THE STUDY OF MICRONUCLUES INDEX IN PATIENTS PRESENTING WITH MALIGNANT LESIONS

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ABSTRACT: Micronucleus 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 malignant oral lesions. 30 patients with malignant lesions from the Department of oral medicine, Vydehi Institute of Dental Sciences and Research Centre were screened for the presence of micronucleus. The buccal scrapings were 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 malignant 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.

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

INTRODUCTION: Micronucleus, 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 is 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 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 exposure & its duration, amount they consume each day were noted. Dietary history, Oral habits, Family history 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 around 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. About 400-500 cells were screened from each slide and then the micronucleus index was calculated.

The micronucleus index was calculated as;

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\text{Micronuclei Index} = \frac{\text{Micronuclei content}}{\text{No of cells screened from each slide}}
\]

![Fig. 1: H & E staining of Buccalsmear, the arrow showing micronucleus](image)
RESULTS:

| Age in years | Malignant |
|--------------|-----------|
|              | No | %   |
| 21-30        | 1  | 3.3 |
| 31-40        | 4  | 13.3|
| 41-50        | 10 | 33.3|
| 51-60        | 4  | 13.3|
| 61-70        | 9  | 30.0|
| >70          | 2  | 6.7 |
| Total        | 30 | 100.0|
| Mean ± SD    | 55.10±13.75 |

Table 1: Age distribution of Malignant subjects

In the malignant group about 33.3% of subjects were seen in the age group of 41-50 yrs, 30% were seen in the age group of 61-70 yrs, 13.3% of subjects were seen in the age group of 31-40 yrs and 51-60 yrs, 6.7% were seen in the age group of >70 yrs. About 3.3% of subjects were seen in the age group of 21-30 yrs.

The Mean±SD was 55.10±13.75.

Samples are age matched with P=0.184.

| Gender | Malignant |
|--------|-----------|
|        | No | %   |
| Male   | 16 | 53.3|
| Female | 14 | 46.7|
| Total  | 30 | 100.0|

Table 2: Gender distribution of Malignant subjects

Out of the Malignant group about 53.3% were males and 46.7% were females.

Samples are gender matched with P=0.795.

| Habits | Malignant (n=30) | P value |
|--------|------------------|---------|
| Smoking| 15(50.0%)        | 0.05    |
| Alcohol| 10(33.3%)        | 0.781   |
| Tobacco| 22(73.3%)        | 0.347   |

Table 3: Comparison of Habits of Smoking, Alcohol and Tobacco between Malignant subjects

33.3% of Alcoholic users were seen in the malignant group.
73.3% were Tobacco users were seen from the malignant group.
In the malignant cases no MN count was seen among 53.3% of subjects. And 1-5 count was seen among 36.7% of subjects, 6.7% of subjects were seen when the count was from 6-10 and > 10 count was seen in 3.3% of subjects.

In the malignant group 53.3% of subjects had no MN index and 36.7% of subjects had the count of <0.01 of MN index, 6.7% of subjects had the count of 0.01-0.02 of MN index. And 3.3% of subjects had the count of >0.02 of MN index. Hence the distribution of MN index is statistically similar with P=0.776.

The mean MN count in the malignant group showed the value of 1.93+3.16 with the p=0.194.
The mean MN index in the malignant group showed the p value of 0.00387 +0.006 with the p=0.194.

**DISCUSSION:** The micronuclei are seen in blood lymphocytes and urinary bladder epithelial cells by Majer BJ et al. 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. Also can be taken by using a cotton swab according to El ahmer. In the present study buccal scrapings were taken by the help of wooden spatula.

The analysis of micronuclei was done by using Rapid Papnicalaon technique instead of fluorescent dyes for staining purpose since it was very simple to use, less time consuming and economical. By Devendra H Palve. 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. 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) and for malignant (n=21). 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.

According to a study done by Konopackastated that the buccal cell MN count was more in the malignant condition when compared with pre malignant and normal condition. In our study the MN count was about 36.7% in malignant group. According to the study done by Stich et al showed an elevated frequency of MN of buccal cells was documented for malignant conditions.

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.

The MN index in the present study was about 36.7% in the malignant group. The comparison of Mean MN index between malignant and pre malignant cases was significant with p<0.05 by Pratheepa Sivasankariet al. In the present study Mean MN index for malignant conditions showed, p value of 0.194 which was not statistically significant.

There was about 56.7% involvement of males in pre malignant and 53.3% in malignant lesions which is in order with the findings given by Butter Worth. This small amount of distribution can be explained on the basis of usage of tobacco or by cigarette smoking.

**CONCLUSION:** The present study was done to observe and compare the Micronucleus Index among malignant subjects. In this study there was no significant difference among patients with malignant oral lesions with respect to age and gender. Similarly there was no significant difference among two groups in alcohol consumption and tobacco chewing. Finally we did not find any statistically significant difference in both the Micronucleus count and Micronucleus index in malignant lesions.
Therefore the study shows that Micronucleus count and Index alteration was observed in malignant oral conditions with no significant difference. This index can be used as a biomarker or as a screening test in patients with malignant and pre malignant lesions. As this is a very simple and feasible method which can be carried out in larger populations.

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