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MICRONUCLEUS ASSAY - SCREENING TOOL IN THE DIAGNOSIS OF ORAL CARCINOMA IN TOBACCO USERS

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ABSTRACT

Cancer is one of the most important non communicable diseases with high mortality rate. Cancer of the oral cavity is common in south Indian male population due to the usage of tobacco in the form of cigarette smoking and smokeless tobacco. The genotoxic risks in tobacco users on buccal mucosa can be assessed by Micronucleus (MN) test. 25 patients with oral carcinoma and 25 patients with various pre malignant diseases were included in our study. They were screened for micronucleus. MN frequency was significant ($p<0.001$) among tobacco users than non users in the cancer group and the MN frequency were not significant among the pre malignant tobacco users , so the present study can be used as a screening test in the population using tobacco.

Key words: Tobacco users, Oral carcinoma, Pre malignancy, Micronucleus.

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INTRODUCTION

Cancer, one among the four non communicable diseases[1], is a complex disease caused by genotoxic effects of chemical carcinogens or environmental pollutants resulting in genomic instabilities at an early stage of cancer. 555,400 national cancer deaths occur in India in 2010. At 30 – 69 yrs, the three most common fatal cancers were oral, stomach, lung in men. Tobacco related cancers represented 42% in male and 18.3% in female cancer deaths.[2] The figures on worldwide tobacco use continue to be so large that they are numbing. There are about 1.2 billion smokers and hundreds of millions of smokeless tobacco users.[3] Cigarettes are the main type of tobacco product consumed throughout the world. Bidis are popular in India and a substantial amount of tobacco is consumed worldwide in the form of smokeless tobacco products, including chewing tobacco, moist stuff, which is placed between the cheek and gum, is used extensively in India.[3] Cigarette smoking causes well over 1 million cancer deaths annually worldwide. Epidemiological studies from the United States, India and Pakistan provide sufficient evidence that smokeless tobacco causes oral cancer in humans. Poly cyclic aromatic hydrocarbons (PAH) is the carcinogen present in tobacco leads to squamous cell carcinoma of oral cavity.[3] To evaluate the genotoxic risks in tobacco users on buccal mucosa, observed as DNA damages can be assessed by MN test. Micronuclei are chromatin containing bodies that represent fragments or even whole chromosomes that were not incorporated into a daughter cell nucleus at mitosis [4] have been used as bio markers for the assessment of DNA damages. There was a significant reduction in the mortality rate in the intervention arm versus the control arm due to the detection of oral cancer at an early stage.[5]

MATERIALS AND METHODS

25 cases of squamous cell carcinoma, attending radiotherapy department for radiotherapy treatment were included in our study. 25 premalignant cases with leukoplakia, erythroplakia, Sub-mucosal fibrosis, lichen planus, attending the department of dental surgery were also included in our study. Detailed history was taken regarding the usage of tobacco in the form of cigarette smoking, chewing pan and betal nut. From all these cases the specimen from the lesion were collected and slides were prepared by the protocol given by Fenech et al.[6] Patient was asked to rinse the mouth and scraping was taken from the buccal mucosa with the help of a wooden spatula. Smear was prepared and was kept in the methanol glacial acetic acid fixative in 3:1 ratio. After 20 minutes the smeared slides were stained with May-Grunwald stain and it was counter stained with Giemsa. After drying 500 cells were screened for micronucleus. MN Scoring criteria:

- the diameter of the MN should be less than one-third of the main nucleus
- MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary
- MN should have similar staining as the main nucleus

Figure 2 is the example for the typical micronucleus, figure 1 and figure 3 should not be included in the scoring.

