Micronuclei frequency amongst smokers having premalignant lesions and correlation with frequency and duration of smoking

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ABSTRACT

Introduction: Cancer is caused by genotoxic effects of chemical carcinogens like tobacco. At early stages of cancer, genomic instability is reflected often as Pre-malignant lesions. Micronuclei (MN) are small, extra-nuclear bodies which have separated from the main nuclei. MN scoring can be used as a biomarker to identify different pre-neoplastic conditions much earlier than the manifestations of clinical features. **Aims & objectives**:1) Compare the MN frequency in normal looking buccal mucosa and potentially premalignant buccal mucosal lesions among tobacco users.2) to assess the effect frequency and duration of use of the tobacco on MN frequency. **Materials and Method:** Two groups, smokers with or without any premalignant lesion were randomly selected. Buccal smears of all participants were taken using wooden spatula and stained with standard Papanicolaou (PAP) stain and Micronuclei frequency per 100 cells were counted. The results were analyzed statistically using "unpaired t-test". **Results**: The Micronuclei frequency in tobacco smokers with normal looking buccal mucosa and PML was 2.27 and 1.93 respectively showing that the genotoxicity can be detected even before onset of premalignant lesion, but no significant difference were found between all 3 premalignant lesion (PML). There was a definite correlation between the occurrence of micronuclei and the frequency and duration of smoking. **Conclusion**: The increase in Micronuclei frequency in tobacco smokers shows that the genotoxicity can be detected even before onset of PML. So MN frequency can be used as a prognostic, educational and interventional tool in the management of patients with smoking habits.

Key-words: Micronuclei, Premalignant lesions, smoking, Tobacco smokers

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INTRODUCTION

Micronuclei (MN) are small, extra-nuclear bodies which have separated from the main fragment, generated during cellular division by late chromosomal fragments because of their association with chromosomal aberrations [1,2,3,4,5,6]. MN scoring can be used as a biomarker to identify different pre-neoplastic conditions much earlier than the manifestations of clinical features [3,4,5,6,7,8,9].

This study was undertaken to compare the MN frequency in normal looking buccal mucosa and potentially premalignant buccal mucosal lesions among tobacco users and also to assess the effect frequency and duration of use of the tobacco on MN frequency in Saurastra region, Gujarat.

MATERIALS AND METHODS

A prospective comparative study was conducted over a two month period in a tertiary care hospital. Two groups, smokers with or without any premalignant lesion (n = 80) and nonsmokers (n = 20) with sound medical history were selected randomly. Criteria for inclusion were 5-20 bidi/cigarette smoking/day for more than 5 years. To avoid confounding factors found in other studies like age, sex and alcohol, only non alcoholic male between 25-45 years were assessed. Alcohol consumption in any form was an exclusion factor for both groups. No attempts were made to balance sex bias as all smokers were male. Consent forms of all participants were taken and approval from the Ethical Committee was obtained.

After a thorough medical history and oral examination, participants were asked to rinse the oral cavity. Using a sterile wooden spatula, buccal smears were taken from right buccal mucosa or from premalignant lesion, spread over a clean glass slide in circular manner from central of slide to periphery and fixed in methyl alcohol. The smears were stained using standard Papanicolaou (PAP) staining protocol. The smears were observed under oil immersion for counting of Micronuclei frequency in zig-zag manner. Total micronuclei in 100 screened cells and micronuclei frequency were counted.

To designate an extra nuclear body as a MN, the criteria given by Tolbert *et al.*[2,24] were used. We had counted Micronuclei frequency as follow.

Micronuclei frequency= Total number of micronuclei per 100 cells / Total number of micronucleated cells

Statistical analysis

Comparison of micronuclei frequency amongst premalignant lesions were done by "Unpaired 't' test". All the Statistical analysis was done by using 'Graphpad online' software. P<0.05 was considered as statistically significant in all results.

RESULTS

Among 80 smokers, 21 had premalignant lesion (9-SMF, 8-Leukoplakia, 4-Erythroplakia), rest 59 didn't have any oral lesion.12 cases couldn't be assessed due to lack of cellularity.

