

COMPARISON OF MICRONUCLEATED CELL IN BUCCAL SMEARS AMONG SMOKERS AND NON-SMOKERS

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ABSTRACT

BACKGROUND

The health complexities caused due to tobacco smoking has not been restricted to any geographic region and has spread worldwide. As the oral mucosal cells, which line the oral cavity are the first barrier, they represent the preferred target site for the early genotoxic events. Tobacco use is one of the most important aetiological factors in initiation of oral cancer as it increases the risk of cancer by exposing the buccal mucosal to the carcinogenic chemicals either through inhalation or by ingestion. Micronuclei are round to oval cytoplasmic chromatin mass, which occurs as a result of segregation defects due to chromosomal instability causing chromatin to be excluded from the reformed nucleus. Micronuclei assay in exfoliated buccal cells is a useful and less invasive method for monitoring genetic damage.

MATERIALS AND METHODS

A total of 100 male subjects (50 smokers, 50 non-smokers) were examined. Buccal smears were wet fixed and stained with pap stain. 100 cells per slide were counted and assessed for micronuclei count. T-test and Pearson correlation was used as a statistical tool for analysis.

RESULTS

Significantly, smokers had higher percentage of micronucleated cells (T-5.865; P (0.000), total number of micronuclei (T-6.713; P (0.000) and mean micronuclei count (T-5.865; P (0.000) than non-smokers. Pack years correlated significantly and positively with mean micronuclei count. However, pack year did not have significant relation with percentage of micronucleated cells and total number of micronuclei.

CONCLUSION

The genotoxic effects of tobacco smoke cause chromosomal damage in the epithelial cells of buccal mucosa and are reflected in the increased micronuclei in smokers. Micronuclei assay can be used as a simple and reliable marker for genotoxic evaluation.

KEYWORDS

Micronuclei, Genotoxic, Tobacco, Oral cancer.

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BACKGROUND

Cancer a modern epidemic among the non-communicable diseases and is the second most common cause of mortality in developed countries and one of the ten most common causes of mortality in developing countries like India.¹ Oral cancer is one of the ten most common human cancers with 5,75,000 new cases and 3,20,000 mortalities per year worldwide.² Various causes includes tobacco consumption,

alcoholism, human papillomavirus, poor diet, etc.¹ Oral cancer prevalence in the world is often correlated with the pattern of tobacco products consumption and a dose-response relationship exist between the prevalence of oral cancer and the level of consumption of tobacco products.²

Over 1 billion people worldwide are tobacco users. Cigarette smoke, which contains more than 4000 chemicals, including nearly 50 known carcinogens is one of the main cancer risk factors.³ Some of the cytotoxic substances present in cigarette are polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines, etc. However, nicotine has been determined to be the major addictive substance present in the cigarette.⁴

The process of aberrant mitosis gives rise to the micronucleus. Micronuclei are a round or an oval chromatin mass, which is visible through a microscope. The chromatin mass is present in the extra vicinity of the nucleus and comprises eccentric chromosomes, chromatin fragments or whole chromosomes, which failed to reach the spindle poles

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during the process of mitosis.⁴ Micronuclei assay in exfoliated buccal cells is a useful and minimally-invasive method for monitoring genetic damage in humans in comparison to obtaining blood samples for erythrocyte and lymphocyte assays or tissue biopsies.⁵ The micronucleus serves an important role as a biomarker for the assessment of damage in DNA of the affected individuals.⁴

AIMS AND OBJECTIVES

1. To assess the cytogenic damage in the form of micronuclei in smokers.
2. To compare the micronuclei score among smokers and non-smokers.
3. To find out the effect of pack years on micronuclei.

MATERIALS AND METHODS

Inclusion Criteria

- Smokers with smoking history of more than 5 years.
- Non-smokers.

Exclusion Criteria

- Females.
- Subjects with history of chewing beetle nut, alcohol consumption.

A total of 100 subjects were included in the study and divided into two groups. Group 1- Nonsmokers (50) and Group 2- Smokers (50). Group 2 comprised of individuals with history of smoking more than 5 years. Study involved only male subjects to avoid sex bias. Age and smoking habits were also noted.

Collection of Sample

Informed consent was taken. Subjects were asked to rinse their mouth with water before obtaining the buccal mucosal cells. The exfoliated buccal mucosal cells were scraped using wooden spatula with gentle pressure and they were spread over clean glass slides. Smears were fixed in 95% alcohol and stained with Papanicolaou stain.

Scoring Criteria

The criteria by Tolbert et al were adopted for micronucleus count, which consists of the following-

- Round and smooth periphery, which indicates a membrane.
- Less than 1/3 of the diameter of the nucleus, but big enough for distinction of shape and colour.

- Colour intensity similar to the nucleus.
- Consistency and texture similar to the nucleus.
- Focal length similar to the nucleus.
- No connection or overlapping with the nucleus.

Cells with distinctive margins and nuclei were counted. Micronuclei were not counted in areas with cell overlap. Finally, data were analysed by T-test and Pearson correlation.

Scoring

The stained smears were viewed under oil immersion at 100x magnification to identify and record the MN count. 100 cells were counted in each stained smears and were examined by three blind examiners.

RESULTS

100 subjects were analysed, which consist of 50 smokers with smoking history of more than 5 years and 50 non-smokers. 100 cells were counted in each slide. Number of cells positive for micronuclei was counted and number of micronuclei in each cell was counted. Average number of micronuclei in each slide was calculated (Figure 1 and 2). T-test was used for statistical analysis and $P < 0.01$ was considered as statistically significant.

It was found that significantly smokers had higher percentage of micronucleated cells with mean 19.1400 than non-smokers with mean 6.1600; ($t=9.754$), ($P < 0.0001$) Table 1.