RESULTS

Table 1
Age and Sexwise distribution of Premalignant and malignant cases

Age	Premalignant		Malignant	
	Male	Female	Male	Female
30-34	-	1	-	-
35-39	1	1	-	-
40-44	3	1	0	1
45-49	4	4	4	1
50-54	4	2	1	1
55-59	2	-	5	2
60-64	1	1	4	3
65-69	-	-	1	2
Total	15	10	15	10

Results were statistically analyzed by unpaired students 't' test. Among 25 pre malignant cases 15 were males and 25 were females. Similarly among 25 malignant cases 15 were males and 15 were females. Majority of the patients were in the age group of 45 – 54 in the pre malignant group. Among malignant group majority of the patients were in the age group of 55- 64. (Table 1)

Table 2
Comparison of Mean MN frequency between tobacco users and non users among pre malignant cases

Tobacco	MN Count/500 cells	x	SD	P Value
Users (n=9)	22	2.4	3.2	≥0.05
Non-users (n=16)	55	3.4	4.7	

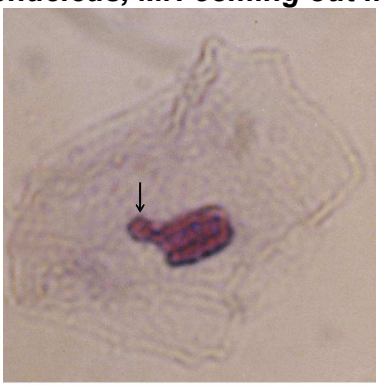
The mean micronucleus frequency among the tobacco users in the pre malignant group is less than that of non users and the p value is not significant between the two groups. (P>0.05) (Table 2)

Table 3
Comparison of Mean MN frequency between tobacco users and non users among malignant cases

Tobacco	MN Count/500 cells	x	SD	P Value
Users (n=24)	142	5.9	6.2	≤ 0.05
Non-users (n =1)	3	3	0	

The mean micronucleus frequency among the tobacco users in the malignant group is more than that of non users and the p value is significant. (P< 0.05) (Table 3)

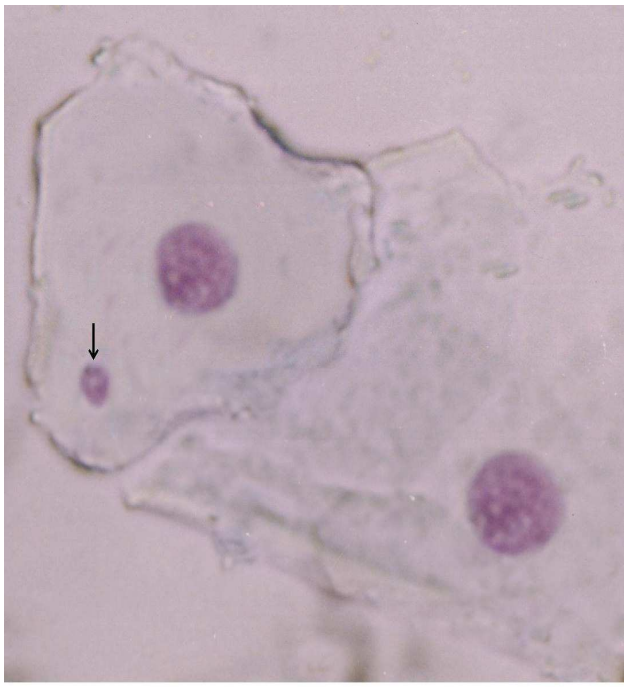
Figure 1
Shows budding micronucleus, MN coming out from the main nucleus



