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Original Research Article

ROLE OF CARICINOGENS IN ORAL CANCER: A MICRONUCLEUS STUDY

Pratheepa Sivasankari. N *[1], Ms. Anjana [2], Sundarapandian S [3].

*[1] Associate Professor, Department of Anatomy, SRM Medical College Hospital and Research centre, Kanchepuram Dt. Tamil Nadu, India.

[2] MBBS 2nd Year Student, SRM Medical College Hospital and Research centre, Kanchepuram Dt. Tamil Nadu, India.

[3] Professor & Head, Department of Anatomy, SRM Medical College Hospital and Research centre, Kanchepuram Dt. Tamil Nadu, India.

ABSTRACT

Background: The three most common fatal cancers were oral, stomach, lung in men. Tobacco related cancers represented 42% in male and 18.3% in female cancer deaths. Poly cyclic aromatic hydrocarbons (PAH) is the carcinogen present in tobacco leads to squamous cell carcinoma of oral cavity.

Context and purpose of the study: To study the genotoxicity of tobacco and alcohol on the buccal mucosa of alchoholics, smokers and betel nut chewers which is indicated by increased Micro nuclei. 20 persons having the habit of consuming alcohol and smoking and betel nut chewing were compared with 20 controls. After getting the informed consent the material was collected and stained for MN Assay.

Results: MN frequency in alcoholic, smokers, betel nut chewers were found to be significant with the 'P' value of <0.05 in our study.

Conclusion: The present study has revealed that there is a correlation of significant increased frequency of micro nucleus present in users of (1) alcohol and smoking in combination (2) betel nut chewers as compared to normal counterparts, indicating strong cytogenetic damage which may lead to cancerous proliferation. Tobacco can be considered as a leading carcinogenic agent for causing DNA damage which is indicated by increased micro nucleus.

Implication: The present micro nuclear study shows a feasible and economical method which could be used as a screening test in population having the habit of alcohol and smoking or betel nut chewing for identifying the effects of genomic instabilities and to introduce timely interventional strategy in order to treat and control the epidemic.

KEY WORDS: Micronucleus, Tobacco, Alcohol, Betal Nut Chewing, Oral Carcinoma.

Address for Correspondence: Dr. Pratheepa Sivasankari. N, Associate Professor, Department of Anatomy, SRM Medical College Hospital and Research centre, Kattankulathur, Kanchepuram dt. Tamil Nadu, India. **E-Mail:** drpratheepa@yahoo.co.in

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BACKGROUND

Cancer, one among the four non communicable diseases [1], is a complex disease caused by genotoxic effect of chemical carcinogens or environmental pollutants resulting in genomic instabilities at an early stage of cancer.

555,400 national cancer deaths occurred in India in 2010. At 30-69 yrs, the three most com

mon fatal cancers were oral, stomach, lung in men. Tobacco related cancers represented 42% in male and 18.3% in female cancer deaths [2]. Poly cyclic aromatic hydrocarbons (PAH) is the carcinogen present in tobacco leads to squamous cell carcinoma of oral cavity [3].

Exposure to both ethanol and tobacco results in an increase in the incidence of their malignant neoplasm [4].

Oral cytology is becoming increasingly important in the early diagnosis of oral cancers, as a procedure for obtaining cell samples that can then be analysed by sophisticated diagnostic techniques [5].

Evaluation of the genotoxic risks in tobacco users and alcoholics on buccal mucosa,observed as DNA damages can be assessed by exfoliative cytology such as micronuclei test. Micronuclei are chromatin containing bodies that represent fragments or even whole chromosomes that were not incorporated into a daughter cell nucleus at mitosis [6], have been used as biomarkers for the assessment of DNA damages.

The study aims to show that this micronucleus assay can be done as a screening test in a large population to find out the development of cancer of the oral cavity at an early stage. There was a significant reduction in the mortality rate in the intervention arm versus the control arm due to the detection of oral cancer at an early stage [7].

MATERIALS AND METHODS

Materials used for this study was Methanol, Glacial acetic acid, Giemsa stain and maygrunwald stain.

Inclusion criteria: 10 persons having the habit of consuming alcohol and smoking, 10 persons having the habit of betel nut chewing, 20 persons not having the habit of alcohol and tobacco were taken as controls.