Significantly, smokers had higher total number of micronuclei (mean-67.8000) than non-smokers (mean-12.3200) with ($t=6.713$); ($P < 0.0001$) Table 2.

Smokers had higher mean micronuclei count (3.1811) than non-smokers (1.8786) with $t=5.865$; $P < 0.0001$, Table 3.

Number of cigarette smoked per day and the duration of smoking was also noted. Pack years was calculated and analysed using Pearson correlation and it was found that pack years correlated significantly and positively with mean micronucleated count ($P < 0.05$). However, pack years did not have significant relation with percentage of micronucleated cells and total number of micronuclei, Table 4.

T-Test (Table 1)

Group Statistics					
	Group	N	Mean	Std. Deviation	Std. Error Mean
Percentage of micronucleated cells	Smokers	50	19.1400	8.06102	1.14000
	Non-smokers	50	6.1600	4.85445	.68652

Independent Samples Test					
		t-test for Equality of Means			
		t	df	Sig. (2-tailed)	Mean Difference
Percentage of micronucleated cells	Equal variances assumed	9.754	98	.000	12.98000

Significantly, smokers had higher percentage of micronucleated cells than non-smokers.

T-Test (Table 2)

Group Statistics					
	Group	N	Mean	Std. Deviation	Std. Error Mean
Total number of micronuclei	Smokers	50	67.8000	57.25988	8.09777
	Non-smokers	50	12.3200	11.69064	1.65331

Independent Samples Test					
		t-test for Equality of Means			
		t	df	Sig. (2-tailed)	Mean Difference
Total number of micronuclei	Equal variances assumed	6.713	98	.000	55.48000

Significantly, smokers had higher total number of micronuclei than non-smokers.

T-Test (Table 3)

Group Statistics					
	Group	N	Mean	Std. Deviation	Std. Error Mean
Mean micronuclei count	Smokers	50	3.1811	1.45372	.20559
	Non-smokers	50	1.8786	.59363	.08395

Independent Samples Test					
		t-test for Equality of Means			
		t	Df	Sig. (2-tailed)	Mean Difference
Mean micronuclei count		5.865	98	.000	1.30246

Significantly, smokers had higher mean micronuclei count than non-smokers.

Correlations (Table 4)

Correlations		
		Pack Years
Percentage of micronucleated cells	Pearson Correlation	.097
	Sig. (2-tailed)	.505
	N	50
Total number of micronuclei	Pearson Correlation	.249
	Sig. (2-tailed)	.082
	N	50
Mean micronuclei count	Pearson Correlation	.281
	Sig. (2-tailed)	.048
	N	50

Pack years correlated significantly and positively with mean micronuclei count. However, pack years did not have significant relation with percentage of micronucleated cells and total number of micronuclei.

DISCUSSION

As we all know, prevention is better than cure, our present study has been directed towards prevention and early diagnosis of oral cancer. In this regard, the present study assessed micronucleus count in buccal mucosa and showed that in smokers the percent of micronucleated cells and the

mean micronuclei count was significantly higher than that of non-smokers.

DNA damage caused due to the use of tobacco and can be assessed by MN test. It is found to be most sensitive when compared with other tests as it neither requires tedious procedure like cell culture and metaphase preparation, nor it requires any specific DNA stains.⁵ MN assay is a better indicator for genotoxicity damage than chromosomal aberrations or sister chromatid exchange. Increased MN frequency has a higher risk for the

development of oral cancer. They may be used as a quantifiable estimate of the extent of recent DNA injury.

Study by Pradeep et al reported that the mean micronuclei count was 3.11 in smokers and 0.50 for non-smokers. Konopacka et al have reported that the mean micronuclei was 1.50 (± 0.47) in smokers and 0.55 (± 0.32) for non-smokers.¹

These findings are in agreement with the present study. Naderi et al study concluded that the mean MN in smokers with smoking history of more than 10 years was higher in comparison with smokers with smoking history of less than 10 years. The percent MN in smokers who smoked either less or more than 10 years was not significant.⁶

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.⁷

The results about the mean number of micronuclei in men and women were controversial; some researches have shown higher micronuclei values in men and the others have reported higher values in women. To omit the effect of sex on the obtained results in the present study, only male subjects were included.

The applied staining methods for micronucleus specification in exfoliated oral mucosal cells have been different in various studies and include Feulgen-Fast green, May-Grunwald Giemsa and Papanicolaou.⁸ Papanicolaou staining has been used in many recent studies^{9,10} including the present study with acceptable results.

In other studies, cigarettes and other forms of tobacco have been compared, while in the present study, only the effect of tobacco smoking on percent of micronucleated cells and mean micronuclei count was assessed. Confounding factors especially alcohol consumption was eliminated and the synergic effect of these two substances on the micronuclei was omitted.

In the present study, percent of micronucleated cells and the mean micronuclei count was significantly higher among smokers than that of non-smokers. Pack years correlated significantly with mean micronuclei count, but not with the percentage of micronucleated cells. The differences regarding the association of pack years with the micronuclei count is mainly because, a very limited number of subjects will reveal the exact duration and frequency of smoking habits.

CONCLUSION

Epithelial tissues are the one, which are more prone and appropriate for determination of any harmful changes in the body due to smoking. 90% of cancers are ascertained to exist in the epithelial tissues, which make their collection as samples easy and necessary as it does not cause discomfort to the patient. In the present study, it is found that smokers had significantly higher micronuclei count than non-

smokers. To come to justifiable and reliable results, large population should be studied and also should be reassessed after habit weans to see whether the micronuclei count decreased after the cessation of smoking. Detection of micronuclei can be used as a prognostic, educational and interventional tool in the management of subjects with smoking habits.

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