Arrow shows Budding MN

Figure 2

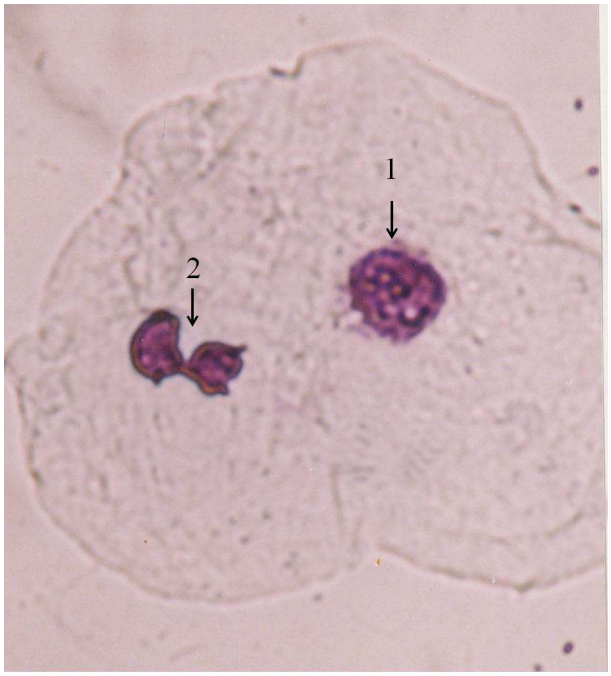
Shows separated out micronucleus from the main nucleus and lies inside the cytoplasm of the main nucleus which has the same colour of the main nucleus and ¼ th of the size of the main nucleus[6]



Arrow shows MN - Separated out from the main nucleus present inside the cytoplasm of the same cell

Figure 3

Shows karyorrhexis and separating micronucleus.



Arrow 1 shows KR; Arrow 2 shows separating micronucleus

DISCUSSION

Cigarette is the main form of tobacco product consumed throughout the world , a substantial amount of tobacco is consumed worldwide in the form of smokeless tobacco products including chewing tobacco , moist stuff pan or betel quid which is placed between the cheek and gum, a product that often contains tobacco and is used extensively in India. Cigarette smoking leads to carcinoma of the oral cavity and lung cancer and the smokeless tobacco chewing leads to cancer oral cavity. The most important Carcinogen in cigarette smoke is PAH.[1] In the present study, oral carcinoma ranked at 88% in patients using tobacco, either in the form of chewing or smoking. Our findings are in accordance with the study conducted by Scully in 2000[7], in which 75% of the patients of oral carcinoma were tobacco users. Similar results were also reported by Matsui in 2006.[8] Hence tobacco can be considered as a leading carcinogenic agent which causes DNA damage by its genotoxicity and leads to cancerous proliferation. 77% of leukoplakia patients were tobacco users either in the form of smoking or chewing as observed in the present study which confirms the results of the study conducted by Saraswathy in the year 2006.[9] Hashibe et.al 2001[10] identified tobacco chewing and alcohol as risk factors for erythroplakia. 100% erythroplakia cases in our study were found to be tobacco users. MN frequency when compared between tobacco users and non-users were found to be significant with the 'P' value of <0.05 in our study. Our findings are in accordance with findings reported by Bloching et al 2000 [11]and Konapacka2003.[12]

Our study confirms the findings of Nair et al in 1991,[13] reported that mean MN micronucleus frequency as 5.2 in tobacco users and 2.9 in non users. In our study, the mean was 5.9 and 3 among users and nonusers respectively. Sarto et.al 1987[14] also observed a statistically significance difference in the MN frequency among smokers and non smokers. Recent study conducted by Beena P. Patel, 2009[15] also stated that there was a significant p value (p=0.001) for the micronucleus frequency between the controls and chewers. According to the study conducted by Sudha Sellappa 2009[16] between chewers, smokers and controls, there was no significant difference between the mean percentage of MN cells among 2 groups (Chewers and Smokers) but the result was statistically significant between tobacco users and controls. The statistically significant increase in the MN count among tobacco users in our study results are in accordance with the study conducted by V. Ramakrishnan 2011[17], according to him MN was significantly higher (15.82 ± 1.31) in chewers than controls (4.82 ± 1.47) (P < 0.001). Hence all these studies confirmed that the carcinogen in the tobacco leads to mutation and important etiological factor for the carcinoma of oral cavity.

CONCLUSION

There is a significant increase in the micronucleus frequency among tobacco chewers in the malignant group. So this MN study can be used as a screening test for the population having the habit of using tobacco in the form of cigarette or smokeless tobacco for identifying the genomic instabilities at an early stage. Red patches (Erythroplakia) need to be properly diagnosed at an early stage because of the 100% malignant transformation.

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