

The Diagnostic Significance of Micronuclei in Tobacco Chewers

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Abstract

The Micronuclei frequency in tobacco chewers seem to increase steadily as the genotoxic damage increases and progresses from premalignant to malignant oral lesions. This can be easily evaluated in exfoliated oral epithelial cells and helps in assessing the severity. This study was taken up with the objective to assess the frequency of micronuclei (MN) in buccal smear of tobacco chewers with healthy mucosa, premalignant and malignant oral lesions and compare all these cases with that of healthy individuals. The study subjects were divided into four groups each consisting of 20 individuals. The groups were cases with healthy oral mucosa, second group with premalignant oral lesions, third with malignant oral lesions in tobacco chewers and last group with normal controls. The exfoliative cytological smears were stained with Papanicolaou stain. The micronuclei frequency was identified using Tolbert's criteria. The micronuclei were found to increase in frequency in cases as compared with controls. There was a significant increase from apparently healthy tobacco chewers to premalignant and malignant oral lesions. Hence, micronuclei can be used as a screening tool in tobacco chewers to assess the potentiality of carcinoma and thus a useful diagnostic as well as prognostic indicator.

Keywords: Micronuclei, Oral exfoliative cytology, Oral cancer, Squamous cell carcinoma, tobacco chewers.

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INTRODUCTION

Cancer is one among the most important and common cause of morbidity and mortality worldwide. Oral cancer is one of the emerging carcinomas because of behavior and lifestyle modifications mainly of smoking and due to smokeless tobacco forms. Oral squamous cell carcinoma has poor prognosis with an overall median survival rate of 56% [1], and the poor prognosis is mainly accounted by the late diagnosis and treatment owing to the ignorance of the patients. Hence early diagnosis and treatment is the key to reduce morbidity and improve the survival rate. Though oral cancers are easy to detect and histopathology of tissue biopsy remains the gold standard diagnostic tool, it is the need of the hour to implement new screening modalities using biomarkers to detect high risk cases. One such biomarker is micronuclei (MN) assay in exfoliative cytology of buccal smears, which can be used as a diagnostic tool as well as prognostic indicator.

Micronuclei are extranuclear cytoplasmic bodies seen in association with chromosomal aberrations. They are induced by many substances like alcohol, tobacco, betel nut and irradiation [2]. Hence the micronuclei assessment in exfoliated buccal cells will turn to be a promising tool in the study of epithelial

damage and to detect the pathogenesis of carcinoma in relation to chromosomal breakage or mitotic interference.

MATERIALS AND METHODS

This case control study was carried out at a tertiary care hospital of South India at Coimbatore Medical College & Hospital over a period of 1 year from July 2013 to July 2014. The age of the patients ranged from 20 to 65 years. The study included 80 participants and was divided into four groups of 20 each, a control group of non tobacco chewers with clinically normal oral mucosa and tobacco chewers with oral malignant lesions, premalignant lesions and healthy oral mucosa. The study was started after Ethical Committee clearance. Before sample collection, written and informed consents were obtained from the patients. A detailed history of tobacco chewing with duration, frequency of usage and any complaints were collected. Also clinical history of existing and previous illness, investigations done and treatment details were obtained from the study population. Buccal smears were taken after rinsing the mouth with saline, then fixed with 95% isopropyl alcohol and stained with Papanicolaou stain. In this study, cellular evaluation of the cytological smears was performed using optic microscope under oil

immersion and 500 cells per smear were examined in zigzag method. The criteria as provided by Tolbert et al for evaluation of micronuclei frequency were followed [3]. Tolbert et al criteria was that the cell should fulfil the following

- Intact cytoplasm and relatively flat cell position
- Little or no overlap with adjacent cells
- Little or no debris
- Nucleus normal and intact, nuclear perimeter smooth and distinct.

The recommended criteria for the identification of micronucleus is

- Rounded smooth perimeter
- Less than one third the diameter of the associated nucleus
- Staining intensity similar to that of the nucleus
- Texture similar to that of nucleus
- Same focal plane as nucleus

Later, tissue biopsy was taken from the respective oral lesions; diagnosis was confirmed and compared with the MN frequency in cytology smears.

RESULTS

In the present study, the incidence of oral carcinoma was higher over the age of 50 years. About 30% were below 50 years, 20% were between 50 - 60 years, 35% between 60 - 70 years and 15% were over 70 years, but 55% of the premalignant lesions were found to be below 50 years. This showed increase in malignancy with age. In this study, higher incidence of malignancy was seen among females than males because of exclusion of smoking and other habits. Moreover, tobacco quid chewing was seen commoner in women (Fig-1). The results showed that involvement of buccal mucosa was 50% than any other sites.

