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RESEARCH ARTICLE

VARIATION OF MICRONUCLEI FREQUENCY WITH RISK FACTORS IN ORAL/OROPHARYNGEAL CARCINOMA IN WEST BENGAL

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ABSTRACT

Objective: The aim of this study is to find out the variation in micronuclei (MN) frequency with various risk factors in oral and oropharyngeal carcinoma in West Bengal, India.
Methods: In this study 107 subjects with oral leucoplakia were grouped according to their exposure to tobacco (both smoked and chewed), alcohol and betel quid. Their buccal smears were obtained on slides, air dried and fixed with 80% methanol and finally stained with Giemsa stain and observed under microscope. The MN frequency was calculated and analysed.
Results: The variation in MN frequency, analysed by Students T test for male subjects exposed to tobacco alone was found to be statistically significant ($p < 0.05$).
Conclusion: The MN frequency was found to vary with risk factor exposure; highest with exposure to tobacco and alcohol and lowest in subjects who were not exposed to any of the three substances. The rise in MN frequency was found to be significant in subjects exposed to tobacco alone.

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INTRODUCTION

The analysis of micronuclei (MN) in buccal cells is a sensitive method for monitoring genetic damage in human populations (Foiles *et al*., 1989; Sarto *et al*., 1990; Kayal *et al*., 1993). The assay is reliable and technically easy to perform. The direct correlation between MN formation and genomic damage makes the MN assay an efficient addition to the metaphase analysis (Fenech *et al*., 1990). Oral carcinoma is a major health problem in India. It is highly prevalent in males than femeles (World Health Report, 2005). The World Health organization lists oral cancer as one of the 10 most common cancers (World Health Report, 2005). About half of all oral cancers are associated with a recognized precancerous oral mucosal lesion, leucoplakia. Previous work suggests that exfoliated buccal mucosal cell MN frequency may be a marker of epithelial carcinogenic progression (Casartelli *et al*., 2000; Halder *et al*., 2004).

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Several risk factors for development of oral cancer are well recognized (Scully, 2000).We looked at the variation in MN frequency in subjects exposed to tobacco (both smoked and chewed), alcohol and betel quid (Adhikari *et al*., 2014).

MATERIALS AND METHODS

Prior to this study, clearance was obtained from the Ethics Committee of the Ramakrishna Mission Seva Pratishthan and Vivekananda Institute of Medical Sciences, Kolkata. 500 patients were screened in the Departments of Otolaryngology and Faciomaxillary Surgery for the presence of oral precancerous lesions. Excluding cases of frank oral carcinoma, 107 patients were found to have oral leucoplakia. The subjects were administered a standard questionnaire, and grouped according to risk factor exposure. The MN assay was conducted on all of them as previously published by the authors (Halder *et al*., 2004). The criteria for selection of binucleated cells were: main nuclei those are separate and of approximately equal size, distinguished main nuclei that touch and even overlap as long as nuclear boundaries, main nuclei

those are linked with nucleoplasmic bridges. The trinucleated, quadranucleated, multinucleated cells and the cells undergoing apoptosis were excluded from the MN score (Fenech *et al.*, 2003).

RESULTS

Table I shows the risk factor exposure of the patients in our study. Apart from exposure to tobacco alone in male subjects, the number of subjects in each category was too small for meaningful statistical analysis. The MN frequency was calculated for each group, except for subjects with exposure to alcohol alone and with betel quid as there was only a single patient in these categories. The variation in MN frequency was analysed by Students T test for male subjects exposed to tobacco alone, and was found to be statistically significant ($p < 0.05$). The results are displayed in Table II and the cell containing micronuclei is depicted in Figure 1.

Table 1. Patient exposure to risk factors

Risk Factors	Male	Female	Total
Tobacco	30	3	33
Alcohol	1	-	1
Tobacco + Alcohol	7	-	7
Betel quid	8	9	17
Betel quid + Tobacco	12	-	12
Betel quid + Alcohol	1	-	1
Betel quid + Tobacco + Alcohol	7	-	7
Non –user	13	16	29
Total	79	28	107

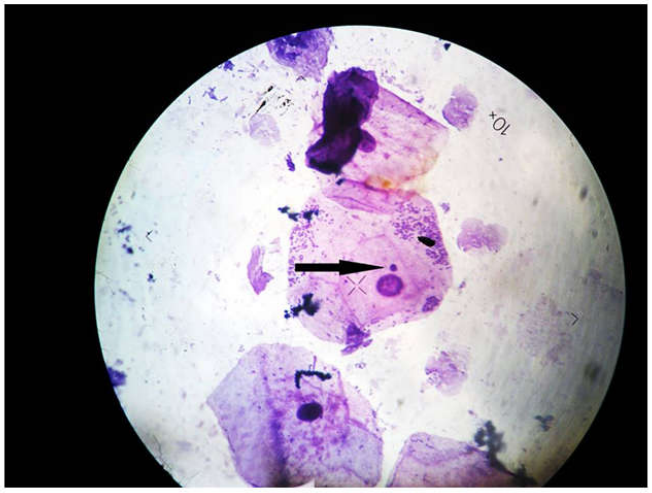

Figure 1. Micrograph of a human buccal cell showing micronuclei after being stained by the method of Pap staining and observed under 10X magnification

