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Original Research

A Comparative Study of Micronuclei in Oral Exfoliated Epithelial Cells in Potentially Malignant and Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Micronuclei are small, additional nuclei formed as a result of exclusion of chromosome fragments or the whole-chromosome lagging at mitosis. Micronuclei indirectly reflect the chromosomal breakage or impairment of mitotic apparatus. Micronuclei in exfoliated oral epithelial cells are widely used as biomarkers of chromosomal damage, genome instability and cancer risk in humans. Micronuclei scoring can be used as a biomarker to identify different preneoplastic conditions much earlier than manifestations of clinical features and might specifically be exploited in screening of high-risk population for a specific cancer. **Aim:** To correlate frequency of micronuclei in oral exfoliated cells in clinically diagnosed cases of potentially malignant disorders (PMDs) and oral squamous cell carcinoma. **Material and Methods:** The study subjects consisted of clinically and histopathologically diagnosed cases of oral squamous cell carcinoma, oral sub mucous fibrosis and leucoplakia. Healthy subjects without any tobacco consumption habits formed the control group. The cytosmear from all the four groups were stained with Papanicolaou stain. Micronuclei were identified according to the criteria given by Tolbert *et al.* (1992). **Result:** The frequency of micronuclei was higher in patients with oral squamous cell carcinoma as compared to the other subject groups and the difference was found to be highly significant. **Conclusion:** This study concluded that there is gradual increase in micronuclei counts from normal oral mucosa to PMDs to oral carcinoma.

KEYWORDS: Micronuclei, Chromosomal fragment, Potentially malignant disorders, Oral squamous cell carcinoma, Papanicolaou stain

INTRODUCTION

Oral cancer is one of the most common causes of morbidity and mortality nowadays. In developing countries, both smoking and smokeless tobacco have cancer-causing behaviour that continues to be increasing the global burden of oral cancer. Oral squamous cell carcinoma (OSCC) is the most common of all oral malignancies that arise directly or is preceded by some benign lesions or conditions which is termed as potentially malignant disorders (PMDs) [1]. PMDs like leucoplakia, oral submucous fibrosis (OSMF), lichen planus and others have unpredictable malignant transformation. Micronuclei are small, oval extranuclear cytoplasmic bodies formed as a result of chromosomal aberrations in oral cells induced by various genotoxic

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agents. It has been established that in the head and neck region, micronuclei are found at increased frequencies from normal mucosa to PMDs to carcinoma, suggesting ever increasing chromosomal instability [2].

MATERIAL AND METHODS

Study sample consisted of 60 subjects and was divided into two groups as follows:

Group I (Controls): control group comprised of 15 healthy subjects with clinically normal oral mucosa.

Group II (Cases): comprised of 45 patients (15 OSMF, 15 leucoplakia and 15 OSCC).

Relevant history of each patient, including their oral habits, was recorded thoroughly. Age- and sex-matched healthy subjects having no obvious oral lesions or habits of consumption of tobacco, other tobacco-related substances or potentially toxic substances were selected as control group. Patients with provisional or confirmed diagnosis of any cancer were included in the study. Written informed consents from these patients were taken for the procedures to be carried out on them.

COLLECTION OF EXFOLIATED CELLS

Procedure

Subjects were asked to rinse their mouth gently with water. Smears were prepared using a moistened wooden spatula, with a gentle scraping motion and exerting little pressure; cells were scraped from the margins of the lesional area in patients with PMDs and OSCC and from buccal mucosa in controls. The scrapings were evenly smeared onto the precleaned microscopic slides. Just prior to drying, one of the smeared slides was fixed in 95% isopropyl alcohol for 15–30 min. Smears were then stained with PAP. All the slides were observed under the microscope using low-power magnification (10×) for screening and high-power magnification (40×) for confirming and counting of the micronucleus (MN). Zigzag method was followed for screening the slides which is the most commonly used method as this will reduce the probability of counting the same cell twice. A number of 500 cells with intact nuclei and cell boundaries were evaluated on each slide for counting the MN.

The following criteria given by Tolbert *et al.* (1992) [9] were considered: Parameters for cell inclusion in the cells to be scored:

1. Intact cytoplasm and relatively flat cell position on the slide.
2. Little or no overlap with adjacent cells.
3. Little or no debris.
4. Nucleus normal and intact, nuclear perimeter smooth and distinct.

Parameters for Identifying Micronucleus

(a) Rounded smooth perimeter suggestive of a membrane.

(b) Less than one-third the diameter of the associated nucleus, but large enough to discern shape and colour.

(c) Staining intensity similar to that of the main nucleus.

(d) Texture similar to that of nucleus.

(e) Same focal plane as nucleus.

(f) Absence of overlap with or bridge to the nucleus.

Data Entry and Statistical Analysis

Ethical clearance was taken by the institute before commencing the study.

All calculations were performed using Microsoft 2007 version for windows for Excel. The data obtained were statistically analysed with the help of ANOVA [Analysis of variance] -test and Tukey's post-hoc test.

RESULTS

The present study comprised of 15 cases of histopathologically diagnosed OSCC and 30 cases of potentially malignant group and 15 healthy control subjects having no obvious oral lesions or habits of

consumption of tobacco, other tobacco-related substances or other such substances. Potentially malignant group comprises of 15 cases of leucoplakia and 15 cases of OSMF.