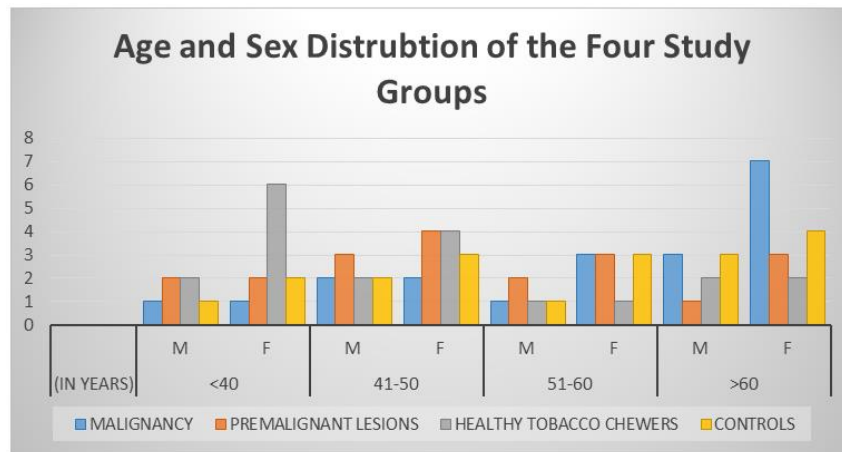


Fig-1: Comparison of age and sex distribution

The data was tabulated, computed and analyzed with SPSS V21 and ANNOVA was performed to find out the variation among the four groups. In this study, it was found out that the mean value of normal controls was 3.35, tobacco chewers with healthy

mucosa was 12.6, those with premalignant lesions was 19.7 and malignancy was 33.75, which showed gradual increase of risk towards malignancy (Table-1, Fig-2). The difference of the mean value was found to be statistically significant and the p value is 0.0001.

