Original Research Article

Effects of alcohol on micronucleus in human exfoliated buccal cells

Jeeva Priya T[1], Vidyasankar[2], Sankaran PK[1], Kishor Kumar C[3*]

[1]Assistant Professor, Department of Anatomy, Madha Medical College and Research Institute, Chennai, Tamil Nadu, India
[2]Associate Professor, Department of Radiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India
[3]Department of Physiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India
[*]Corresponding author email: **sehejan@gmail.com**

International Archives of Integrated Medicine, Vol. 2, Issue 8, August, 2015.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 25-07-2015 **Accepted on:** 03-08-2015
Source of support: Nil **Conflict of interest:** None declared.

Abstract

Background: Oral cancer is the most common malignancy in nearly half of Indian population. The main causes of oral carcinoma are tobacco, alcohol, poor diet and infective agents. These agents damage the chromosomes to form several secondary nuclei known as micronuclei. This study identifies the occurrence of micronuclei and also evaluates the frequency of micronuclei in stained smears of oral exfoliative cells from healthy subjects and alcoholic subjects.
Materials and methods: A total number of 60 alcoholic subjects were referred to the Department of Anatomy, SRM Medical College and Research Center, SRM Nagar for micronucleus assay from the Department of Dentistry. Equal numbers of controls were included with normal looking oral cavities.
Results: Out of 60 alcoholic subjects 43 showed presence of micronuclei and out of 60 control subjects, only 6 showed micronuclei. With these observations, alcohol is one of the predisposing factors of oral carcinoma.
Conclusion: It is evident from our present study, it is clear that in alcohol consumption, the buccal mucosa, which are at high risk for development of oral cancer, show an increase in the MN frequencies.

Key words

Alcohol, Micronucleus, Exfoliative cells, Oral cancer, Oral carcinoma.

Introduction

Oral cancer is the most common malignancy in 40% of Indian population [1]. The primary sites of oral cancer are buccal mucosa, tongue, alveolus, palate, lips and floor of the mouth [2]. The main causes of oral carcinoma (acting on a genetically susceptible individual) include

tobacco use, bidi leaf, tobacco plus spices, shaked lime, areca nut, alcohol consumption, diet poor in fresh fruit and vegetables, infective agents (candida viruses) immune deficiency and exposure to sunlight [3].

Micronuclei are extra-nuclear cytoplasmic bodies; they are induced in cells by numerous genotoxic agents that damage the chromosomes. The damaged chromosomes, in the form of a centric chromatids (or) chromosome fragments, lag behind in anaphase when centric elements move towards the spindle poles. After telophase, the undamaged chromosomes, as well as the centric fragments, give rise to regular daughter nuclei. The lagging elements are included in the daughter cells, but a considerable proportion is transformed into one (or) several secondary nuclei, much smaller than the principle nucleus and is therefore called micronuclei [4, 5, 6].

Micronuclei are induced in oral exfoliated cells by a variety of substances, including genotoxic agents and carcinogenic compound in tobacco, betal nut and alcohol [7]. With this view in mind, the present study was carried out to assess the occurrence and compare the levels of micronuclei in oral exfoliative cytology of healthy control subjects and subjects presenting with habit of alcohol consumption.

Material and methods

The present descriptive study was undertaken in subjects present with habit of alcohol consumption. Subjects were grouped in the following two categories.

- Control group – subject without any habits like smoking, tobacco chewing and alcohol consumption.
- Subjects present only with habit of alcohol consumption.

A total number of 60 alcoholic subjects were referred to the Department of Anatomy, SRM Medical College and Research Center, SRM Nagar, Chennai, for micronucleus assay from the Department of Dentistry. Equal numbers of controls were included with normal looking oral cavities.

Collection of specimens

A subject was asked to rinse the mouth thoroughly. The material was collected from the oral cavity by scraping the buccal mucosa on the cancer prone area using a clear wooden spatula, scraped material was spread on the precleaned slide and smeared. After air-drying the slides were kept in freshly prepared fixative in the proportion of 3 parts of methanol and 1part of glacial acetic acid for 20 minutes. These fixed slides were stained with May- Grunwald and Giemsa.

