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Metanuclear Abnornmalities in Oral Premalignant and Malignant Lesions.

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ABSTRACT

Cancer is a complex disease with altered expression, abnormal growth and disruption of normal function of cells caused by genotoxic effects of chemical carcinogens or environmental pollutants. The three most common fatal cancers were oral, stomach, lung in men. The aim of the study is to identify the occurrence of meta nuclear abnormalities (KL, KR, BN) and to identify the occurrence of meta nuclear abnormalities in different stages of malignancy and to compare the incidence of metanuclear abnormalities in pre malignant and malignant conditions. A Total number of 25 pre malignant cases and 25 malignant cases and equal number of controls were included in our study. The material was collected and stained for Metanuclear abnormalities. Statistical analysis was done by standard t test. Metanuclear abnormalities in patients with oral carcinoma and pre malignant lesions in comparison with controls were found to be significant with the 'P' value of <0.05 in our study.

Keywords: Karyolysis(KL) , Karyorrhexis (KR), Binucleated cell (BN) ,Broken egg, Oral carcinoma, Pre malignant lesions.

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INTRODUCTION

Early detection and follow-up of oral precancerous lesions is important for predicting their potential for malignancy. [1] The primary risk factors for the development of these lesions are comparable worldwide. [1,2] Many reports have described various cytoplasmic and nuclear changes in oral malignant and premalignant lesions and after radiation therapy. These changes include cellular enlargement, vacuolization, cytoplasmic granulation, nuclear enlargement, pyknosis, karyorrhexis, karyolysis, multinucleation, micronucleation, nuclear budding, and binucleation. [3,4]. Nuclear changes: karyorrhexis signifies nuclear breakup into smaller fragments (fig 1) . karyolysis signifies a progressive dissolution of chromatin (Fig 2) . Multinucleation (Fig 3) is caused by membrane damage associated with accelerated proliferation of the nucleus ,resulting in an inability of the membrane to keep up with the nuclear division.[5] The aim of the study was to evaluate the frequency of karyorrhexis ,karyolysis ,broken egg and binucleated cells from the buccal mucosa of malignant and pre malignant lesions.