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

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 [8]. 500 cells were screened in each person from the slides prepared and the incidence of micronucleus was recorded and the collected data was subjected to unpaired Student's 't' test.

RESULTS

The present study showed increased amounts of micronuclei present in both alcohol and smoking and betel nut chewers. Our present findings showed a Mean micronuclei index count in alcoholic and smokers was 68.90. There is a significant p value between alcohol and smokers and control (Table 1).

Table 1: Comparison of duration and Micronucleus (MN) between people taking alcohol and smoking with non-users.

		GROUP STATISTICS					
	Group	N	Mean	SD	SE	Independent t test	P value
Duration	Alcohol and Smoking	10	12.1	4.408	1.394	12.5	0
	Control	20	0	0	0		
MN	Alcohol and Smoking	10	68.9	23.812	7.53	12.692	0
	Control	20	1.7	3.097	0.692		

There is a significant increase in MN count among betel nut chewers 74.60. (excluding persons suffering from oral malignant and premalignant lesions) The mean in controls without any habit of using tobacco in any form was found to be 1.70 (Table 2).

Table 2: Comparison of duration and Micronucleus between people taking betel nuts with non-users.

		GROUP STATISTICS					
	Group	N	Mean	SD	SE	Independent t test	P value
Duration	Betal nut chewing	10	17.1	5.174	1.636	15.053	0
	control	20	0	0	0		
MN	Betal nut chewing	10	74.6	26.09	8.251	12.54	0
	Control	20	1.7	3.097	0.692		

Mean MN Frequency between smokers and alcoholics and betal nut chewers was not significant (Table 3).

Table 3: Comparison of duration and Micronucleus between people taking alcohol and smoking with people taking betel nuts.

		GROUP STATISTICS					
	Group	N	Mean	SD	SE	Independent t test	P value
Duration	Alcohol and smoking	10	12.1	4.408	1.394	2.326	0.032
	Betal nut chewing	10	17.1	5.174	1.636		
MN	Alcohol and smoking	10	68.9	23.812	7.53	0.51	0.616 NS
	Betal nut chewing	10	74.6	26.09	8.251		

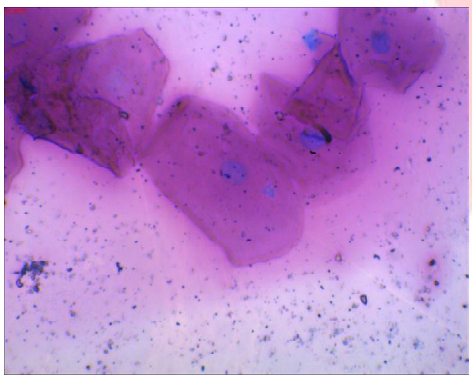
MN frequency in all the three comparisons between alcoholic, smokers with non-users betel nut chewers with non-users alcoholics , smokers and betel nut users with non-users were found to be significant with the 'P' value of <0.05 in our study (Table 4).

The present study has revealed that there is a correlation of significant increased frequency of micronucleus present in users of (1) alcohol and smoking in combination (2) betel nut chewers as compared to normal counterparts, indicating strong cytogenetic damage which may lead to cancerous proliferation (Table 4).

Table 4: Comparison of Micro nucleus between people taking alcohol and smoking in combination, people taking betel nuts with non users.

Group statistics					
	Group	N	Mean	SD	P Value
MN	Alcohol and smoking	10	68.9	23.812	0
	Betal nut chewing	10	74.6	26.09	
	Control	20	1.7	3.097	

Fig. 1: Micronucleus



Above photographic image shows a micro nucleus which can be appreciated in the buccal smear of a person consuming alcohol and smoking.

Figure 1 Shows the micronucleus present inside the cytoplasm of the same cell and has the same stain and one fourth of the main nuclei.

DISCUSSION

Oral carcinogenesis is a multistep process of accumulated genetic damages leading to cell dysregulation with disruption in cell signaling, DNA-repair, and cell cycle events, which are fundamental to hemostasis. These events can be conveniently studied in the buccal mucosa, which is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects [9].

Casartelli et al. [10] 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.

In the present study, making use of exfoliated buccal cells for assaying MN can be arguably explained on two bases. First, since approximately 90% of human cancers originate from epithelial cells, they represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body by way of inhalation and ingestion. The second reason is that the collection of buccal cells is certainly the least invasive method available for measuring the DNA damage in humans. This holds especially true in comparison to obtaining blood samples (for lymphocyte and erythrocyte assays) or obtaining tissue biopsies.

