



Original Research Article

The Study of Micronucleus Index in Patients Presenting with Pre Malignant Lesions

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ABSTRACT

Micronucleus (MN) is the nucleus that expresses the genotypic alterations caused in the process of malignancy. It is characteristically seen in exfoliated epithelial cells like Buccal Mucosa and urinary bladder wall during pre-cancerous and cancerous conditions in less and large proportions respectively. It is commonly used as a Biomarker to assess the stage and severity of neoplasm. Aim of our study is to observe the micronucleus Index in patients presenting with pre-malignant oral lesions. 30 patients with pre-malignant lesions from the Department of oral medicine, Vydehi Institute of Dental Sciences and Research Centre were screened for the presence of micronucleus in the buccal scrapings obtained from the site of the lesion in the oral cavity by conventional methods. The obtained slides were stained by using Haematoxylin & Eosin stains and the micronucleus index was calculated. The results showed that the alteration of micronucleus count was observed in premalignant conditions with respect to age and gender. Hence it can be concluded that the micronucleus index can be used as a biomarker or as a screening test in patients presenting with pre malignant conditions.

Key Words: Micronucleus Index; Squamous cell Carcinoma; Leukoplakia; Erythroplakia; Sub mucous fibrosis; Tobacco.

INTRODUCTION

Micronucleus is a microscopically visible, round or oval cytoplasmic chromatin mass in the extra nuclear vicinity, originated from aberrant mitosis. It consists of eccentric chromosomes, chromate fragments or whole chromosomes which failed to reach spindle poles during mitosis and has been used as biomarkers for assessment of DNA damages.^[1]

Micronuclei are derived from chromosomal fragments and whole

chromosomes lagging behind in anaphase. The Micronuclei assay can be used to show both clastogenic and eugenic effects. Exfoliated epithelial cells have traditionally been used for cancer screening and bio-monitoring of genotoxic effects in humans. The frequencies of micronuclei observed in the exfoliated cells of oral mucosa are an appropriate Index to monitor the genotoxicity because these cells are in direct contact with the carcinogen. Micronucleus is

the erratic nucleus that is formed during the anaphase of mitosis or meiosis.^[2]

The analysis of micronuclei has gained increasing popularity as in vitro genotoxicity test and as a biomarker assay for human genotoxic exposure and effect. The main reasons for this development are that in comparison with chromosomal aberrations, the scoring of micronuclei is simple, requires shorter training and is less time consuming. It is expected to be more sensitive than chromosomal aberration assay, because of the increased statistical power brought out by the fact that the number of cells analyzed can easily be increased to thousands when only a hundred or a few hundred cells are usually scored for chromosomal aberrations.^[3]

MATERIALS AND METHODS

A Case control study was conducted in the Department of Anatomy, Vydehi Institute of Medical Sciences and Research Centre Bangalore from December 2008 to April 2010. 30 patients of which 17 males and 13 females in the age group between 25-65 years clinically proven with pre-malignant cases were chosen from the Department of Oral Medicine, Vydehi Institute of Dental Sciences and Research Centre Bangalore. Informed consent was obtained from each patient. Detailed history regarding the patient's personal habits of various exposures & its duration, amount they consume each day were noted. Dietary history, Oral habits, Family history

The micronucleus index^[1] was calculated as:

$$\text{Micronucleus index} = \frac{\text{Micronuclei count}}{\text{No. of cells screened from each slide}}$$

regarding oral diseases & oral carcinomas were noted down.

Materials

Wooden Spatula for scraping the lesion, Marker pencil for numbering the slides, Clean Glass Slides for taking the smears, Plastic Jars for storing the fixed slides, Coplin jars for staining, Absolute Alcohol as a fixative, Haematoxylin (Harris) and Eosin stain, Light Microscope 10x, Differential counter for calculating the cells & DPX solution.

Methods

Detailed history of each patient was obtained, after the patients rinsing the mouth with water properly the scrapings were taken by the slide marked with diamond marker, from the lesion by using a dry wooden spatula. The scraped material was directly placed on a clean glass slide 1cm from the end of the slide. Then another slide with a smooth edge-spreader is taken and is placed at the edge over the smear kept 30-40 degree angle and smear is made with a forward movement of the spreader.

The obtained smears are air dried & fixed in 90% absolute alcohol & stained with haematoxylin and eosin. Later these stains were observed for the micronucleus under a delta microscope. Then the total numbers of cells from each slide were counted by using the differential counter machine by the ZigZag method.^[4] About 400-500 cells were screened from each slide and then the micronucleus index was calculated.



FIG No. 1: H&E staining of Buccal smear , the arrow showing micronucleus.

RESULTS

Table 1: Age distribution of Pre-malignant subjects.