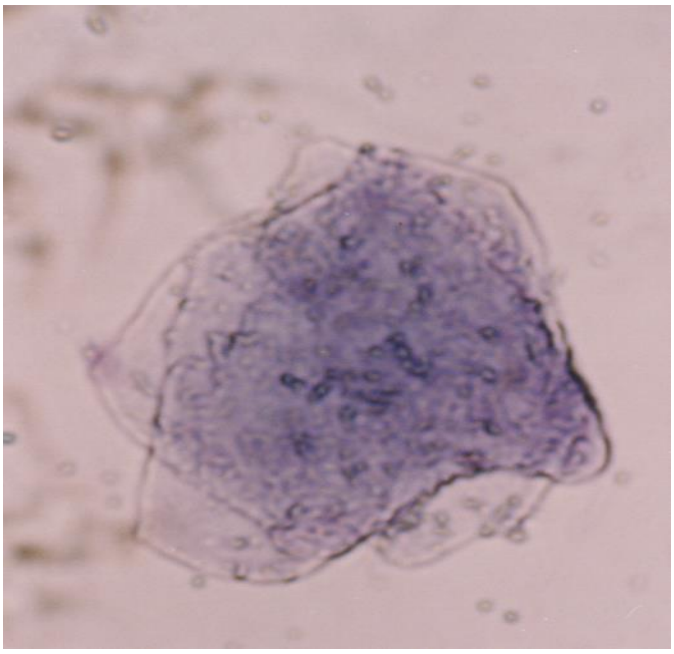

Fig 1 : Cell shows disintegrated chromatid material – KR

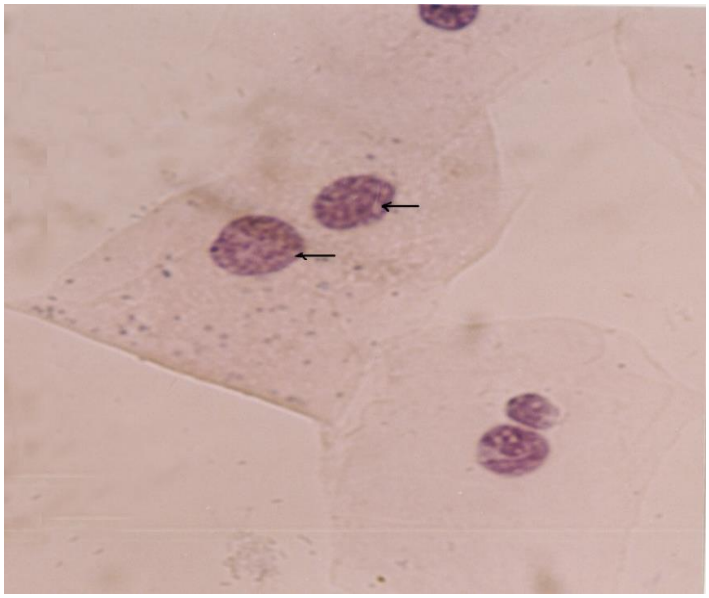

Fig 2: Arrow shows KR progressed to complete dissolution of nucleus - KL

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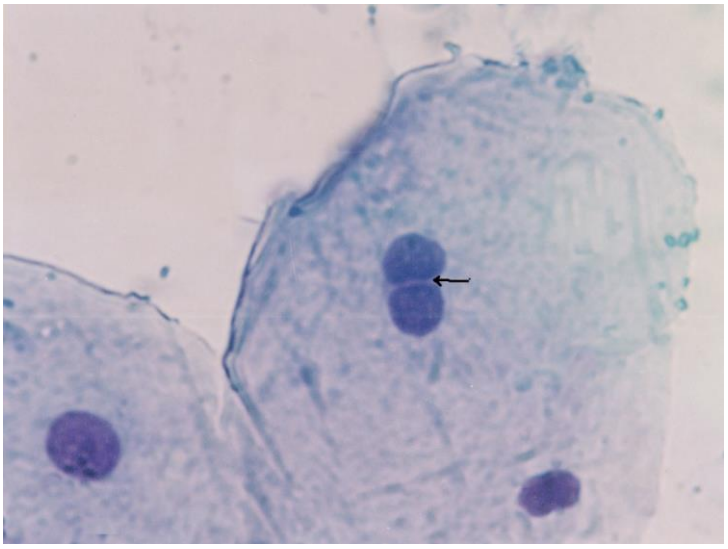

Fig 3: Arrow shows metanuclear abnormality – BN Cell

MATERIALS AND METHODS

Materials: Methanol, Glacial acetic acid, Giemsa stain and maygrunwald stain.

Inclusion criteria: Persons suffering from oral malignant and premalignant lesions.

Methodology: After getting the informed consent, the person was asked to rinse the mouth and the material was collected from the oral cavity by scraping the buccal mucosa using a clean wooden spatula. Scrapped material was spreaded on cleaned slides and smeared. After air drying, the slides were kept in the methanol glacial acetic acid fixative in the proportion 3:1 for 20 minutes. There fixed slides were stained with May Grunwald and Giemsa stain. They were observed for nuclear abnormalities under Bright Field Nikon microscope under 10 x 100 magnifications. Observations were recorded and tabulated. 500 cells were screened in each person from the slides prepared and the incidence of meta nuclear abnormalities (KL, KR, BN) were recorded and the collected data was subjected to unpaired Student's 't' test. [6]

RESULTS

Some of the metanuclear anomalies such as karyorrhexis (KR), Karyolysis (KL), broken egg were noted in all oral carcinoma patients and premalignant cases.

The occurrence of KL, KR increased abruptly after the exposure to alcohol, but steadily decreased thereafter. The increase in the prevalence of karyorrhexis and pyknosis was even more 3.7 to 4.6 times higher than control values. But karyolysis was 7 fold increased in controls.

The higher number of broken eggs in patients not exposed to tobacco and alcohol suggested that BE might be associated with DNA repair or a healthy mucosa.

Out of 50 referred cases ,25 were in the age group of 30-69 years presenting with various pre-malignant lesions and the rest 25 were in the similar age group and presenting with various stages of oral squamous cell carcinoma. Fifty controls (ie) the persons without any oral lesions and no personal habits like tobacco chewing, smoking and alcohol in the same age group between 30-69 years were also recruited for comparison (Table 1)

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Table 1:Age and sexwise distribution of premalignant and malignant cases

Age group	Control group			Study group		
	Male	Female	Total	Male	Female	Total
30- 34	-	1	1	-	1	1
35 - 39	1	1	2	1	0	1
40-44	5	3	8	3	2	5
45-49	8	1	9	8	2	10
50-54	5	3	8	5	5	10
55-59	7	3	10	7	4	11
60-64	6	5	11	5	3	8
65-69	0	1	1	1	3	4
Total	32	18	50	30	20	50
	52.2	53.1	52.5	53.0	54.7	53.7
SD	7.4	10.1	8.4	7.5	9.2	7.5

