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MICRONUCLEUS ASSAY OF BUCCAL MUCOSAL CELLS IN SMOKERS AND NON-SMOKERS

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ABSTRACT The micronuclei assay (MA) in exfoliated buccal cells is an innovative technique which holds promise for the screening of epithelial carcinogens/mutagens. Micronucleus is the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. The micronucleus is thought to be an indicator of DNA damage consequent to possible carcinogen exposure.

The objectives of our study were to evaluate the frequency of micronuclei in exfoliated buccal mucosal cells of smokers and non-smokers and to correlate the mean micronuclei with period of smoking.

Buccal smears obtained from 68 age matched male subjects (40 smokers, 28 non-smokers) were included in the study. Papanicoloau stained smears were screened for micronuclei as per Tolbert et al criteria, with 1000 cells being examined in both categories.

Results showed a statistically significant difference in the mean micronuclei count in buccal cells of smokers as compared to non smokers.

Micronucleus assay can be used as a simple bio-marker for screening of pre-malignant changes in cells.

KEYWORDS : Buccal Cells, Micronuclei Assay, Smokers

INTRODUCTION:

The collection of buccal cells is arguably the least invasive method available for measuring DNA damage in humans, especially in comparison to obtaining blood samples for lymphocyte and erythrocyte assays, or tissue biopsies. The buccal cell micronuclei assay was first proposed in 1983 [1] and continues to gain popularity as a biomarker of genetic damage in numerous applications. Micronucleus is the name given to the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. The number of micronuclei is related to the increasing effects of carcinogens. This event happens before clinical symptoms of cancer appear hence study of micronuclei by exfoliative cytology may serve as a screening tool for diagnosis of precancerous conditions similar to cervical cytology. [2,3,4]

In the present study, 68 males with 28 controls non-smokers and 40 smokers with similar mean ages were enrolled for DNA damage analysis in buccal cells by Micronucleus assay (MA).

AIM and OBJECTIVES:

The aim of the study was to determine the frequency of micronuclei in buccal mucosal cells in smokers and non-smokers. The objectives were to correlate the mean micronuclei in buccal mucosal cells of smokers with period of smoking and to compare the mean micronuclei in buccal mucosal cells of smokers with mean micronuclei in non-smokers.

MATERIALS AND METHODS:

This was a prospective study where a total of 68 age matched males (40 smokers and 28 non users/non-smokers) were selected from among the outpatients who attended our hospital. Smokeless tobacco chewers were not included . The study was cleared by the institutional ethics committee.Before collecting the samples, a written consent of each individual was taken. Each subject was asked about his lifestyle, food consumption, infectious diseases, X-ray exposure and medication history.Individuals who had had a recent viral infection or had been exposed to X-rays in the past month or those who had been under medication, obvious oral malignancy, diabetes, betel nut chewers and tobacco chewers were excluded from the study. The lifestyle (except the habit of smoking cigarettes) and dietary habits of the controls were similar to those of the smoker category.

CYTOLOGICAL PREPARATION AND SCREENING:

Before sampling, each individual rinsed his mouth thoroughly with tap water. The exfoliated cells were obtained by scraping the buccal mucosa with a moistened wooden spatula. The wooden spatula containing the exfoliated cells was then smeared onto pre-cleaned glass slides. These slides were immediately fixed with isopropyl alcohol in a Coplin jar. The slides where then stained with Papanicolaou (Pap stain) using standard method. After staining, 1000 cells were examined under the 1000 X magnification. The slides were randomized and scored by two observers. The criterion which was developed by Tolbert et al [5] was used for counting the micronuclei. Tolbert criteria for micronucleus include the following:

(1) Rounded smooth perimeter suggestive of a membrane
(2) Less than a third the diameter of the associated nucleus, but large enough to discern shape and colour
(3) Staining intensity similar to that of the nucleus
(4) Texture similar to that of nucleus
(5) Same focal plane as nucleus and
(6) Absence of overlap with, or bridge to, the nucleus.

Keratohyaline granules and bacteria which could interfere with the counting of the micronuclei in the buccal cells in the oral cavity were identified and excluded.

The quantum of smoking exposure was calculated by:
The number of packs × Years of exposure

RESULTS:

Buccal smears from 40 male smokers and 28 male non smokers where examined for presence of micronuclei. Alcohol fixed buccal smears were stained with Papanicolaou stain and 1000 cells were studied in each smear for number of micronuclei. Amongst the controls 2 smears were found to be unsatisfactory and excluded from analysis.

All the 68 males were in the age group of 17 to 30 years.
Amongst the 40 male smokers, majority of 22/40 (55%) were smoking for the past 2-4 years. None of the males had history of smoking for more than 10 years. (Chart1)

All 68 males were social alcohol drinkers on a mixed diet. No family history of oral cancer, regular medications or exposure to radiation in the past month was noted in both the categories.