RESULT OF THE STUDY

Group	*N*	Mean	Std. Deviation	*F* Value	*P* Value
1	15	3.00	1.254	62.876	.000
2	15	8.00	2.104		
3	15	9.00	1.852		
4	15	13.00	2.591		
Total	60	8.25	4.091		

1. Healthy group, 2. Osmf, 3. Leucoplakia, 4. OSCC.
One Way ANOVA

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
1	2	-5.000*	.734	.000
	3	-6.000*	.734	.000
	4	-10.000*	.734	.000
2	1	5.000*	.734	.000
	3	-1.000	.734	.527
	4	-5.000*	.734	.000
3	1	6.000*	.734	.000
	2	1.000	.734	.527
	4	-4.000*	.734	.000
4	1	10.000*	.734	.000
	2	5.000*	.734	.000
	3	4.000*	.734	.000

*Tukey's post-hoc comparison.

The mean number of micronuclei cell distribution was compared in control, OSMF, leucoplakia and OSCC groups which was recorded as 3.00 ± 1.254, 8.00 ± 2.104, 9.00 ± 1.852 and 13.00 ± 2.591, respectively. The mean difference between the number of micronuclei in OSMF, leucoplakia and OSCC groups was statistically significant (*P* = .000).

DISCUSSION

Micronucleus (MN), a recently upgraded topic, especially in the field of oral cancer takes its origin from chromosome fragments or whole chromosomes, which lag behind at anaphase during nuclear division. MN in the exfoliated oral epithelial cells (Figure 1a and 1b) represents a preferred target site for early genotoxic events induced by carcinogenic agents.

The International Collaborative Project on Micronucleus Frequency in Human Populations/) [humanities] (HUMN) was organised to collect data on MN frequencies in different human populations and different cell types to determine the extent to which MN frequency is a valid biomarker of ageing and risk for diseases such as cancer [3].

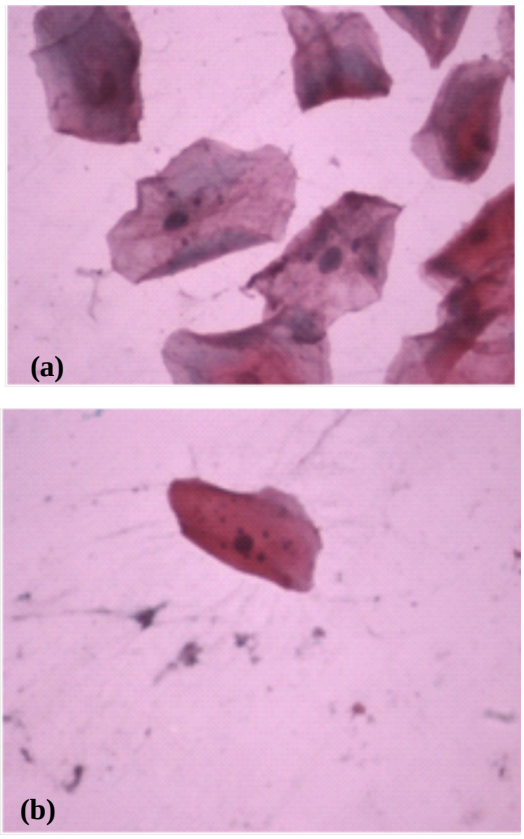

Figure 1a and 1b: Photomicrograph showing micronuclei in exfoliated epithelial cells

The use of the MN test on exfoliated cells from oral epithelium with the aim of undertaking biomonitoring on human populations exposed to genotoxic agents was first proposed by Stich *et al.* [4].

The present study evaluated the mean number of micronuclei in healthy controls, OSMF, leucoplakia and OSCC groups. Micronuclei frequency was seen increasing from the control (3.00 ± 1.254) to OSCC groups (13.00 ± 2.591) and the mean difference was statistically significant ($P < .0001$). These observations indicate cytogenic damage of the epithelial cells. According to the literature, there are studies [5,6] that are being conducted in past and there was a statistical significance between the mean percentage of micronuclei in controls, OSMF, Leucoplakia and OSCC. The same results were observed in our study. According to Samantha and Dey, the reason for micronuclei formation includes chromosomal loss or breakage, chromosomal aberrations, mitotic apparatus dysfunctions, aneuploidy and genetic instability. Hence, with this, we can say that micronuclei is an important upcoming marker of tumerogenesis [7]. In a study by Saran *et al.*, there was a stepwise increase in percentage of micronucleated cells and micronuclei from control to precancer patients, and from precancer to cancer patients [8].

To summarise, micronuclei in exfoliated cells are an innovative technique, which holds promise for the study of epithelial carcinogens. Micronuclei in exfoliated cells reflect genomic instability in individuals exposed to carcinogenic mixtures. Various studies conducted have found that micronuclei in exfoliated cells are a sensitive method for monitoring genetic damage in human population. Hence, micronuclei are a useful assay as a screening and early detection technique for cancer susceptibility.

CONCLUSION

MN test is also an important method for monitoring preneoplastic oral lesions, thereby guiding management strategies to be adopted. It is a very simple, inexpensive and non-invasive screening technique for diagnosing individuals who are at risk of developing cancer. From the present study, it is evident that the individual cancer risk can be predicted on the basis of increased percentage of micronuclei in the oral epithelial cells which helps in identifying the PMD patients who are at high risk of developing cancer.

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