



EVALUATION OF MICRONUCLEI IN EXFOLIATED BUCCAL CELLS OF SMOKERS AND NON SMOKERS - A COMPARATIVE STUDY

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ABSTRACT

Cigarette smoking, tobacco chewing and their derivatives are considered a major risk factor for cancers involving the oral cavity. Cigarette contain several carcinogens that induces an array of genetic aberrations, including gene mutations, chromosome aberrations, micronuclei, sister chromatin exchanges, DNA strand breaks, and oxidative DNA adducts.

The micronucleus test is used as an indicator of genotoxic exposition, since it is associated with chromosome aberrations. The buccal cell micronucleus assay provides information on the cytogenic damage in the tissues that are targets of human carcinogens and from which carcinomas can develop.

Aim and Objective: The present study assesses the micronuclei in the exfoliated cells from the buccal scrapings in smokers and non smokers using Giemsa and Acridine Orange stains.

Material and Methods: 31 smears were taken from both smokers and nonsmokers and stained with Giemsa and Acridine Orange. After the evaluation of the slides the results were compiled and subjected to statistical analysis.

Result: A significant increase in the frequency of micronuclei in smokers was noted compared to non smokers. The mean micronucleus frequency in the oral exfoliated cells was significantly increased in smokers than non smokers ($p < 0.001$).

Conclusion: The present study concludes that, the micronucleus assay in exfoliated buccal cells holds promise as a specific biomarker of genotoxicity, for screening of oral cancer and as prognostic indicator.

KEYWORDS : Smokers, Non smokers, Micronuclei, Acridine Orange, Giemsa.

INTRODUCTION

Oral cancer is major health problem which accounts for 50-70% of all cancers diagnosed and are correlated with tobacco chewing.¹ It has been established that there is a dose-response relationship between the amount of tobacco product used and the development of oral cancer.² Recently cigarette smoking has been recognized as an important risk factor for several types of cancer, mainly oral cancer.³

The carcinogenic effect of cigarette smoking is driven largely by the mutagenicity of various chemicals in the smoke. Tobacco smoke induces an array of genetic aberrations, including gene mutations, chromosome aberrations, micronuclei, sister chromatin exchanges, DNA strand breaks, and oxidative DNA adducts in various models.⁴

Elaborate invasive as well as non invasive techniques have been discovered and at present used for early cancer detection and mass screening. These include routine staining procedure, use of special stains, micronucleus assay, AgNOR count, image cytometry, electron microscopy examination of cytology smears. The procedure employed should be ideally simple, easy to perform and less traumatizing to the patient. One such reliable and sensitive diagnostic tool is exfoliative cytology. It is a useful screening method for detection of oral cancer.⁵ The study of DNA damage in exfoliated cells collected from the oral cavity holds great promise as a minimally invasive method for monitoring populations exposed to genotoxic agents.⁵ Micronuclei are extranuclear cytoplasmic DNA bodies which are induced in cells by numerous genotoxic agents that damage chromosome.⁶ Buccal cell micronuclei are a putative biomarker for oral cancer risk; evidence suggests that micronuclei are elevated in buccal mucosal cells of persons who harbor precancerous lesions and in cancer patients.⁴

The micronucleus (MN) assay in exfoliated buccal cells is a useful and minimally invasive method for monitoring genetic damage and serves as a tool for early detection of cancerous and precancerous lesions, which is really the need for the hour.⁷

The present study was undertaken to assess micronucleus frequency in the smears taken from the exfoliated buccal mucosal cells of smokers and non smokers.

MATERIAL AND METHODS

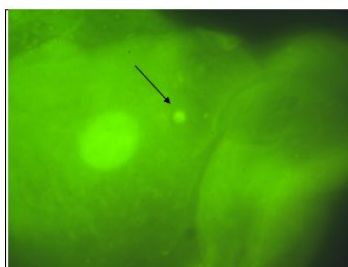
31 Patients with a history of smoking were selected as the study group and 31 healthy patients without any habits were selected as control group depending upon the fulfillment of inclusion and exclusion

criteria. Patients with existing oral mucosal lesions were excluded from the study.