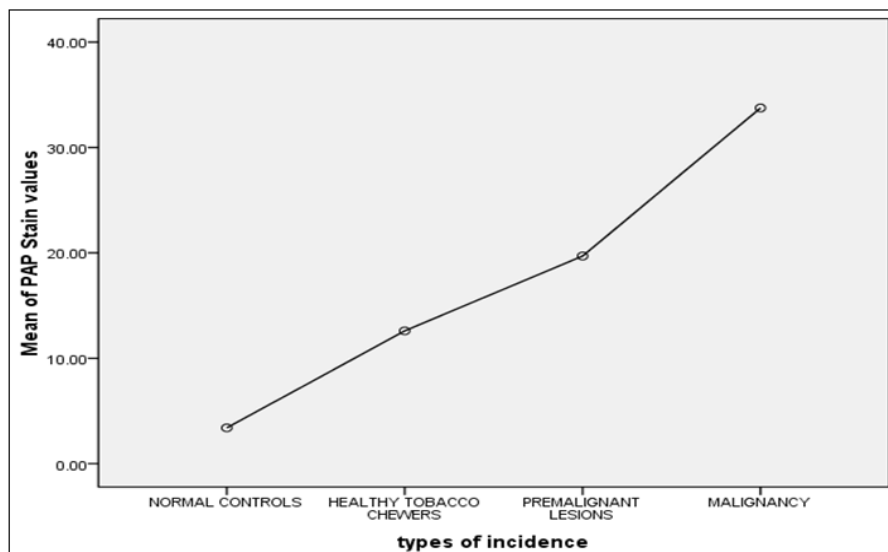
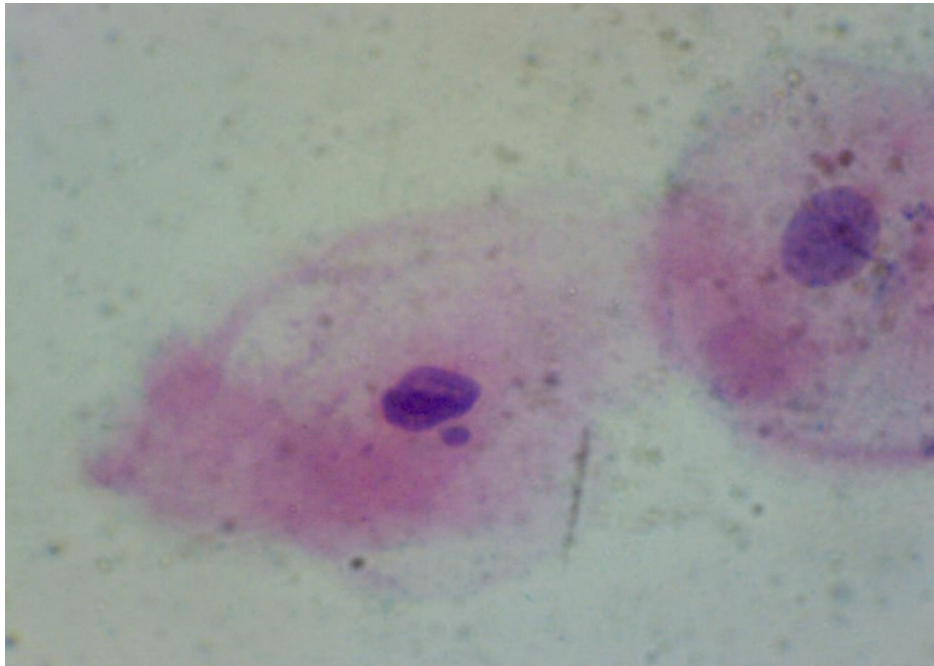
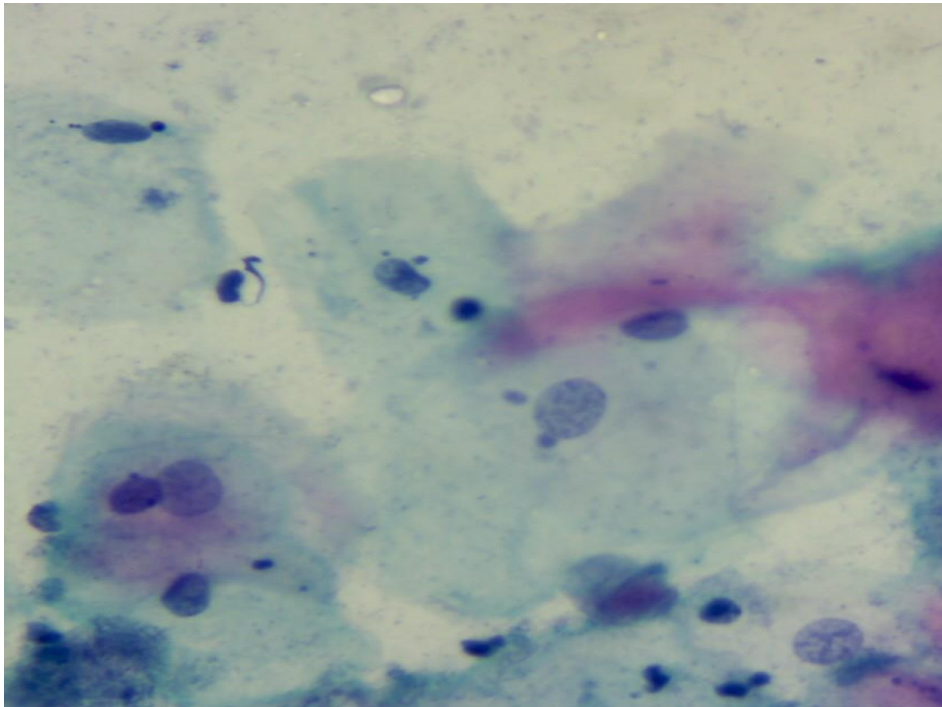


Fig-2: Comparison of Micronuclei frequency in study groups

Table-1: Comparison of micronuclei frequency in four groups

Group	N	Mean	Variance	Std. Deviation	Std. Error
Controls	20	3.35	0.9763	0.9881	0.2209
Tobacco chewers with healthy mucosa	20	12.6	5.2	2.2804	0.5099
Premalignant oral lesions	20	19.7	7.9053	2.8116	0.6287
Malignant oral lesions	20	33.75	42.7237	6.5363	1.4616

The micronuclei in premalignant and oral malignant lesions are shown in Fig 3-6.

**Fig-3: Buccal epithelial cell with one micronuclei in Leukoplakia (100X, Pap Stain)****Fig-4: Buccal epithelial cell with many micronuclei in Carcinoma Tongue (40X, Pap Stain)**

