures resembling nuclei.[8] Micronuclei are induced in oral exfoliated cells by a variety of substances, including genotoxic agents and carcinogenic compound in tobacco, betel nut and alcohol.[5]

Unstable chromosome aberrations can be studied in the epithelial cells by the detection of micronuclei and other nuclear aberrations in exfoliated interphase cells. Micronuclei have been used since 1937 as an indicator of genotoxic exposure due to their association with chromosomal aberrations.[9] They can be detected in exfoliated cells and used as an indicator of recent DNA injury within oral mucosa.[10] The frequency of micronucleated exfoliated cells elevates in human tissues, which appear to be the main targets of carcinogens, and from which carcinomas arise.[11] The exfoliated cell micronucleus assay involves microscopic analysis of epithelial smears to determine the prevalence of micronucleation, an indicator of structural or numerical chromosome aberrations.[12] The assay is reliable and technically easy to perform, non-invasive and sensitive with limited cost.[2]

Research in this field is quite promising; hence this study was conducted to assess the levels of micronuclei with increasing risk of Cancer.

Materials and Method

The study was conducted in the Department of Oral Medicine & Radiology, Rishiraj College of Dental Sciences & Research Centre, Bhopal. A total of 50 patients with oral lesions and 50 age and sex matched healthy controls were selected for the study. All the subjects were administered a standardized questionnaire to obtain any history of relevant risk factors and addiction. Patients were grouped according to the following criteria:

1. Group I: 50 Healthy subjects with no oral lesions as control
2. Group II : 50 Patients with potentially malignant and malignant oral disorders

(a) Potentially malignant disorders

1) Oral Submucous Fibrosis (n=12)
2) Leukoplakia (n=10)
3) Oral lichen Planus (n=8)

(b) Malignant disorders

1) Oral Squamous Cell Carcinoma (n=20)

Those patients were excluded from the control group who had any history of tobacco intake or alcohol consumption. Recurrent cases of oral cancer were also excluded.

Incisional biopsy was done for the Group II subjects from the representative sites after performing relevant investigations and taking all aseptic precautions and the specimen was submitted for histopathological diagnosis. Subjects were asked to rinse their mouth gently with water for collection of exfoliated cells. Mucosal cells were scraped from buccal mucosa of controls and mucosa surrounding the lesion of study subjects using a slightly moistened wooden spatula, which were placed in tubes containing 25 ml of neutral buffer solution (0.1M EDTA, 01M Tris and 02M NaCl). The cells were washed in the buffer solution by centrifugation at 800 rpm for 5 minutes and slides were prepared for microscopic analyses. The slides were then fixed in 80% cold methanol. 10% Giemsa solution was used for staining the cells and slides were then mounted with cover glass using DPX mountant.

The micronuclei frequency was evaluated by scoring 1000 cells on each slide, according to criteria established by Tolbert et al[12]. The suggested criteria for identifying MN are:[12]

a. Rounded smooth perimeter suggestive of a membrane.
b. Less than one-third diameter of the associated nucleus, but large enough to discern shape and color.
c. Staining intensity similar to that of nucleus.
d. Texture similar to that of the nucleus.
e. Same focal plane as nucleus.
f. Absence of overlap with, or bridge to, the nucleus.

Results

Data analysis was done using Statistical Package for Social Sciences (SPSS) v.21 for windows. Shapiro-Wilk test showed that values of Micronuclei/ Thousand Cells did not follow normal distribution hence non parametric test namely, Kruskal Wallis test (for more than two groups) and Mann Whitney U test (for two groups) were used for comparison between different groups.