The present study showed increased amounts of micronuclei present in both alcohol and smoking and betel nut chewers. Our present findings showed a Mean micronuclei index count in alcoholic and smokers was 68.90 and in betel nut chewers was 74.60. (Excluding persons suffering from oral malignant and premalignant lesions) The mean in controls without any habit of using tobacco in any form was found to be 1.70 this might be due to the mutation caused by the environmental pollution. Our study is in correlation with Gabriel et al [11] who showed increased micronuclei frequency in exfoliated buccal mucosal cells (EBMC) of tobacco (i.e., cigarettes) smokers. Similarly Suhas et al [12] showed increased micronuclei frequency in smokers of bidi [an indigenous cigarette in which

low-grade tobacco is hand-rolled in a tendu (Diospyros melanoxylon) leaf and tied with a cotton thread.

According to the recent studies of Sellapa et al [13] and Patel et al [14] where the MN count in smokeless tobacco users were higher than that in the control group. Similarly, increase in frequencies of MN in pan masala and gutkha consumers (a form of betel nut) have also been reported by Gandhi and Kour [15]. Nair et al., [16] have reported that the mean frequency of MNCs among paan masala chewers was 0.303±0.058 and was found to be significant when compared with the control group (mean frequency was nil), but non-significant among the different age groups of paan masala chewers. Similarly Trivedi et.al [17] have reported a significantly higher frequency of micronuleated cells in exfoliated buccal mucosa in users of both plain panmasala and panmasala with tobacco when compared with control population.

MN frequency in all the three comparisons between (1)alcoholic, smokers with non-users (2)betel nut chewers with non-users (3) alcoholics , smokers and betel nut users with non-users were found to be significant with the 'P' value of <0.05 in our study. Our findings are in accordance with findings reported by Bloching et al 2000 [18] and Konapacka2003 [19].

Recent study conducted by Beena P. Patel, 2009 [20] also stated that there was a significant p value (p=0.001) for the micronucleus frequency between the controls and chewers. According to the study conducted by Sudha Sellappa 2009 [21] between chewers, smokers and controls, there was no significant difference between the mean percentage of MN cells among 2 groups (Chewers and Smokers) but the result was statistically significant between tobacco users and controls. The statistically significant increase in the MN count among tobacco users in our study results are in accordance with the study conducted by V. Ramakrishnan 2011 [22], according to him MN was significantly higher (15.82 ± 1.31) in chewers than controls (4.82 ± 1.47) ($P < 0.001$)

Hence we show that tobacco users are more prone to formation of micro nucleus which as mentioned above is a bio marker for genotoxicity and epithelial carcinogenic progression. Our findings are in accordance with the study conducted by Scully et al [23] in 2000 who reported that 75% of the patients of oral carcinoma were tobacco users. Accoording to the alcohol abuse the group contain alcoholics and smokers had a significant micronuclear index. These results are in correlation with stich HF et al stated that an elevated frequency of micronucleated cells were observed in alcoholics and smokers [24].

Hence it is put forward that tobacco and alcohol can be considered as a leading carcinogenic agent for causing DNA damage which is indicated by increased micronucleus. Thus micronuclear assay can be regarded as a screening test in large scale population to predict the relative risk of occurrence of cancer.

CONCLUSION

The present study has revealed that there is a correlation of significant increased frequency of micro nucleus present in users of (1) alcohol and smoking in combination (2) betel nut chewers as compared to normal counterparts, indicating strong cytogenetic damage which may lead to cancerous proliferation. The present micro nuclear study shows a feasible and economical method which could be used as a screening test in population having the habit of alcohol and smoking or betel nut chewing for identifying the effects of genomic instabilities and to introduce timely interventional strategy in order to treat and control the epidemic.

The methodology used in the study is simple, rapid and least invasive. Such a method is cost effective and can be done in the rural hospitals for the early detection and diagnosis of the oral carcinoma.

The limitation of this study was the sample size which could have been larger. This technique is primitive and further research by using relationship between years of use of tobacco and alcohol in combination and betel nut and the frequency of nuclear aberrations is recommended.

ABBREVIATIONS

MN - Micronucleus

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