Age in years	Pre-malignant	
	No	%
21-30	3	10.0
31-40	6	20.0
41-50	8	26.7
51-60	8	26.7
61-70	2	6.7
>70	3	10.0
Total	30	100.0
Mean \pm SD	50.40 \pm 13.36	

In the pre malignant group highest about 26.7% subjects were from the age group of 41-60yrs and 6.7% of subjects were in the age group of 61-70 yrs. The Mean \pm SD was 50.40 \pm 13.36. Samples are age matched with p=0.184

Gender distribution of Pre-malignant subjects: Out of the 30 pre malignant cases about 56.7% of subjects were males and 43.3% were females. Samples are gender matched with p=0.795

Table 2: Comparison of Habits of Smoking, Alcohol and Tobacco in Pre-malignant subjects Habits are statistically similar with p>0.05 and there is no significant difference.

Habits	Pre-malignant(n=30)	P value
Smoking	15 (50.0%)	0.05
Alcohol	9(30.0%)	0.781
Tobacco	25(83.3%)	0.347

Table 3: Mean MN count and MN Index in Pre-malignant Subjects.

	Pre-malignant (n=30)	P value
MN Count	3.70 \pm 6.64	0.194
MN Index	0.0074 \pm 0.013	0.194
Inference	No significant difference was seen with p=0.194	

Micronucleus count in Pre-malignant subjects was nil in 46.7%, of count 1-5 in 33.3%, of count 6-10 in 10.0% & of count >10 in 3(10.0%). Distribution of MN count was similar with p=0.776

Micronucleus Index in Pre-malignant subjects was Nil in 46.7%, of <0.01 in 33.3%, of 0.01-0.02 in 10.0% & >0.02 in 10.0%. Distribution of MN index is similar with P=0.776

Mean MN count and MN Index in Pre-malignant Subjects was that there was no significant difference with p=0.194.

DISCUSSION

The micronuclei are seen in blood lymphocytes and urinary bladder epithelial cells by Majer BJ et al.^[5] In the present study the analysis of micronuclei is done exclusively by taking the buccal mucosal scrapings.

The buccal cells were scraped by the help of a wood tongue depressor by Moore et. al.^[6] Also can be taken by using a cotton swab according to Ahmer et. al.^[7] In the present study buccal scrapings were taken by the help of wooden spatula.

The analysis of micronuclei was done by using Rapid Papanicolaon technique instead of fluorescent dyes for staining purpose since it was very simple to use, less time consuming and economical. By Devendra H Palve.^[4] The levels of MN were increased in a study reported by Kumar V et al the reason is that they had followed a fluorescent acridine orange staining method and the analysis was done under fluorescence microscope, increasing the specificity to identify DNA containing

structures. This technique is a time consuming method and requires costlier chemicals and equipment.^[8] In the present study micronucleus were stained with Heamatoxylin and Eosin stains.

In a Study done by Casartelli the evaluation of MN documented a significant increase in MN of pre malignant lesions (n=47).According to his study the MN frequency did not vary with sex or age of patients although it did vary with the anatomic site of the lesions.^[9] The MN count of pre malignant lesions (n=30) showed the p value of 0.776 which was not statistically significant. The percentage of micronuclei frequency in pre-cancerous lesions was 3.2+0.873 and about one fold increase of micronucleus was seen in a study done by Sumanachaterjee.^[10] In the present study it was not significant. In the present study it was not significant, which coincides with the study done by Casartelliet al.^[9]

According to a study done by Konopacka stated that the buccal cell MN count was more in the malignant condition when compared with pre malignant and normal condition.^[11] In our study the MN count was about 33.3% in the pre malignant group.

The evaluation of micronuclei number in buccal cells and peripheral blood lymphocytes showed no significant difference $p>0.05$ when compared between smokers and non-smokers which was done by Yildirim et al.^[12] In the present study there was no such statistical difference was seen when smoking and tobacco was compared with $p>0.05$ which is coinciding with study done by Yildirim et al.^[12]

In a study done by Pratheepa Sivasankari et al who said that in their study the MN index was observed to be two folds more in malignant lesions when compared with the pre malignant lesions.^[1] The MN index in the present study was about 33.3% in the pre malignant group.

The comparison of Mean MN index in pre malignant cases was significant with $p<0.05$ by Pratheepa Sivasankari et al.^[1] In the present study Mean MN index of pre malignant showed, p value of 0.194 which was not statistically significant which does not coincide with the above studies.

The mean percentage of micronucleated (MN) cells was significantly higher in non-smokers/non-users ($P < 0.01$). The mean percentage of MN cells was 1.86 ± 0.26 in users and 1.99 ± 0.30 in smokers. There was no difference between the mean percentage of MN cells in these two groups in a study conducted by Yusufzukul et al in 1997.^[13] The Mean Micronucleus count in pre malignant lesions in our study showed the count of 3.70. The Mean MN Index was about 3.08 as observed by Buajeeb et al.^[14] The Mean MN index of pre malignant lesions in present study was 0.0074.

CONCLUSION

In this study there was no significant difference among patients with pre malignant lesions with respect to age and gender. Similarly there was no significant difference among the pre malignant group in alcohol consumption and tobacco chewing. Finally we did not find any statistically significant difference in both the Micronucleus count and Micronucleus index in pre malignant lesions.

Therefore the study shows that Micronucleus count and Index alteration was observed in pre malignant oral lesions. This index can be used as a biomarker or as a screening test in patients with pre malignant lesions. As this is a very simple and feasible method which can be carried out in larger populations.

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