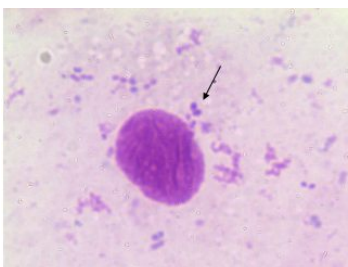
Collection of cells:

Subjects were asked to rinse their mouth gently with water. Mucosal cells were scraped from the buccal mucosa using a slightly moistened wooden spatula. The cells were immediately smeared on microscopic slides. Just prior to drying, the smears were fixed with commercially available alcohol spray fixative (BIOFIX). The slides were coded and were fixed in 100% alcohol.

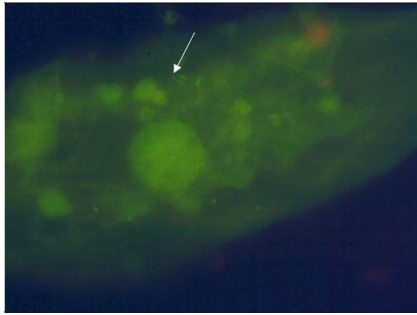
The fixed smears were stained with Acridine orange and Giemsa stains and the stained cells were focused under fluorescent microscope for Acridine Orange and under light microscope for Giemsa and the number of micronucleated cells (MN) were counted. (Photomicrograph 1,2,3,4)



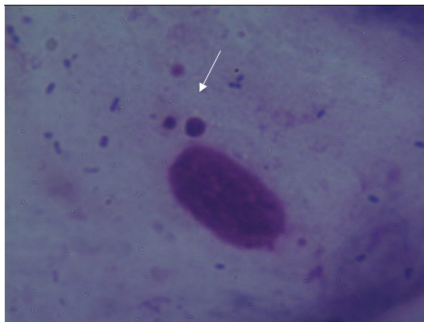
PHOTOMICROGRAPH 1: MICRONUCLEI IN NON SMOKER USING ACRIDINE ORANGE (100X)



PHOTOMICROGRAPH 2: MICRONUCLEI IN NON SMOKER USING GIEMSA (100X)



PHOTOMICROGRAPH 3: MICRONUCLEI IN SMOKER USING ACRIDINE ORANGE (100X)



PHOTOMICROGRAPH 4: MICRONUCLEI IN SMOKER USING GIEMSA (100X)

The scoring of micronuclei was done according to the criteria established by Countryman et al.^{8,9,10,11,12.}

MN must

- Be less than 1/5th to 1/3rd diameter of the main nucleus
- Be on the same plane of focus with main nucleus
- Have the same colour, texture and refraction as the main nucleus
- Have smooth oval or round shape
- Be clearly separated from the main nucleus

Normal Values For MN Frequencies In Epithelial Cells

The average MN frequency reported in healthy population is 1-3 per 1000 cells, with no significant variation between different types of exfoliated cells.¹³

Results and Observation

The results obtained were compiled using MS Excel Worksheet and analyzed using statistical software SPSS version¹⁷.

The mean value of the micronuclei frequency in smokers after staining with Acridine Orange was 505.26 and the mean value of the micronuclei frequency in non smokers was 206.52. The micronuclei frequencies scored are significantly higher in smokers than in non smokers. Standard deviation in smokers was 33.59 and that in non smokers was 28.14. The mean percentage of micronuclei frequency in smokers was 505.26± 33.59 and that in non smokers was 206.52± 28.14. t- Test was done for the given values and a result of 37.96 was obtained. The values were found to be statistically significant (p < 0.001). (Table 1)

Group	N	Mean	Standard Deviation	T
Smokers	31	505.26	33.59	37.96 P < 0.001
Non smokers	31	206.52	28.14	

Table 1: Table showing the mean values of micronuclei in smokers and non smokers using Acridine Orange stain.