The patients randomly selected for the study were in age range of 17-66 years and were divided into 5 groups: 17-26 years (33%), 27-36 years (21%), 37-46 years (15%), 47-56 years (20%) and 57-66 years (11%). Out of 100 subjects, 64% were males and 36% were females. In the study population, controls were 50% and oral disorders patients were 50%. Oral disorder patients group was further divided into 2 groups which included 30% premalignant oral disorders and 20% malignant oral disorders (Table 1). Age wise comparison for number of micronuclei for control, potentially malignant and malignant oral disorders group showed no significant difference for control and malignant oral disorders group. In potentially malignant oral disorders group it was seen that in age groups of 47-56 yrs and 57-66 years micronuclei count was significantly higher than 17-26 yrs, 27-36 yrs and 37-46 yrs (P value < 0.05) (Graph 1). No significant difference between males and females for number of micronuclei was seen in control, potentially malignant

and malignant oral disorders group (P value > 0.05) (Graph 2). Micronuclei frequency was significantly higher in malignant oral disorder group as compared to potentially malignant disorder group and control group. Also, it was significantly higher in potentially malignant group as compared to control group (P value < 0.001) (Table 2) (Graph 3).

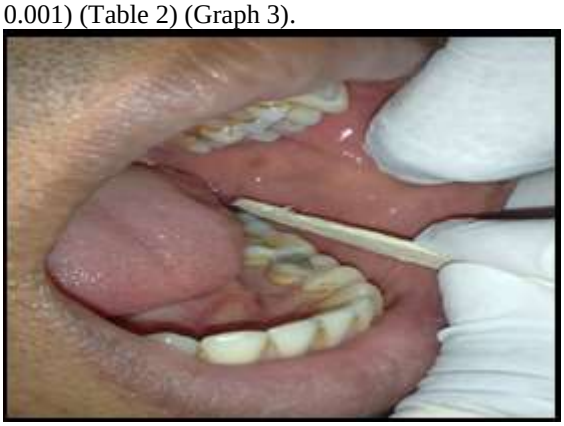


Fig. 1: Exfoliation of cells from buccal mucosa

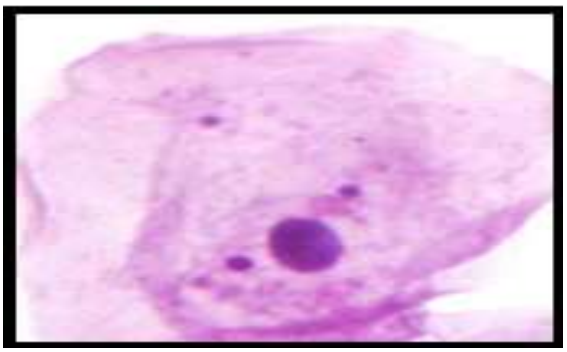


Fig. 2: Photomicrograph of cell with micronuclei

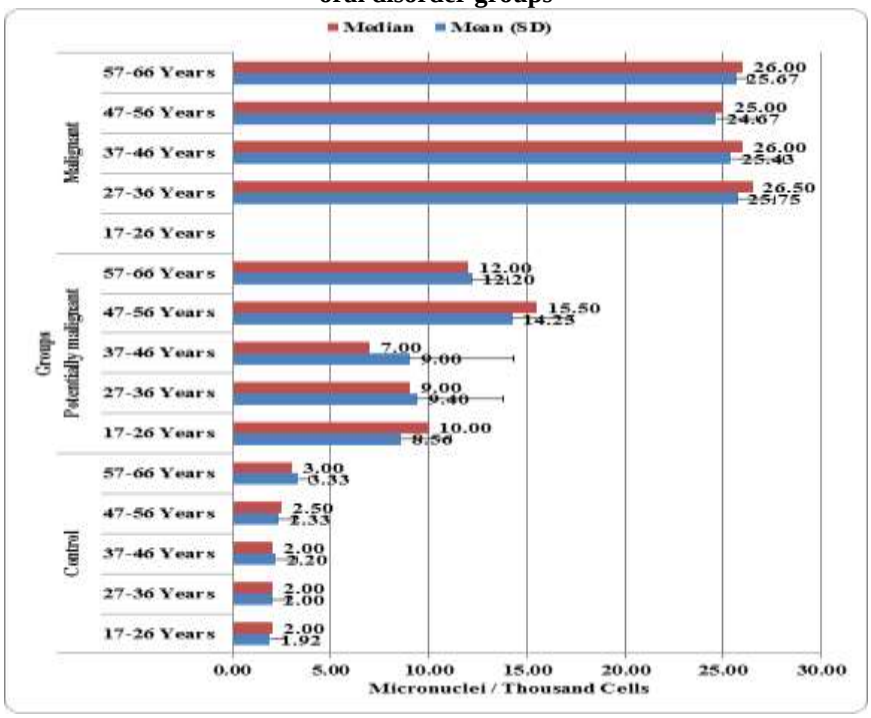
Table 1: Characteristics of Study Population (n=100)

