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MICRONUCLEI—A PROGNOSTIC INDICATOR IN ORAL CANCER

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ABSTRACT Among the various non-invasive early cancer detection tools, micronucleus (MN) assay can be used as a diagnostic and prognostic marker to genotoxic and carcinogenic agents. The objective of this study was to compare and correlate MN frequency between pre-operative and post-operative (lesional & non-lesional) area in oral cancer patient. The study analyzed 36 patients with oral squamous cell carcinoma (OSCC) which were clinically diagnosed and histopathologically graded. The smear was taken from the mucosa surrounding lesion as well as from the opposite side; before and after surgical intervention. Collected smears were stained with acridine orange (AO) and examined for presence of MN in the exfoliated cells. Mean MN frequency was significantly higher in peri-lesional area as compared to non-lesional area. It was concluded that, micronuclei (MNi) frequency can be considered as a good prognostic indicator following treatment outcomes in oral cancer.

KEYWORDS : Oral cancer; Micronucleus assay; Acridine Orange

INTRODUCTION

Oral cancer is among the ten types of malignant neoplasia of highest incidence worldwide and remains one of the ten commonest causes of mortality in developing countries.[1] In a developing country like India nearly about 75,000 to 80,000 new oral cancer cases have been identified every year and this proportion will increase further by 2025.[2] Various invasive as well as non-invasive techniques have been discovered for early cancer detection and mass screening.[3,4] Without doubt it's an established fact that the detection of micronuclei (MNi) in a malignancy is predictable with a sensitivity of 94% and specificity of 100%.[5,6]

Micronuclei (Mni) are extra nuclear cytoplasmic bodies, caused due to double strand chromosomal aberrations and detected through appropriate staining procedure, which are much smaller than the principal nucleus and are therefore called ***micronuclei***.[7,8,9]

It's presence or absence in a cell can be ticked as an "internal dosimeter"[10,11, 12] to estimate the ongoing chromosomal instability which might be due to the existing molecular carcinogenesis.

In this study, we have tried to test if the use of the MN assay would be a better prognostic indicator in pre- and post-surgical treatment of oral cancer patients.

AIMS AND OBJECTIVES:

Present study was intended to compare and correlate MN frequency between preoperative and postoperative (lesional & non-lesional) area in oral cancer patients.

MATERIALS AND METHODS:

Patient Selection

Among OSCC patients, samples were collected in the form of exfoliated cells from peri-lesional and non-lesional areas, before and after surgical intervention.

Collection of exfoliated cells

The smears were fixed with commercially available alcohol spray fixative (available as BIOFIX). Then slides were coded and fixed in 100% alcohol.

Clinical staging

The clinical staging of patients with oral cancer was done based on tumor node metastasis (**TNM)** classification given by the American Joint Committee for Cancer Staging (AJCC).[15,16]

Histo-pathological Grading [11]

Histopathological grading of cancer for the initial biopsy and resected tumor tissue as per **Border's** classification

- Well differentiated (75%-100%)
- Moderately differentiated (50%-75%)
- Poorly differentiated (25%-50%)
- Anaplastic variety (0-25%)

Cytological staining and evaluation

The smears were fixed and stained with 0.1% aqueous acridine orange (AO). 500 epithelial cells from each smear were focused under fluorescent microscope and numbers of micro-nucleated cells were counted and expressed as percentages.

Scoring Criteria

According to **Heddle** &**Countryman et al**[18] **Mni** should fulfill following criteria-

1. Diameter should be less than 1/3rd the main nucleus.
2. Non-refractility (to exclude small stain particles).
3. Colour same as or lighter than the nucleus (to exclude large stain particles).

4. Location within 3 or 4 nuclear diameters of a nucleus; and not touching the nucleus (to make frequency measure ments meaningful).
5. No more than 2 MNi associated with one nucleus.

The statistical evaluation of the data obtained was done using analysis of variance (ANOVA) & student unpaired *t* test.

RESULTS

The study included a total of 36 patients with clinically and histo-pathologically diagnosed OSCC. There were 2 (5.6%) cases of oral cancer in the below 3rd decade, 1 (2.8%) case in the 3rd decade, 12 (33.3%) cases in the 4thdecade, 12 (33.3%) cases in the 5th decade and 9 (25%) cases over 6th decade. Out of 36 patients, 27 (75%) patients were male and 9 (25%) were female.

Clinical staging

This study comprised of 2 (5.6%) cases of stage I, 5 (13.9%) cases of stage II, and 21 (58.3%) cases of stage III, 8 (22.2%) cases of stage IV OSCC.

Histopathological grading

18 (50%) patients had well differentiated, 16 (44.4%) patients had moderately differentiated and 2 (5.6%) patients had poorly differentiated OSCC.