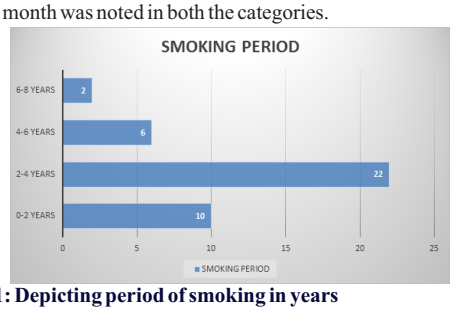

Chart 1: Depicting period of smoking in years

Results of micronuclei assay revealed that the micronuclei were noted in higher numbers ranging from 5 – 20 /1000 buccal cells in smokers. In the 26 control buccal smears, the micronuclei numbers ranged from 2-12/1000 cells.(Chart 2)

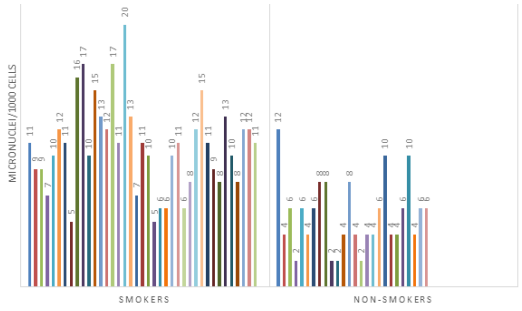

Chart2: Depicting micronuclei counted in 1000 cells in both categories

The mean micronucleus count was higher in smokers as compared to non smokers (Table 1 and Table 2) and this was found to be statistically significant with p value < 0.05

Table 1: Mean micronucleus count in non-smokers

Non smokers	Mean micronucleus count(1000 cells)	Median
26	4.9	4

Table 2: Mean micronucleus count in smokers

Smokers	Mean micronucleus count 1000) cells)	Median
40	10.8	11

DISCUSSION:

Micronuclei are biomarkers of genotoxic events and chromosomal instability and present after genomic damages to the cell caused by cigarette smoking, chewing tobacco, betel nut etc. Such cytogenetic biomarkers are the most frequently used endpoint in human bio monitoring studies and are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability[2,3].

The buccal cell micronucleus is defined as a microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus.[5]

Cigarette smoking has been recognized as an important risk factor for several types of cancer, mainly oral cancer. [3] Cigarettes contain several carcinogens which activate in different tissues and cause DNA adducts products. [6,7]

It has been believed that the number of micronuclei is related to the increasing effects of carcinogens which happens before clinical symptoms of cancer appear. A number of micronutrients, including beta-carotene and other vitamins, have been shown to significantly decrease MN levels (1.4–4-fold) in healthy tobacco users, as well as in individuals with precancerous lesions [8-11].

Kamboj and Mahajan have indicated that evaluation of buccal mucosa epithelial cells is a reliable biomarker for early detection of premalignant and malignant lesions. With increasing micronuclei number, the risk of chromosomal alterations will become higher.[9]

Naderi et al [10] studied the mean number of micronuclei in buccal mucosa of smokers and non-smokers and concluded that non-smokers had significantly lower number than non-smokers.

The increase in number of smokers and the prevalence of oral cancers in them has led to resurfacing of interest in this topic.

Smokers on average had almost tripled the micronucleus frequency compared with non-smokers[2].

The pattern of genetic damage associated with the number of cigarettes smoked per day shows that only subjects who smoked the most (i.e., 40 cigarettes per day) had a significant increase in MN over non-smokers.[12]

In this study after careful review of literature, Pap (Papanicolaou's) stain was used. Pap is considered a better stain over MGG (May Grunwald's Giemsa) for the micronucleus assay screening of the buccal cells according to some studies.[13]

Variation in the number of micronuclei in both categories in our study may be explained due to non long term exposure to smoke amongst the smokers where none were smoking for more than 10 years. Some of the control group also had a relatively higher count.This was found to be their possible exposure to passive smoking.

This being a prospective study with no follow up, the smokers who actually develop fulminant cancer could not be ascertained. Although there was a correlation to the number of packs of cigarettes smoked to the micronuclei the result was not statistically significant.

The micronuclei count in smokers was predominately higher compared to that of non-smokers and this was statistically significant indication increase in micronuclei count with smoking.

CONCLUSION:

Micronucleus assay is a simple non invasive technique using exfoliative cytology to count the micronuclei. Using Papanicolau stain micronuclei are visualised easily. The micronuclei are comparatively higher in smokers as compared to non smkokers and correlate to the quantum of smoking. Larger sample size and follow up studies are required in order to use micronucleus assay as a screening tool for determining genetic damage following exposure to mutagens.

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