Results and Discussion

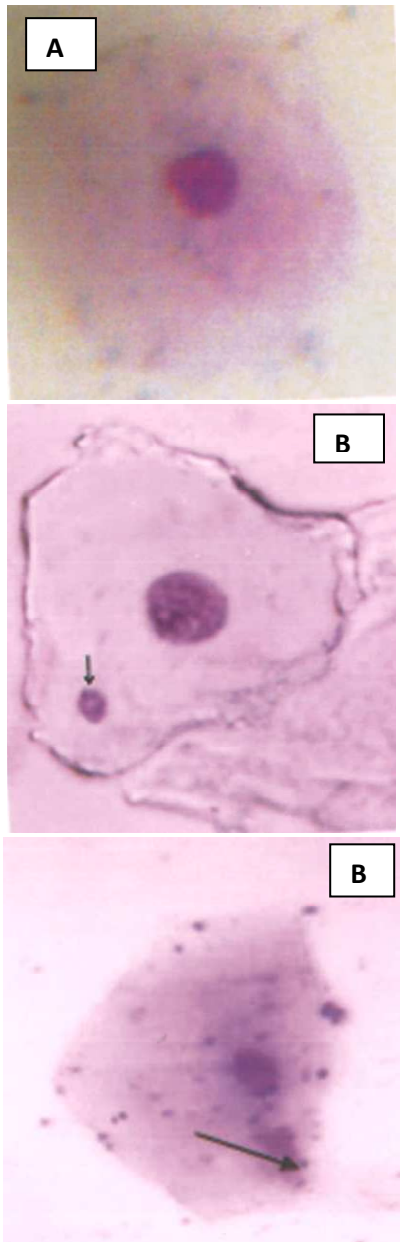
Observations were recorded on the personal history. The observed data were tabulated for analysis. Out of 120 referred cases, 60 were in the age group of 25-70 years, presenting with habit of only alcohol consumption and the rest 60 subjects were in the similar age group, without any personal habits like smoking, tobacco chewing and alcohol consumption.

Out of 60 alcoholic subjects, 43 showed presence of micronuclei (**Figure - 1A, 1B**) and out of 60 control subjects only, 6 showed presence of micronuclei. Mean MN frequency was 1.6 in study groups and 0.2 in control groups which was shown in the **Figure - 1**.

Micronuclei has been proposed as a useful biomarker to assess cytogenetic damage in biomonitoring studies using peripheral lymphocytes and epithelial cells [8, 9]. Segregational defects are considered to be one of the causes for chromosomal instability, where centric and a-centric micronuclei are formed during telophase [3, 9]. Mammalian tissue exposed to various chemical or physical carcinogens show a great variety of nuclear anomalies and mitotic irregularities producing extra-nuclear DNA containing bodies like micronuclei formed by classical mechanisms [10]. The sensitivity of the micronucleus test is

comparable to that of scoring chromatid breaks and exchanges [11, 12]. A reasonable relationship between the carcinogenicity of chemicals and their capacity to induce micronuclei stimulated the application of the micronucleus test to exfoliated human cells. The MN assay can be used for exfoliated cells, which offers the advantage of conducting a genotoxicity test on material from an intact organism with its multitude of defense systems [13].

Figure – 1: Presence of micronuclei in non–alcoholic and alcoholic subjects.
A – Absence of Micronuclei in Control Group.
B – Presence of Micronuclei in Study Group.



Common sites of oral cancers include the buccal mucosa, tongue, palate, alveolus, gingival, lip, floor of mouth and maxillary sinus [14, 15]. By using the micronucleus test on the buccal mucosa we were able to examine an early cellular response to any carcinogens which has been linked to an increase in oral cancer incidence. This study clearly demonstrated that MN frequencies were significantly increased in alcoholic subjects with increased incidence or oral cancer.

Only 6 out of 60 subjects in control group showed micronuclei in their buccal smear because all individuals are continuously exposed to many different genotoxic agents producing micronuclei like drugs, viral infections, environmental pollutants, etc [16]. 43 smears out of 60 alcoholic subjects showed micronuclei indicating higher risk of developing oral cancers. The increase in MN may be due to induction of cytogenetic damage in the epithelium by carcinogenic agents released from alcoholic consumption. Hence the micronucleus assay can be used as a biomarker of genotoxicity in predicting effects of carcinogenic agents.