Results of cytological evaluation:

500 intact epithelial cells were counted from each slide for identifying MN **(Figure 1).**

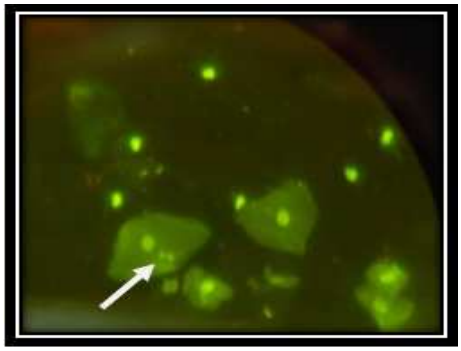
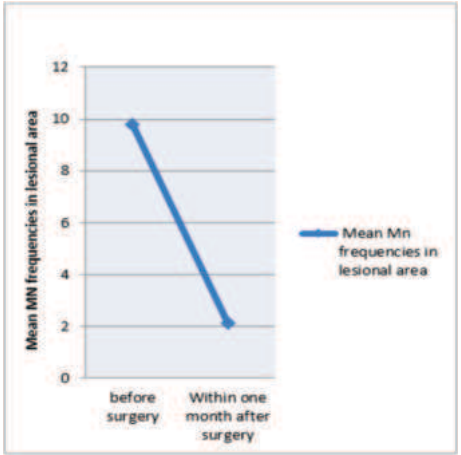


Fig 1 Cell showing micronuclei in Acridine orange stain under Fluorescent Microscope

From **lesional area**, the comparison of the mean MN frequency before and after surgery was summarized in **(Graph 1).** Mean MN frequency was reduced to 2.1389± 0.79831. Within one month after surgical intervention. Statistically the result was highly significant (p<0.001).

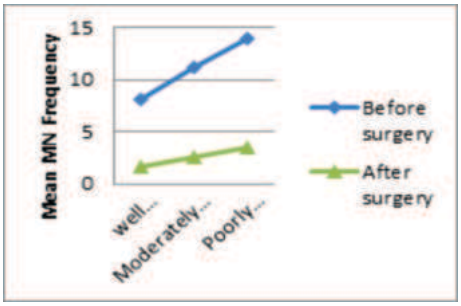


Graph 1: Comparison Of Mean Micronucleus Frequency From Lesional Area Before & After Surgery

In this study, before and within one month after surgery among 36 patients, comparisons of mean MNi frequency between lesional area and non-lesional area were summarized in (Table 1).

BEFORE SURGERY	N	MEAN MN± SD	p value	AFTER SURGERY		
				MEAN MN		p value
LESIONAL AREA	36	9.7778± 2.04396		2.1389± 0.79831		
NON-LESIONAL AREA	36	1.0833± 0.73193		0.5556± 0.50395		
PAIRED DIFFERENCE (LESIONAL AREA V/S NON-LESIONAL AREA)	36	8.6944± 1.73731	<0.001 vhs	1.58333± 0.69179		<0.001 (very highly signi ficant)

In this study, the frequencies of mean MNi in different histological grades of oral cancer before and within one month after surgery are tabulated in (Graph 3). The mean MNi frequencies were found to increase from well differentiated to poorly differentiated SCC. The difference was statistically highly significant (P<0.001).



Graph 2- Comparison Of Mean Micronucleus Frequency In Lesional Area Before And After Surgery Among Different Histo-pathological Grades Of Oral Cancer

The mean MN frequency was observed to increase from stage I to stage IV, but the difference was not statistically significant (F= 0.436, p= 0. 729), since there were only 2 cases in stage I. Thus, MN frequency was not correlated well with the clinical stages of oral cancer. (Table 2)

Stages N	Before Surgery (MEAN MN± SD)		After Surgery (MEAN MN± SD)	
	Lesional Area	Non-lesional Area	Lesional Area	Non-lesional Area
Stage I	29.0000± 1.41421	1.5±0.707	2.5± 0.707	1.000
Stage II	59.2000± 1.92354	1.25±0.500	1.4±0.5477	1.000
Stage III	219.7619± 2.23394	1.4375±0.5128	2.142± 0.7064	1.000
Stage IV	810.3750± 1.84681	1.334±0.5167	2.000± 0.756	1.000

DISCUSSION

Oral cancer is an important cause of morbidity and mortality worldwide. In India, it represents a major health problem constituting up to 40% of all cancers.[12]

Certain biomarkers are used to identify genetic damage in cancer. Among these, MNi are suitable ***internal dosimeters*** for revealing tissue specific genotoxic damage in individuals exposed to carcinogenic mixtures.[10,11,12]

In this study, there was a significant reduction in frequency of MN in post-operative case (lesional area) with a p value (p<0.001) as shown in earlier study done by **A. Haldar and**

T. Chakraborty (2004) et al.[10]

Here, a significant ($p<0.001$) increase in frequency of MNi in peri- lesional area was observed when compared to non-lesional are as shown in earlier study done by **Lav´ınia T´ercia Magalh˜aes D´ orea (2012).**[12]

There is a significant correlation of MN frequency with histopathological grading among oral cancer being observed as in earlier study done by **Devandra palve et al (2008)**[5] and **Uma A. Natarajan et al (2014).**[3]

Here, MN frequency did not correlate well with the clinical stages of oral cancer. However, **de Carvalho et al** studied the frequency of MN of the oral mucosa among 27 untreated patients with carcinoma of the oral cavity and oro-pharynx. A higher MN frequency in the stages T3 and T4 ($P = 0.01$) as compared to stages T1 and T2.[5]

It indicated that, MNi could be used as a biomarker for assessing the risk of cancer development. So, MNi assay could be a valuable tool for diagnosis and prognosis of disease progression.

CONCLUSION

From this study we conclude that, MN assay in exfoliated cells can be used as an early diagnostic tool for identifying chromosomal damage in individuals with a developing oral cancer but can also act as an internal dosimeter to assess effects of ongoing treatment in a patient with invasive cancers.

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