Comparision between males in control and males in study group= z = 0.475=p >0.05
Comparision between females in control and females in study group=t = 0.512=p >0.05
Comparision between the total no.of control and total no. of study group=Z=0.75=P> 0.05

Out of 25 pre malignant cases, 9 (36%) were using tobacco either in the form of smoking or chewing and 16 (64%) were not using tobacco. Out of 25 malignant cases ,24 (96%) were tobacco users and only one patient (4%) was a non-user. (Table 2)

Table 2:Distribution of personal habits among malignant and pre malignant cases

Personal habits	Premalignancy	Malignancy
Tobacco users	9	24
Non – users	16	1

Out of 25 pre malignant cases ,18 (72%) patients had leukoplakia, 3(12%) had erythroplakia, 2 (8%) had lichen planus and the rest (8%) had sub mucosal fibrosis. (Table 3)

Table 3: Distribution of cases among pre malignant lesions

Pre malignant lesions	No .of patients	Percentage
Leukoplakia	18	72
Erythroplakia	3	12
Lichen planus	2	8
Sub mucosal fibrosis	2	8

The mean and SD of KL is more than that of KR and BN and P Value was highly significant ($p < 0.001$) for the meta nuclear abnormalities between the study group and the control group (Table 4)

Table 4 : Comparision of metanuclear abnormalities between study and control group

Metanuclear abnormalities	Study group Mean ±SD	Control group Mean ±SD	P Value
BN	0.76± 2.3	0	< 0.01
KR	2.48± 2.16	0.3±0.61	< 0.001
KL	3.12±2.2	0.12±0.4	< 0.001

Study group = pre malignant and malignant group

DISCUSSION

Epithelial cells are highly proliferative and are the origin of more than 90% of all human cancers. The leukoplakia group showed increased broken eggs compared to the alcohol/tobacco group ($p = 0.0172$). Similarly, the carcinoma group had more broken eggs compared to the alcohol/tobacco group ($p = 0.0104$) [7]

The results obtained from the present study indicate a 60% involvement of males in premalignant as well as malignant condition. This equal distribution can be explained by the usage of tobacco either in the form of pan masala or with betal nut or cigarettes by both males and females.

In the premalignant group of our study, 77.7% of leukoplakia patients were tobacco users either in the form of smoking or chewing as observed in the present study which is in correlation with the study conducted by Saraswathy[8]

100% involvement of buccal mucosa in Erythroplakia confirms the findings of Reichart et al [9]. Hashibe et [10] al identified tobacco chewing and alcohol as risk factors for Erythroplakia .100% cases of Erythroplakia in our study were found to be tobacco users, which leads to epithelial dysplasia and thereby causing premalignant lesions.

Incidence of lichen planus and submucosal fibrosis was found to be very low accounting for 8% of total cases of premalignancy each, in present study, the incidence was 0.15% and it is in correlation with the study conducted by Sarawathy et al. [8]

In the present study ,68% of the cases were in stage iii and stage iv squamous cell carcinoma at diagnosis ,which confirmed the findings of Iype et al[11] rating the incidence as 60.9%

Common meta-nuclear abnormalities as observed in the oral carcinoma have been identified as karyorrhesis, karyolysis and binucleated cells. Presence of BN cells in malignant conditions could be the result of rapid and irregular proliferation of tumor cells.

In this study 76% of BN in malignant cases. KR and KL showed highest index as much as 250% and 312% respectively in the study group with 30% and 12% in controls.

Our results were similar to the results reported by Major et al[12] for two parameters KR(Fig 1) and KL(Fig 2) . According to his study KR, KL values were 446% and 350% respectively. But, his findings on BN cells were not matched with our results. He found 117% of BN cells in his study. BN (Fig 3) cells were comparatively less as 76% in our study and our results were in correlation with the results given by Neisesyan et al [13],also 30% and 12% of KR and KL as observed in controls can be attributed to DNA damages as a normal phenomenon with aging. Cytological atypia in our study is correlation with Hussain et al [14].

CONCLUSION

Chronic exposure to tobacco either in the form of chewing or smoking is associated with carcinogenic cytological changes in oral mucosa. A comparison of metanuclear abnormalities between cases and controls showed a significant difference. This test can be used in premalignant and malignant cases not only as a screening test but also as a prognostic indicator.

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ABBREVIATIONS

KL – Karyolysis
KR – Karyorrhexis
BN – Binucleated cell

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