

Comparative Study of Exfoliated Oral Mucosal Cell Micronuclei Frequency in Normal, Precancerous and Malignant Epithelium

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ABSTRACT Analysis of exfoliated buccal cell micronuclei (MN) is a sensitive method of monitoring genetic damage in the human population. In the present study 50 patients with precancerous or malignant oral epithelial lesions from the Departments of Facio-maxillary and ENT Surgery of RKMSP Hospital, Kolkata were compared with 50 age and sex matched healthy controls without any oral lesions. The MN frequency was increased in preoperative cancer cases and decreased in postoperative cases, while in pre cancerous cases it was higher than in the controls.

INTRODUCTION

Oral cancer is one of the 10 most common cancers as stated by WHO and each year 5,75,000 new cases and 3,20,000 deaths occur worldwide.

In India oral cancer is a major health problem, which accounts for 50-70% of all cancer diagnosed (Jayant et al. 1997).

About half of all cases of oral cancer have associated leucoplakia. Other potentially malignant lesions or conditions include erythroplakia, lichen planus, submucous fibrosis and chronic immunosuppression (Scully and Porter 2000).

WHO distinguishes between oral precancerous lesions and oral precancerous conditions. A precancerous lesion consists of morphologically altered tissue that is more likely to be transformed into cancer than its normal counterpart, such as leucoplakia, erythroplakia and the palatal changes associated with reverse smoking of cigarettes (chutta). A precancerous condition is a state associated with a significantly increased risk for cancer, such as syphilis, sideropenic dysplasia and oral submucous fibrosis.

Since the formation of micronuclei in the eukaryote cells is an end point of chromosomal damage or segregation errors (Geard *et al* 1990) the presence of micronuclei reflects a genotoxic or carcinogenic exposure. Due to its association with chromosomal aberrations, micronuclei have

been used since 1937 as an indicator of genotoxic exposure, based on the radiation studies conducted by Brenneke and Mather (Heddle et al. 1983). The assay is reliable and technically easy to perform. The direct correlation between the micronuclei formation and genomic damage make the micronuclei assay an efficient alteration to the metaphase analysis (Fenech 1990)

The present study was carried out by the Department of Genetic Toxicology, in collaboration with the Departments of ENT and Facio-maxillary surgery, of the Ramakrishna Mission Seva Pratishthan Hospital, Kolkata.

MATERIALS AND METHODS

Fifty patients with oral lesions from the Departments of Facio-maxillary and ENT surgery, and fifty age and sex matched healthy controls obtained from rural camps and Hospital staff were the subjects of this study.

All subjects were administered a standardized questionnaire interview to obtain any history of relevant risk factors and addictions. After a thorough clinical examination the subjects were divided into the following groups:

1. Healthy subjects (n=50) with no oral lesions.
2. Patients with precancerous lesions (n=32)
3. Patients with histopathologically proven oral squamous carcinoma (n=10), preoperative cases
4. Patients with histopathologically proven oral

squamous carcinoma (n=8), post operative cases
 Oral smears were obtained from the subjects as follows:

The subjects were asked to rinse their mouths with water and a premoistened wooden spatula was used to sample cells from the oral mucosa. The spatula was applied to a precleaned microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Giemsa solution and the MN frequency was scored using the criteria described by Sarto et al. (1990) and Tolbert et al. (1992). The same person scored 1000 cells blindly in each case to determine the MN percentage.

RESULTS

The results of our study are summarized in Tables 1 and 2.

Of the fifty controls, i.e., the persons without any oral lesions, 29 were males and 21 were females. Forty-three persons were aged between 15-35 years, 5 persons were in the age group of 36-55 years and 2 persons were in the 56-75 years age group. Five of them had chewed various tobacco preparations, 5 were smokers and one

drank alcohol. As indicated in Table 2 the average percentage of micronuclei in this group was 0.35%. In case of males the average was 0.39%, while in females it was 0.32%.

Of the 32 patients with precancerous lesions (erythroplakia and leucoplakia), 21 were male and 11 were female. Six were in the age group of 15-35 years, 16 were in the age group of 36-55 years and 10 of them were in the 56-75 years age group. Various forms of tobacco chewing were found in 15 of them, 14 were smokers, 2 drank alcohol and no addiction was found in 9 cases. As indicated in Table 2, the average percentage of micronuclei in this group was 0.63%. In case of male the average was 0.55%, whereas, in case of females it was 0.75%.