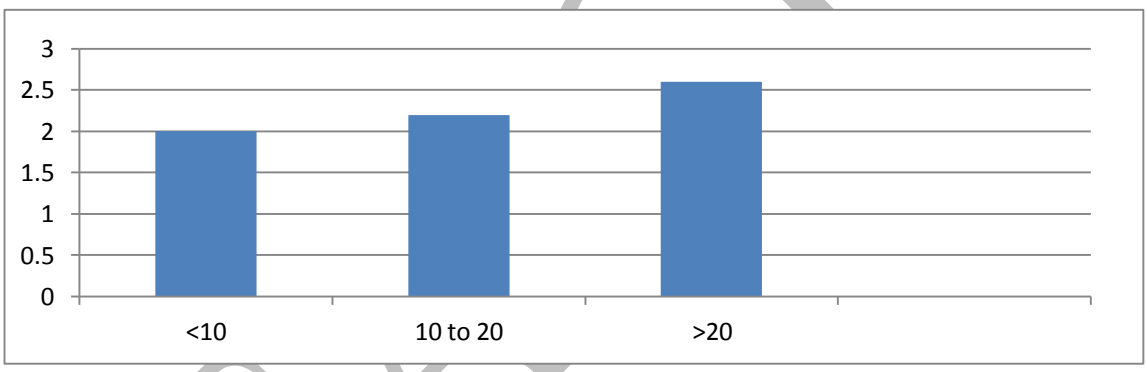
Table 1: MN amongst cases having SMF, Leukoplakia & Erythroplakia.

Oral lesions	Case no.	MN	Average	Variance	ANOVA	Remarks
SMF	6	145	24.5	646.6	F=0.3 *F crit=3.7* P=0.78	NS
Leukoplakia	7	172	24.6	425.0		
Erythroplakia	4	63	15.8	203.0		
		Mean MN				
SMF	6	12.0	2.0	0.3	F=0.8 *F crit = 3.7* P=0.45	NS
Leukoplakia	7	15.1	2.2	1.1		
Erythroplakia	4	5.8	1.5	0.9		

No statistically significant difference was found in MN and Mean MN between the 3 PML. However a trend was seen where leukoplakia showed the highest values, closely followed by SMF and lowest values were seen in erythroplakia.

Table 2: Frequency of smoking and mean MN in tobacco smokers.

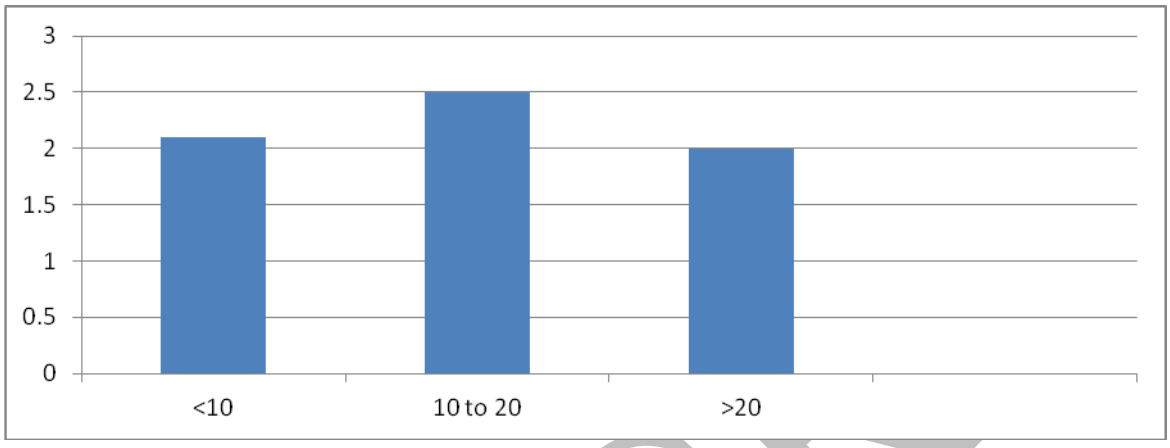
Frequency of smoking	**Case No.**	**MN**	**Average**	**Variance**	**ANOVA**
<10	17	34.6	2.0	0.5	F=1.1
10-20	10	22.1	2.2	1.1	*F crit = 3.3*
>20	11	28.4	2.6	1.2	P=0.33



As frequency of smoking increases, the mean micronuclei count increased marginally in tobacco smokers, but it was not found to be statistically significant.

Table 3: Duration of smoking and mean MN in tobacco smokers

Duration(years)	**Case No.**	**MN**	**Average**	**Variance**	**ANOVA**
<10	8	16.7	2.1	0.3	F=0.95
10-20	16	39.8	2.5	0.9	*F crit = 3.3*
>20	14	28.6	2.0	1.2	P=0.39



The mean micronuclei frequency in tobacco smokers showed no increase with duration of smoking.