Characteristic	n (%)
Age Groups	
17-26 years	33 (33.00)
27-36 years	21 (21.00)
37-46 years	15 (15.00)
47-56 years	20 (20.00)
57-66 years	11 (11.00)
Gender	
Male	64 (64.00)
Female	36 (36.00)
Study subjects	
Control	50 (50.00)
Potentially malignant	30 (30.00)
Malignant	20 (20.00)

Table 2: Comparison of number of Micronuclei between control, potentially malignant and malignant oral disorder groups

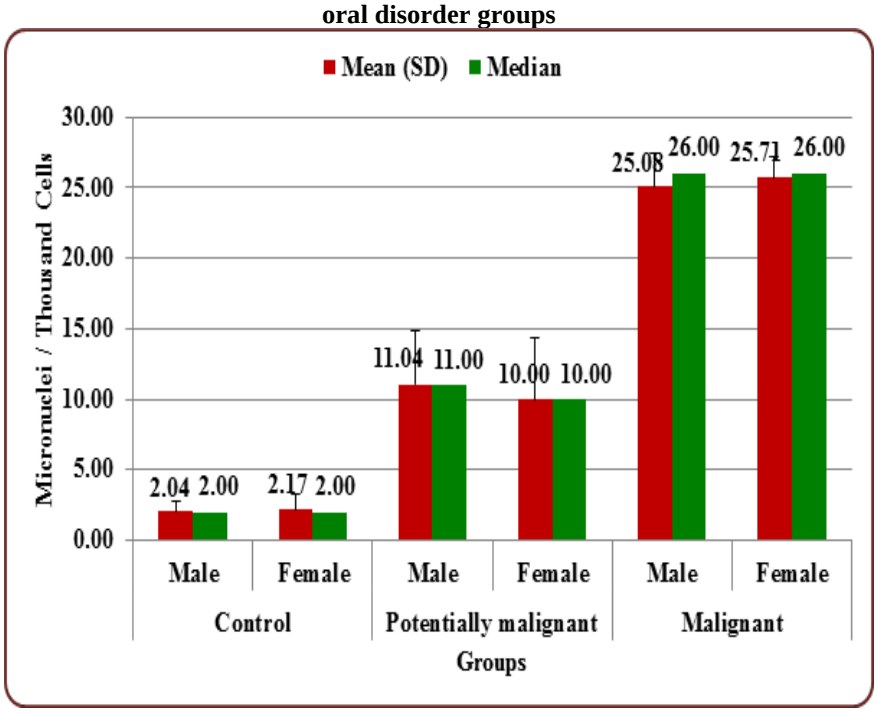
Groups	Micronuclei/ Thousand Cells		
	Mean ± SD	Median	Min-Max
Control	2.10 ± 0.89	2.00	0.00-4.00
Potentially malignant	10.87 ± 3.86	10.50	5.00-17.00
Malignant	25.30 ± 2.06	26.00	21.00-29.00
Kruskal Wallis Test	KW= 84.540, P= 0.000 (<0.001), Sig. Diff.		
Mann Whitney U test	Malignant > Potentially Malignant> Control		