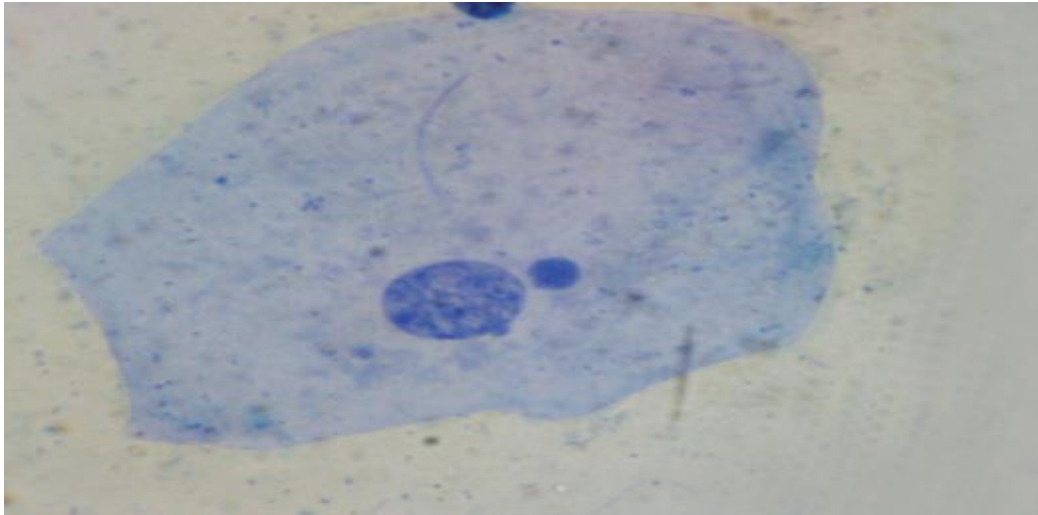


Fig-5: Buccal epithelial cell with one micronucleus in Carcinoma of Soft palate (100X, Pap Stain)

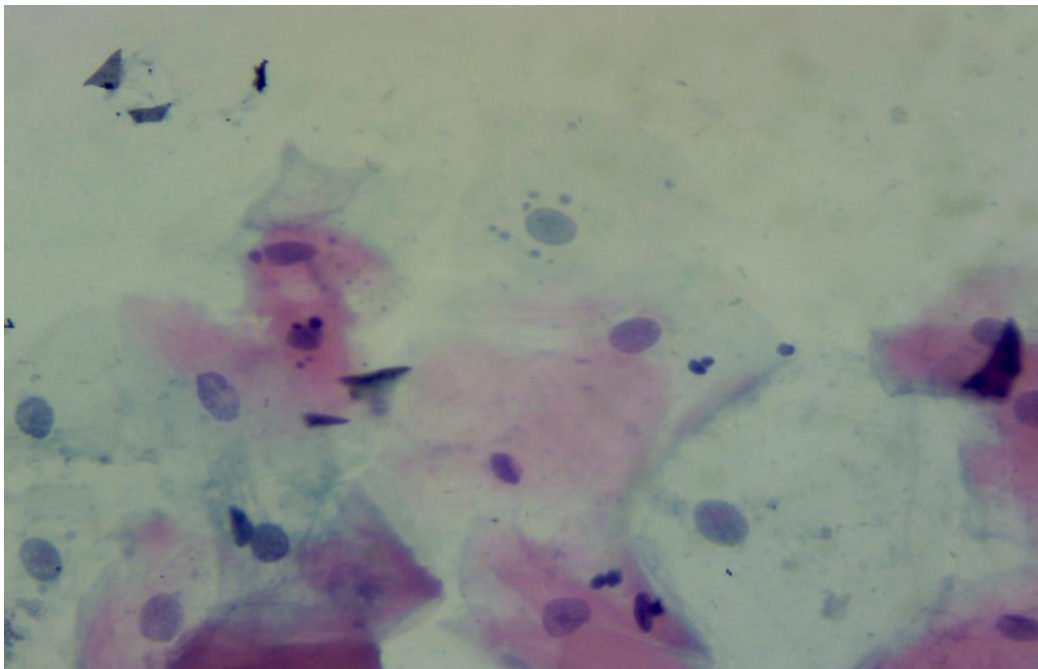


Fig-6: Buccal epithelial cell with many micronuclei in Carcinoma of Buccal mucosa (100X, Pap Stain)

DISCUSSION

Oral cancer is the sixth common malignancy in the world. Annually about 5,00,000 new cases of oral cancers are being diagnosed, of which three fourth of them occur in developing countries. In India, about 75,000 to 80,000 cases are reported annually and the age adjusted incidence rate is 20 per 100,000, which is very high. The overall incidence among all cancers is 30% in India. The oral cancer is mainly concentrated in the SEAR (South East Asian region) where the smokers and smokeless tobacco users are distributed equally, each constituting for about 250 million people. In India, tobacco chewers are about 26% and smokers are 14%. About 65% of the overall cancer cases are related to tobacco usage in India. Annually, tobacco alone contributes to 1,50,000 cancer cases, 42,00,000 heart diseases and 37,00,000 lung diseases in India. Cancer

deaths in India are estimated to be around 56,00,000/yr, of which tobacco is responsible for one third of the cases, with 2500 deaths/day. Global mortality of tobacco related diseases is said to be 22% per year [1].