Of the 10 patients with cancerous lesions (pre-operative), 4 were male and 6 were female. Five were in the age group of 36-55 years and 5 of them were in the 56-75 years age group. Five chewed tobacco preparations, 2 were smokers and no addiction was found in 3 cases. As indicated in Table 2, the average percentage of micronuclei in this group was 1.36%. In males the average was 1.66% whereas in females it was 1.16%.

Of the 8 patients with cancerous lesions (post-

Table 1: Detailed History

Type	Sex		Total no. of samples	Age group (in yrs)			Drinking water		Tea			Addiction			
	Male	Female		15-35	36-55	56-75	Tube well	Corp. water	No	With milk	Without milk	Tobacco chewing	Smoking	Alcohol	None
Control	29	21	50	43	5	2	46	4	25	16	11	5	5	1	43
Pre-cancerous	21	11	32	6	16	10	25	7	3	26	6	15	14	2	9
Cancerous (Pre operative)	4	6	10	-	5	5	10	-	3	6	1	5	2	-	3
Cancerous (Post operative)	4	4	8	2	2	4	6	2	1	8	1	5	3	-	2

Table 2: Frequency of micronuclei

Group	% of micronuclei (Sex wise avg)		% of micronuclei (avg)
	Male	Female	
Control	0.39	0.32	0.35
Precancerous	0.55	0.75	0.63
Cancerous (Pre-operative)	1.66	1.16	1.36
Cancerous (Post-operative)	0.51	0.38	0.44

operative), 4 were male and 4 were female. Two of them were in the age group of 15-35 years, 2 were in the age group of 36-55 years and 10 of them were in the 56-75 years age group. Five of them chewed tobacco preparations, 3 of them were smokers and no addiction was found in 2 cases. As indicated in the table2 the average percentage of micronuclei in this group was 0.44%. In case of males the average was 0.51% while in case of females it was 0.38%.

DISCUSSION

About 92% of human cancers are derived from the external and internal epithelium, i.e., the skin, the bronchial epithelium and the epithelia lining the alimentary canal. No effective techniques have yet been developed for making direct chromosome preparation from epithelial tissues. Unstable chromosome aberrations can be studied in epithelial cells by the detection of MN and other nuclear aberrations in exfoliated interphase cells (Picker and Fox 1996).

The induction *in vivo* and *in vitro*, of micronucleated cells by carcinogens and mutagens is a sign of the genotoxic effect of such substances (Mandard et al. 1987). The MN assay in exfoliated cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens (Tolbert et al. 1992). Various groups have found analysis of MN in buccal cells to be a sensitive method for monitoring genetic damage in human populations (Foiles 1989; Sarto 1990; Kayal 1993). Micronuclei are suitable internal dosimeters for revealing tissue specific genotoxic damage in individuals exposed to carcinogenic mixtures (Stich et al. 1990; Burgaz 1995).

Casartelli et al. (2000) observed MN frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. They concluded that the gradual increase in MN counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression.

To the authors' best knowledge the only previous study of oral epithelial MN in eastern India is the work of Ghosh and Parida (1995). They obtained 50 smears from various tribes in Orissa. All subjects were smokers and drank alcohol. The MN frequency was reported to be 7.37% in males and 5.9% in females.

In our present study the oral mucosal MN frequency in the control population was 0.35% (males 0.39%, females 0.32%). In subjects with precancerous lesions, the MN frequency was 0.63% (males 0.55%, females 0.75%). In cancer patients (pre-operative), the MN frequency was 1.36% (males 1.66%, females 1.16%). In cancer patients (post-operative), the MN frequency was 0.44% (males 0.51%, female 0.38%).

It is evident that our findings agree with those of Casartelli et al. (2000), namely, a gradual increase in micronuclei frequency from normal to precancerous to cancerous lesions.

There is a marked difference between the MN frequency in our control group and that found by Ghosh and Parida (1995) in their study group. However the population from which their subjects were drawn was held to be at high risk of oral cancer.

To conclude, although our numbers are small, the oral mucosal MN frequency may be a marker of epithelial carcinogenic progression. Further studies are required for determining its usefulness in this role.

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