



COMPARATIVE EVALUATION OF MICRONUCLEI AMONG 18-50 YEARS OLD SMOKERS, NON-SMOKERS AND ARRESTED SMOKERS - AN INVIVO STUDY

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ABSTRACT

Objective: Comparison of micronuclei count in exfoliated oral mucosal cells among 18–50-year-old smokers, non-smokers and arrested smokers. **Methods:** The study comprised of three groups namely smokers, non-smokers and arrested smokers. At the start of the study, intraoral examination was conducted for assessing the oral mucosal lesions using WHO oral health assessment forms for adults 2013 on subjects aged 18-50 years belonged to both the gender. Written consent was taken from all the study participants based on inclusion and exclusion criteria. The study included swab collection and microscopic examination. Exfoliated cells were obtained by scraping the buccal mucosa with a moistened wooden spatula and the scraped cells were placed on clean glass slides and smears were prepared. The examination of micronuclei was conducted under a 1000X magnification. The criterion which was developed by Tolbert et al was used for counting the micronuclei. **Results:** The mean values for the groups were 1.5, 1.98, and 2.25 respectively, showing small numerical differences with highest micronuclei count amongst smokers. The analysis revealed an F-value (2, 87) of 0.190 and a p-value of 0.827, indicating no significant differences among the three groups ($p > 0.05$). **Conclusion:** The micronuclei count was found to be slightly higher in smokers (2.25 ± 4.45) compared to non-smokers (1.98 ± 3.27) and arrested smokers (1.5 ± 3.99). The comparison of micronuclei count between smokers, non-smokers and arrested smokers was found to be statistically non-significant ($p=0.827$). Therefore, it was advised that monitoring people over time can provide light on how smoking cessation and commencement affect the frequency of micronuclei. In order to encourage tobacco cessation efforts, it was our responsibility as public health dentists to educate people about the detrimental effects of smoking and the cellular consequences of stopping, particularly among current and former smokers.

KEYWORDS : Micronuclei, Smoking, Exfoliated Oral Mucosal Cells, Cytogenetic Damage, Tobacco Cessation

INTRODUCTION

One of the leading causes of morbidity and death in the modern era is cancer, with oral cancer ranking among the top three cancers in India and accounting for 30% of all cancer cases. The most common type of oral cancer is primarily caused by tobacco use in any form, which is strongly linked to both the disease's growth and a dismal prognosis. Evidence reveals that buccal cell micronuclei (MNs) are significantly higher in the buccal mucosal cells of individuals who have precancerous lesions and in cancer patients. This makes MNs a potential biomarker for oral cancer risk.

Hence this study was conducted to evaluate the compare the micronuclei count in exfoliated oral mucosal cells among 18-50 yr old smokers, non-smokers and arrested smokers.

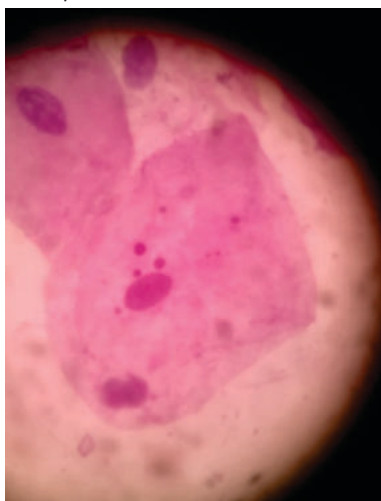


Fig 1 Illustrates the Micronuclei

MATERIALS AND METHODS

Study subjects were selected from OPD of Coorg Institute of Dental Sciences, Virajpet; OPD of peripheral dental units of CIDS, Virajpet; and OPD of Govt. Hospital, Virajpet. The study subjects were grouped into smokers, non-smokers and arrested smokers based on inclusion and exclusion criteria. Written informed consent was obtained prior to sample collection from each participant. Every person shall thoroughly rinse his / her mouth with tap water prior to sampling. Using a moistened wooden spatula, buccal mucosa was scraped to extract the exfoliated cells. Smears were prepared using clean glass slides once the cells have been scrapped off. 95% alcohol was used to fix the smears. A commercial staining kit called RAPIDPAP was used to apply the Papanicolaou procedure to all of the cytological smears. Approximately 1000 cells from each slide were analysed at a 400X magnification. MN cells were viewed at 1000x magnification.



Fig 2 Illustrates the Armentarium Used For Sample Collection



Fig 3 Illustrates The Sample Collection of Exfoliated Buccal Mucosal Oral Cells

RESULTS

Among the study population, there were 14 arrested smokers, 40 non-smokers and 36 smokers. Results show that 18 (20%) subjects belong to the age group 18-25 years, 22 (24%) belong to the age group 26-36 years and 50 (55.6%) belong to the age group 37-50 years; 17 (18.9%) 23 subjects were female and 73 (81.1%) subjects were male; 15 (16.7%) subjects were not working and 75 (83.3%) subjects were working.