Buccal cells serve as preferred sites and target for early genotoxic events induced by carcinogenic agents. The oral epithelium is capable of continuous renewal of cells. Hence new cells formed in the basal layer by mitosis, upon time migrate to the surface and replace the shed cells. The stem cells expressing genetic damage are present in the basal layer. The cells formed will differentiate into the keratinized superficial layer and are exfoliated into the buccal cavity. The biomarkers of genomic damage like micronuclei, nuclear buds and those of cell death like apoptosis and karyolysis are identified in both the lymphocytes and buccal cells [4]. Micronucleus in oral exfoliated cells is

a marker of chromosomal damage caused by genotoxic agents from tobacco and tobacco-related substances, radiation and alcohol [5]. The MN assay has been used to assess the genotoxic damage in oral squamous cell carcinoma and premalignant lesions [6, 7]. Thus micronuclei assay is a newer, novel technique in oral exfoliative cytology.

Stich *et al.*, was the first to develop a protocol for micronucleus assay in exfoliated buccal mucosa cells in 1983 [8, 9]. It was used widely in occupational and lifestyle studies. Many studies have been published in the past 25 years using micronucleus assay in epithelial cells from oral mucosa, nasal mucosa, cervix, bronchus, bladder and oesophagus. The human micronucleus (HUMN) project in 1997 was an international collaborative program aimed to standardize micronucleus assay in peripheral blood lymphocytes and to assess the effects of protocol and scoring criteria on the values obtained. HUMN project published the results in 2001 [10].

The micronuclei can be demonstrated in lymphocytes, erythrocytes and exfoliated cells like oral, nasal and also urothelial cells. Micronucleus can be identified by acridine orange, Fielgen, 4',6-diaminido-2-phenylindole (DAPI), Papanicolaou, Giemsa, May Grunwald Giemsa, crystal violet, propidium iodide stains and also by FISH [11, 12].

Kamboj and Mahajan pointed out that assessment of micronuclei in buccal mucosa epithelial cells was a valuable biomarker for early detection of premalignant and malignant lesions of various sites [13]. The present study was aimed at evaluating the differences in micronuclei frequencies. The incidence of oral malignant lesions is found to be common after the age of 50 years. This was disputed that as the frequency and duration of tobacco chewing increased, there was significant increase in the malignant transformation. In the current study, the incidence was observed to be higher in females and was probably due to tobacco quid chewing predominantly seen in women and also because of the exclusion of other lifestyle habits like smoking and alcohol [14].

Casartelli *et al.*, assessed micronuclei frequencies in exfoliated buccal cells in premalignant lesions and malignant lesions of oral cavity. They concluded an increase in micronuclei frequency in order from normal mucosa to premalignant lesions to carcinoma [6].

Pratheepa Sivasankari *et al.*, 2008 evaluated 25 cases of chronic tobacco users with premalignant and malignant oral lesions. They found similar results in micronuclei frequency [15]. Desai *et al.*, noted similar results in his study on the exfoliated buccal cells of patients with precancerous oral lesions including leukoplakia, oral submucous fibrosis, and lichen planus

[16]. They predicted an increase in MN frequency in the study group compared to the healthy individuals. This also agreed with the findings of Saran *et al.*, [17]

Ahmad *et al.*, pointed out a good correlation of increased micronuclei frequency in gutkha users with oral submucous fibrosis. They also observed that gutkha chewing induced OSMF in a shorter duration of 4 years when compared to other causes. This is probably explained by various ingredients of the quid and frequency of quids per day [18]. Halder *et al.*, analyzed 50 cases of oral premalignant and malignant lesions and compared them with healthy controls. They observed that MN frequency seem to be increased preoperatively and tend to decrease in postoperative patients [19]. Similarly, the MN frequency was increased in premalignant lesions than in healthy controls.

CONCLUSION

Thus, micronuclei in oral exfoliative cells can be an effective tool in detecting cytogenetic damage and helping in early diagnosis, treatment and prognosis. However, the buccal micronuclei assay right from sample collection, staining, diagnostic criteria and evaluation needs to be standardised. Further, the whole of the smear should be screened for obtaining MN frequency and preferably at least 1000 cells should be validated. As this is time consuming and has high chance of inter observer variations, MN assay needs to be automated. Many studies are being done in the scenario and more valuable improvements are expected.

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