Graph 1: Age wise comparison of number of Micronuclei in control, potentially malignant and malignant oral disorder groups

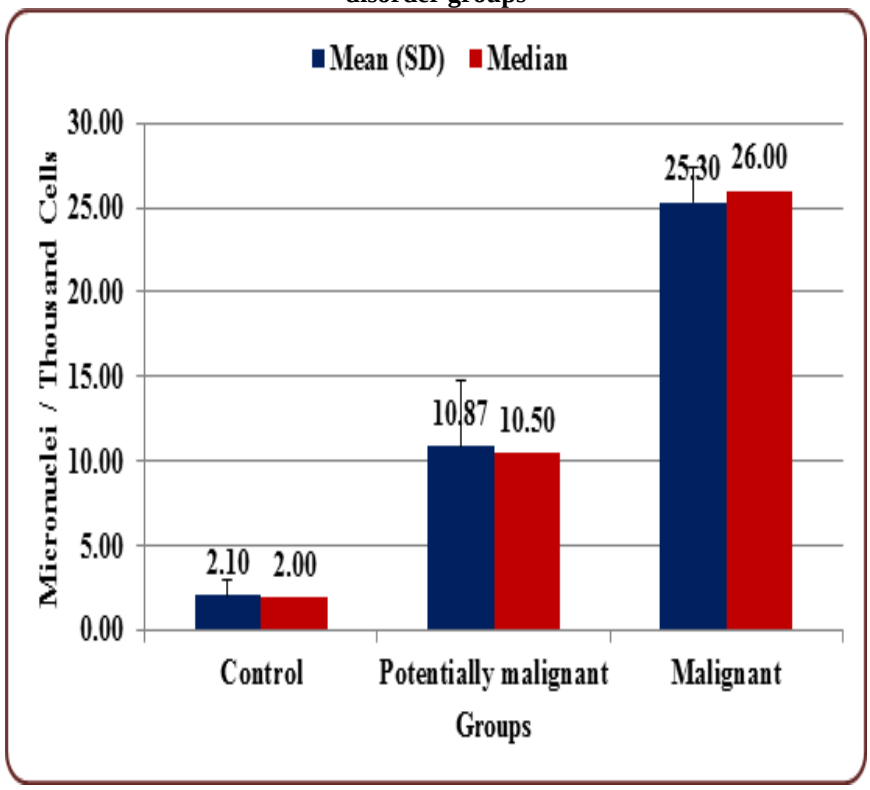


Note: Lines above mean bars shows Standard deviation

Graph 2: Gender wise comparison of number of Micronuclei in control, potentially malignant and malignant oral disorder groups



Graph 3: Comparison of number of Micronuclei between control, potentially malignant and malignant oral disorder groups



Discussion

Oral carcinogenesis is a multi-step process of accumulated genetic damage leading to cell dysregulation with disruption in cell signalling, DNA-repair and cell cycle which are fundamental to homeostasis.[13] The assessment of molecular changes may become the primary means of diagnosis and may guide management.[3] To evaluate genetic instability, there are biomarkers that predict if a premalignant lesion or condition is likely to develop into an aggressive metastasizing tumor.[2] Molecular epidemiology research focuses on three types of biomarkers: biomarkers of exposure (e.g., cytogenetic endpoints- chromosomal aberrations, micronuclei, and sister chromatid exchanges), biomarkers of susceptibility (e.g., genetic polymorphisms), and biomarkers of disease (eg, tumor biomarkers).[14]

Micronuclei are one of such biomarkers that are cytoplasmic chromatin masses with the appearance of small nuclei that arise from lagging chromosomes at anaphase or from acentric chromosome fragments.[13] The use of the micronucleus test on exfoliated cells from oral epithelium with the aim of undertaking biomonitoring on human populations exposed to genotoxic agents was first proposed by Stich et al.[15] The efficacy of this test for this purpose has been highlighted in many studies.[2, 5, 9, 16]