Table 1: Distribution of Study Subjects Based on Age in Study Groups

Study Groups	18-25 yrs	26-36 yrs	37-50 yrs	Total	Mean Age
Arrested smokers	0 (0%)	2 (14.3%)	12 (85.7%)	14 (100%)	43.21±7.55
Non smokers	11 (27.5%)	10 (25%)	19 (47.5%)	40 (100%)	34.33±11.53
Smokers	7 (19.4%)	10 (27.8%)	19 (52.8%)	36 (100%)	36.56±9.84
Total	18 (20%)	22 (24.4%)	50 (55.6%)	90 (100%)	

Table 2: Distribution of Study Subjects Based on Gender in Study Groups

Study Groups	Female	Male	Total
Arrested smokers	0 (0%)	14 (100%)	14 (100%)
Non smokers	12 (30%)	28 (70%)	40 (100%)
Smokers	5 (13.9%)	31 (86.1%)	36 (100%)
Total	17 (18.9%)	73 (81.1%)	90 (100%)

Table 3: Distribution of Study Subjects Based on Occupational Status in Study Groups

Study Groups	Non-working	Working	Total
Arrested smokers	1 (7.1%)	13 (92.9%)	14 (100%)
Non smokers	11 (27.5%)	29 (72.5%)	40 (100%)
Smokers	3 (8.3%)	33 (91.7%)	36 (100%)
Total	15 (16.7%)	75 (83.3%)	90 (100%)

Table 4: Comparison of Micronuclei Count Among Study Groups Using One-way Anova

Groups	N	Min	Max	Mean	SD	SEM	CI		One-way ANOVA
							LB	UB	
Arrested smokers	14	0	15	1.50	3.99	1.06	-0.81	3.81	F = 0.19 p = 0.827 (NS)
Non smokers	40	0	12	1.98	3.27	0.51	0.93	3.02	
Smokers	36	0	20	2.25	4.45	0.74	0.74	3.76	
Between groups	Sum of squares = 5.76; df= 2; Mean squares= 2.88								
Within groups	Sum of squares= 1321.22; df=87; Mean squares=15.18								

S: Significant at p value <0.05 using ANOVA; NS: Not Significant.

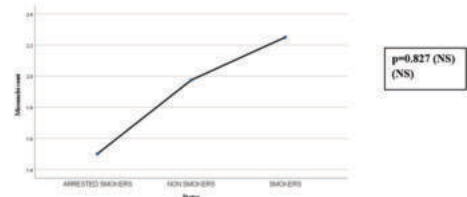


Fig 4: Comparison of Micronuclei Count Among Arrested Smokers, Non-smokers, and Smokers Using One-way Anova- Means Plot

The micronuclei count was found to be slightly higher in smokers (2.25 ± 4.45) compared to non-smokers (1.98 ± 3.27) and arrested smokers (1.5 ± 3.99). The comparison of micronuclei count between smoker, non-smokers and arrested smokers was found to be statistically non-significant ($p=0.827$).

DISCUSSION

In the present study, the mean number of micronuclei count was found to be slightly higher in smokers (2.25 ± 4.455) compared to non-smokers (1.98 ± 3.278) and arrested smokers (1.5 ± 3.995). The results are in accordance with the study conducted by Yusuf Ozkul et al in Turkey, where the mean percentage of micronuclei cells was seen higher in smokers (1.99 ± 0.30) than non-smokers/non-users (1.86 ± 0.26). The results are also in accordance with the studies, where they have also used smokeless tobacco chewers / combined tobacco users as other study group. In a study conducted by Himanta Bansal et al in Punjab, India, the mean of micronuclei counts which was found to be more in smokeless tobacco chewers (24.13 ± 10.68) as compared with smokers (11.96 ± 4.23) and control (4.17 ± 2.99). In a study conducted by Meenakshi Upadhyay et al in Modinagar, Uttar Pradesh (UP), the micronuclei was found to be highest in the smokers and chewers (21.996 ± 9.916) followed by chewers (10.413 ± 3.865), smokers (7.589 ± 5.672) and controls (1.033 ± 1.265). In a study conducted by Sangeeta Palaskar et al in Ambala, Haryana, the mean number of micronuclei was found to be higher in smokeless tobacco users (45 ± 6.18) than in smokers (22.07 ± 5.88) and non-smokers/non chewers (6.13 ± 2.29) by using PAP stain; while by using MGG stain it was observed that mean number of micronuclei was higher in smokeless tobacco users (38.6 ± 6.51) than in smokers (17.67 ± 5.76) and non-smokers/non-chewers (3.53 ± 1.407).

In the present study, the comparison of mean number of micronuclei count between three study groups (smokers, non-smokers and arrested smokers) was found to be statistically non-significant ($p>0.05$). The results are in accordance with the study conducted by Yusuf Ozkul et al, wherein comparison of mean of micronuclei count between smokers, non-smokers/non users was found to be statistically non-significant ($p>0.05$). The results are in contrast with study conducted by Himanta Bansal et al, wherein comparison of mean of micronuclei count between smokeless tobacco chewers, smokers and control was found to be statistically significant ($p<0.001$). The findings are also in contrast with the study conducted by Meenakshi Upadhyay et al, wherein comparison of micronuclei count between smokers and chewers, smokers and controls were found to be statistically significant ($p<0.001$). The results are also in contrast with the study conducted by Beena P Patel et al, wherein comparison of mean of micronuclei count between smokeless tobacco users, smokers, non-smokers and non-chewers was found to be statistically significant by using PAP stain ($p<0.001$) and also by using MGG stain ($p<0.001$). The results are also in contrast with the study conducted by Beena P Patel et al, wherein comparison of mean of micronuclei count between smokeless tobacco users, smokers, non-smokers and non-chewers was found to be statistically significant by using PAP stain ($p<0.001$) and also by using MGG stain ($p<0.001$).

CONCLUSION

Study showed that it contributes valuable data to the understanding of tobacco related cytogenetic damage, which is relevant for cancer risk assessment and prevention strategies. Smoking causes double stranded breaks in DNA, which is thought to be the most severe sort of DNA damage. According to prior studies, there was reduction in double stranded breaks a month after quitting smoking. Since research on arrested smokers is limited, the results of present study provide a positive reinforcement and emphasize the need of making smoking cessation. Therefore, it is our duty as a public health dentists to raise awareness about the harmful effects of smoking/ effects of quitting smoking at the cellular level, especially among current and former smokers which might stimulate tobacco cessation efforts.

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