DISCUSSION

Oral cancer is the 11th most common cancer worldwide and the most common in India. Cancer is caused by genotoxic effects of chemical carcinogens or environmental pollutants resulting in genomic instability. At an early stages of cancer, genomic instability is reflected often as leukoplakia, erythroplakia sand submucous fibrosis(SMF)[1].

A cigarette contains numerous cytotoxic substances, suchas polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, heavy metals, poisonous gases and pesticide residues [2]. These elements exert a genotoxic effect on the oral tissues. It has been established that there is a dose-response relationship between the amount of tobacco product used and the development of oral cancer[3].

The MN assay in exfoliated buccal cells is a useful and minimally invasive method for monitoring genetic damage in humans. The level of baseline chromosome damage in untreated cancer patients and also in various Premalignant lesions is much higher than in cancer-free controls. Therefore, Micronuclei scoring can be used as a biomarker to identify different preneoplastic conditions much earlier than the manifestations of clinical features and might specifically be utilized in the screening of high-risk population for a specific cancer[10].

Table 4: Comparison of MN in tobacco users having either no oral lesion or any of the PML.

Study name	**n = NOL/PM`L**	**Stain**	**Mean MN in NOL**	**Mean MN in PML**	**Remark**
Dindire et al[1] 2012	10/ 20	PAP	2.85	4.85	Significant
Our study	59/ 21	PAP	2.27	1.93	NS

Even after extensive literature search, only single study was available for this comparison which had used Pap stain. Dindire et al[1] found that MN frequency was more in PML compared to no oral lesion, however our result shows that tobacco users with no oral lesion have more MN. Our findings can be attributed to the fact that cells with more chromosomal damage are lost at a higher frequency than those with less damage and hence micronucleated cells are also lost[11].

Table 5: Comparison of MN in tobacco users having PML.

Study name	n= S/L/E	Stain	SMF	Leukoplakia	Erythroplakia	Remark
Dindire et al[1] 2012	9/6/3	PAP	5.10	3.00	8.66	Significant
Our study	9/8/4	PAP	1.99	2.16	1.45	NS

In the study of Dindire et al[1] lowest values of Mean MN are seen in leukoplakia followed by SMF, and highest values are seen in Erythroplakia which is different from our results. The overall value of MN is higher in their study.

Table 6: Comparison of MN with duration of smoking.

Study name	Stain	MN in <10 year	MN in 10-20 year	MN in >20 year	Remark
Naderi et al[11]	Feulgen	1.89	2.01		NS
Kamath et al[3]	PAP	65.57	45.83		NS
Our study	PAP	2.1	2.5	2.0	NS

Table 7: Comparison of MN with frequency of smoking.

Study name	Stain	MN in <10 bidi/day	MN in 10-20 bidi/day	MN in >20 bidi/day	Remark
Kamath et al[3]	PAP	55.74	68.00		NS
Our study	PAP	2.09	2.48	2.04	NS

One study done by PAP stain and another by feulgen are available for comparison of duration and frequency of consumption and MN.

Naderi et al[11] found no significant association between MN and duration of smoking. Study of Kamath et al[3]has shown interesting results. Their study shows that MN increases initially and there after decreases with duration and only marginally increases with frequency of smoking.

The present study shows that there is a marginal increase in MN in with 10-20 years and with 10-20 bidi/day. In the >20 years and >20 bidi/day group the levels of MN fall back, comparable to <10 years and <10 bidi/day groups. No reason could be attributed to this fall. However these results are comparable with Kamath et al[3].It appears that smoking has ability to manifest chromosomal alterations from initial stages of tobacco usage. Other confounding factors may also affect induction of MN count in individuals.

CONCLUSION

The Micronuclei frequency in tobacco smokers with normal looking buccal mucosa and PML was 2.27 and 1.93

respectively showing that the genotoxicity can be detected even before onset of premalignant lesion. The mean micronuclei frequency remained same amongst the 3 premalignant lesion- submucosal fibrosis, leukoplakia and erythroplakia. So which premaligant lesion will develop cannot be predicted on the basis of different micronuclei frequency.

There was a direct correlation with increased MN in smokers and increased correlation of presence of MN with duration and frequency of smoking.

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Abbreviations

MN - Micronuclei

PAP- Papanicolaou stain

PML- Pre-malignant Lesion

NOL- No Oral Lesion

SMF- Submucosal fibrosis