There is evidence that deficiency in dietary folate and vitamin B_{12} may also increase in occurrence of MN [12] compared to that of localized folate deficiency induced by alcohol consumption in buccal mucosa cells which is independent of the plasma folate level [17]. Plasma and buccal mucosal levels of folate and vitamin B_{12} were however, not assessed in the present study.

It is evident from our study, that in alcohol consumption, the buccal mucosa are at high risk for development of oral cancer, showing an increase in the MN frequencies, which may be correlated to the beedi smoking and tobacco chewing habit. However, it cannot be concluded that oral carcinoma will arise from these sites. The possibility to quantitate genotoxic damage in various tissues will make this approach applicable for a wide spectrum of studies on human population groups.

Conclusion

There is a high increase in micronuclear count from controls to study subjects. This concludes that MN assay can be used as a biomarker for detecting the progression of cancer and can be used as a screening test in the population at risk.

References

1. WHO Project of the National Cancer Registration Programme. Development of an atlas of cancer in India. First All India Report 2001-2002. Available from tm.http://www. Canceratlasindia.org/Chapter 3-1-h.
2. Moshberg , Merletti F, Boffetla P, Gandolfo S, Ozzellon F, Pracchia F, et al. Appearance, site of occurrence, and physical and clinical characteristics of oral carcinoma in Tornio, Italy. Cancer, 1989; 63(12): 2522-2527.
3. Browne RM, Camsey MC, Waterhouse JAH, Manning GL. Etiological Factors in oral squamous cell carcinoma. Comm Dent Oral Epidemiol, 1977; 5(6): 301-306.
4. Fenech M., Browne RM, Camsey MC, Waterhouse JAH, Manning GL. Etiological Factors in oral squamous cell carcinoma. Comm Dent Oral Epidemiol, 1977; 5(6): 301-306.
5. Garewal HS, Ramsey L, Kaugars G, Boyle J. Clinical experience with the micronucleus assay. J Cellul Biochem, 2004; 53(17): 206-212.
6. Halder A, Chakraborty T, Mandal K, Gure PK, Das S, Ray Chowdhury R, et al. Comparative study of Exfoliated oral mucosal cell micronuclei frequency in normal. Precancerous and malignant epithelium. Int J Hum Genet, 2004; 4(4): 257-260.
7. Konopacka M. Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells. Neoplasma, 2003; 50(5): 380-382.
8. Matsui W. ADAM Health illustrated Encylopedia, Baltimore, 2006.
9. Pratheepa Sivasankari N, Kaur S, Reddy KS, Ramachandra Rrao K, Madhan Kumar SJ. Micronucleus Assay - Screening tool in the diagnosis of oral carcinoma in tobacco users. Int J Pharm Bio Sci, 2012; 3(4): 646–651.
10. Proja NK. Paszkuioizc GM, Nasca MA, Franke GE, Pauly JL. Smoking and smokeless tobacco- associated human buccal cell mutations and their association with oral cancer - A Review. Cancer Epidemiol Biomark Prev, 2006; 15(6): 1061-1077.
11. Sinha R, Anderson DE, McDonald SS, Greenwald P. Cancer risk and diet in India. J Postgrad Med, 2003; 49: 222-228.
12. Yadaw JS, Chadha P. Genotoxic studies in Pan Masala chewers: A high cancer risk group. Int J Hum Genet, 2002; 2(2): 107-112.
13. Hashibe M, Mathew B, Kuruvila B, Thomas G, Sankaranarayana R, Parkin DM. Chewing tobacco, alcohol and the risk erythroplakia. Cancer Epidemiol Biomark Prev, 2000; 9: 639-645.
14. Ramirez A., Saldhaha P. Micronucleus investigation of alcoholic patients with oral carcinoma. Genetics and Molec Res, 2002; 3: 246-260.
15. Ogden GR. Alcohol and oral cancer. Alcohol, 2005; 35: 169-173.
16. Pawitan JA. Biological tests for mutagenesis in man. A Clinical Review. Medical progress, 1999; 16-18.
17. Stich HJ, Curtis JR, Parida BB. Application of the micronucleus test to exfoliated cells of high cancer risk groups; tobacco chewers. Int J Cancer, 1982; 30(5): 553-559.