Table 2. Variation in MN frequency

Risk Factor	MN % (male)	MN % (female)	t value (male)
Tobacco	0.543+/-0.467	0.620+/-0.072	3.1
Tobacco + Alcohol	0.643+/-0.445	-	-
Betel quid	0.333+/-0.405	0.447+/-0.425	-
Betel quid + Tobacco	0.390+/-0.218	-	-
Betel quid + Tobacco + Alcohol	0.482+/-0.349	-	-
Non-user	0.200+/-0.243	0.164+/-0.143	-

DISCUSSION

The induction by carcinogens of micronucleated cells, both *in vivo* and *in vitro,* is a sign of the genotoxic effect of such substances (Mandard *et al*., 1987). Micronuclei are small extranuclear bodies formed as a result of breaking off of whole or a part of chromosomes or chromatids during mitosis, visible as a separate extra nucleus in one of the daughter cells. The micronuclei can also form as a byproduct of cellular defense, by a double strand break in the DNA creating a separate linear fragment, or may be from breakage of an anaphase bridge. The MN assay in exfoliated cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens (Tolbert *et al*., 1992). The authors observed MN frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma of Indian subjects and concluded that the MN frequency may be a marker of epithelial carcinogenic progression (Halder *et al*., 2004). This was in agreement with the previous work of Casartelli *et al*. (2002).

There is a high incidence of, and mortality from, squamous cell carcinoma of the upper aero digestive tract in South Asia, parts of France and central Europe. Tobaccos (smoked and smokeless), heavy alcohol consumption, and areca nut are major risk factors (Johnson *et al*., 1996). Oral squamous cell carcinoma is a major health problem in India. It ranks first among all cancers in males, and is third most common among females. Cigarette smoking, alcohol drinking, and betel quid chewing are significantly associated with the risk of oral cancer, and genetic polymorphism of Cytochrome P-450 and Glutathione S-transferase may further increase this (Sreelekha *et al.,* 2001; Hung *et al.,* 1997). Early detection and quantification of cytogenetic damage in oral premalignancy or malignancy may help in assessment and hence management of the disease and finally improve survival rates (Katarkar *et al*., 2014). The extent of DNA damage can be easily detected by simple yet reliable markers like- micronuclei (MN) assays, apoptosis frequency.

The micronuclei frequency has reported to be higher in people with multiple risk factors, then on a decreasing note from smoking tobacco to smokeless oral tobacco and finally to non users (Sreelekha *et al*., 2001). On the contrary, research in this field has also come out with the results that smokeless tobacco causes more damage than the smoked form (Motgi *et al*., 2014). However, few studies have also confirmed that tobacco in any form is genotoxic, but smokers are reportedly at a higher risk (Guruprasad *et al*., 2014). The extent of cytogenetic damage has been detected much higher in cases of frank oral carcinoma than in premalignant conditions, where data ranges from a higher trend in lichen planus, then oral submucous fibrosis and finally lesser in leukoplakia (Katarkar *et al*., 2014). The premalignant conditions are advised to be checked regularly on the basis of MN assays to get an idea of the extent of cytogenetic damage, in order to increase the survival rates (Sanchez-Siles *et al*., 2014). Moreover, there is no significant difference in the cytogenetic parameters in case of males and females. Individuals with habits of being exposed to risk factors for a longer time has a higher chance of getting the DNA damage than those exposed for lesser time, easily

detected through such assays (Naderi *et al*., 2012). So, biological monitoring and proper care is very essential for the exposed individuals (Singaravelu *et al*., 2014). In the present study the MN frequency in subjects with oral leucoplakia was found to vary with their exposure to different risk factors. The mean MN frequency was found to be highest in those exposed to tobacco and alcohol, followed by exposure to tobacco alone, exposure to all three substances, exposure to betel quid and tobacco, and exposure to betel quid alone. It was lowest in subjects not exposed to any of these substances. As the total number of subjects in each group was small, statistical analysis was only performed in the case of exposure to tobacco, which was found to be significant. Although our numbers are small it would appear that exfoliated buccal mucosal MN frequency varies with subject exposure to recognised risk factors, namely tobacco (smoked and chewed), alcohol and betel quid.

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