In the present study mean number of micronuclei frequency for control group was 0.21% (males 0.20%, females 0.21%). In potentially malignant group the micronuclei frequency was 1.08% (males 1.10%, females 1.0%). In malignant disorder group the micronuclei frequency was 2.53% (males 2.50%, females 2.57%). Micronuclei frequency was significantly higher in malignant oral disorder group as compared to potentially malignant disorder group and control group. Also, it was significantly higher in potentially malignant group as compared to control group (P value < 0.001). It was observed that a stepwise increase in percentage of micronuclei was seen from control to potentially malignant and from potentially malignant to malignant patients.

A similar study conducted by Halder et al (2004)[9] and Khanna S et al (2014)[17] found that micronuclei frequency in the control group was 0.35%, 0.32%; in precancerous lesions it was 0.63%, 0.61% and for cancerous lesions was 1.36%, 1.03% respectively. These results were similar to our study. As compared to our study, a marked difference was seen in the micronucleus frequency found by Ghosh et al (1995)[18], where buccal mucosa scrapings from 50 individuals who were active tobacco and alcohol users belonging to tribes of Koraput district in Orissa State (India), were smeared by Feulgen technique and fixed. Micronuclei frequency was 7.37% in males and 5.90% in females which was much higher as compared to our study may be because the population from which their subjects were drawn was held to be at higher risk of oral cancer.

In the present study age wise comparison for number of micronuclei showed no significant difference for control and malignant oral disorders group. In potentially malignant oral disorders group it was seen that in age groups of 47-56 yrs and 57-66 years, micronuclei count was significantly higher than17-26 yrs, 27-36 yrs and 37-46 yrs where P value was <0.05. Gender wise comparison showed no significant difference in the micronuclei count between males and females in control, potentially malignant and malignant oral disorders group (P value > 0.05).

In our study, micronuclei frequency in the oral exfoliated cells in the control group was observed to be in range of 0% to 0.4% with a mean micronucleus frequency of 0.21% which is quite similar with those reported by Palve et al (2008)[5] and Kassie et al (2001)[19] where micronuclei frequency was 0.21% and 0.20% respectively.

Micronuclei frequency in the oral exfoliated cells in the potentially malignant disorder group was observed to be in range of 0.50 % to 1.70 % with a mean micronucleus frequency of 1.08%. The micronuclei frequency levels in potentially malignant disorder group in our study were similar with those reported by Bloching et al (2000)[20] and Grover et al (2012)[21] with 1.90% and 1.68% respectively.

Similarly, micronuclei frequency in the oral exfoliated cells in the malignant disorder group was observed to be in range of 2.10 % to 2.90 % with a mean micronucleus frequency of 2.53%, which was quite similar with those reported by Bloching et al (2000)[20] and Palve et al (2008)[5] with 2.05% and 1.86% respectively.

It is evident that our findings agree with the studies done by Casartelli et al (2000)[3], Bloching et al (2000)[20], Halder et al (2004)[9], Palve et al (2008)[5], Devi et al (2011)[2], Dindgire et al (2012)[22], which showed a gradual increase in micronuclei frequency from normal to precancerous to cancerous lesions. These observations indicate cytogenetic damage of the oral epithelium. Micronucleated cell indexes reflect genomic instability. Micronuclei are suitable internal dosimeters for revealing tissue specific genotoxic damage in individuals exposed to carcinogenic mixtures.[2]

Conclusion

Micronucleus test is also an important method for monitoring preneoplastic oral lesions, thereby guiding management strategies to be adopted. It is a very simple, inexpensive and non-invasive screening technique for diagnosing individuals who are at risk of developing cancer. From the present study, it is evident that the individual cancer risk can be predicted on the basis of increased percentage of micronuclei in the oral epithelial cells which helps in identifying the potentially malignant disorder patients who are at high risk of developing cancer.

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