The mean value of the micronuclei frequency in smokers after staining with Giemsa was 496.13 and the mean value of the micronuclei frequency in non smokers was 119.35. The micronuclei frequencies scored are significantly higher in smokers than in non smokers. Standard deviation in smokers was 32.98 and that in non smokers was 25.51. The mean percentage of micronuclei frequency in smokers was 496.13 ± 32.98 and that in non smokers was 119.35± 25.51. t- Test was

done for the given values and a result of 39.63 was obtained. The values were found to be statistically significant (p < 0.001). (Table 2)

Group	N	Mean	Standard Deviation	T
Smokers	31	496.13	32.98	39.63 P < 0.001
Non smokers	31	119.35	25.51	

Table 2: Table showing the mean values of smokers and non smokers using Giemsa stain.

The present study showed that the frequency of micronuclei is increased in smokers when compared to non smokers, implicating that smoking causes genotoxic damage to epithelial cells.

Discussion

Oral squamous cell carcinomas are characterized by complex karyotypes that involve many chromosomal deletions, translocations and structural abnormalities. Cells from these types of tumors often have errors in chromosome segregation that lead to the formation of a lagging chromosome or chromosome parts that become lost during the anaphase stage of cell separation and are excluded from the reforming nuclei. The laggards are observed in the cytoplasm as micronuclei.¹⁴

The presence of micronuclei (MN) and other nuclear anomalies within these cells has been shown to be associated with genetic defects in genome maintenance, accelerated ageing, exposure to genotoxic agents, oral cancer risk and neurodegenerative diseases.¹⁵

MN are small fragments of extranuclear DNA formed during cell division, which provide a nonspecific but quantifiable marker of DNA damage, so it is used to identify cellular damage caused by carcinogenic agents. Smoking is a well-known source of carcinogenic influence in humans.¹⁶

The buccal cell micronucleus (MN) assay was first proposed in 1983 and it continues to gain popularity as a biomarker of genetic damage in numerous applications. MN assays provide information on the cytogenetic damage in the tissues, that are targets of human carcinogens and from which carcinomas can develop.¹⁴

The major advantage of exfoliative cytology is the noninvasive character of the technique, which allows a simple and pain-free collection of intact cells from different layers in the epithelium for microscopic examination and quantitative evaluation.¹⁷ In the present study the analysis of micronuclei is done exclusively by taking the buccal mucosal scrapings.

The aim of the present study was to use micronucleus assay as a genotoxic marker in smokers and non smokers. The present study showed that the frequency of micronucleus was significantly higher in smokers than in non smokers and the mean difference between the two was statistically significant. (Table 1, 2)

In a study by Suhas et al on buccal cell changes which are associated with smoking by using the micronucleus assay, there was found to be a significant correlation between the habit of smoking and the frequency of the micronucleated oral mucosal cells.¹⁸ The results of present study are in accordance with this study.

Piyathilake CJ et al in 1995, in their study showed that smokers are three times more likely to have micronucleated buccal mucosal cells.¹⁹

Naderi et al in 2013 evaluated the micronuclei frequency in the exfoliated buccal cells in smokers. The micronuclei frequency in smokers was found to be significantly high in smokers compared to nonsmokers.³ The findings are in agreement with the present study. Wu et al have reported the positive relation between micronuclei frequency and smoking intensity. The micronuclei frequency in buccal cells was higher in heavy smokers.²⁰

Elevated frequencies of micronuclei cells reveal the genotoxic action of carcinogens and may indicate an elevated probability for the formation of particular chromosome changes, which in turn, via the effect of such alterations on oncogene expression could be associated with neoplastic transformation. Early detection of a premalignant or cancerous oral lesion would improve the survival to a greater extent and also will reduce the morbidity associated with the treatment to a considerable extent.

From the present study it can be inferred that individual cancer risk can be predicted on the basis of increased percentage of micronuclei in oral epithelial cells and helps in identifying individuals with high risk of developing oral cancer.

Thus, micronucleus assay is valuable in showing genotoxic damage even before phenotypic changes are attributed to the healthy mucosa of people with high risk of developing oral cancer, but it cannot predict when such transformations will occur.

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