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## Elevated frequencies of micronuclei and other nuclear anomalies in alcoholic subjects

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### Abstract

The present study was aimed to investigate the frequency of micronuclei and other nuclear anomalies in alcoholic subjects. The present study included 100 subjects out of which 54 were alcoholics (23 smokers and 31 non smokers) and 46 subjects (non smokers) were healthy controls. The average age of alcoholics was of  $44.185 \pm 4.434$  years, very close to that of control individuals which was  $41.281 \pm 6.424$  years. Nuclear anomalies were assessed using micronuclei assay in exfoliated buccal epithelial cells. The result of micronucleus assay showed an increase in the frequency of micronuclei (MN). In alcoholics the frequency of MN was found to be higher ( $3.707 \pm 0.379$ ) as compared to healthy controls ( $0.869 \pm 0.141$ ) the difference was significant at ( $p < 0.001$ ). Similarly the frequency of MN in alcoholic smokers subjects was found to be increased  $4.608 \pm 0.585$  as compared to healthy controls ( $0.869 \pm 0.141$ ). The significant difference was observed at  $p < 0.001$ , like MN other nuclear anomalies binucleate (BN), karyolysis (KL) and karyorrhexis (KH) also showed a significant difference ( $p > 0.001$ ).

**Keywords:** Alcoholics, micronuclei, binucleate, karyolysis, karyorrhexis.

### 1. Introduction

Genomic damage is probably the most important and fundamental cause of the development of anomalies and degenerative diseases. It has been found that genomic damage is produced by genotoxins, various medical procedures that include radiation and chemicals, lifestyle factors and genetic factors such as inherited defects in DNA metabolism or repair<sup>[1]</sup>. Obe and Ristow<sup>[2]</sup> found that alcohol has mutagenic, carcinogenic and teratogenic effects. Alcoholic beverages allow other carcinogenic substances to pass into target cells and lead to decrease in cell metabolism thus producing immune deficiency<sup>[3]</sup>. Micronuclei (MN) and other nuclear abnormalities are biomarkers of genotoxic events and chromosomal instability<sup>[1]</sup>. Micronuclei originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division<sup>[4, 5]</sup>. These are induced in oral exfoliated epithelial cells by various substances, including genotoxic agents and carcinogenic compounds found in tobacco, betel nut and alcohol<sup>[6]</sup>. These are also observed in exfoliated buccal epithelial cells, from people who are exposed to organic solvents, diesel derivatives, polycyclic aromatic hydrocarbons and lead-containing paints<sup>[7]</sup>. In the present study the micronuclei and other nuclear anomalies such as binucleate cell (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH) in alcoholic subjects as well as healthy subjects were observed in different areas of Haryana.

### Materials and methods

The present study included 100 subjects selected from different areas of Haryana out of which 54 were alcoholics (23 smokers and 31 non smokers) and 46 subjects (non smokers) were healthy controls, matched with respect to their lifestyle. The average age of alcoholic was of  $44.185 \pm 4.434$  years, very close to that of control individuals which was  $41.281 \pm 6.424$  years. The subjects who smoked  $>5$  cigarettes/day at least for 1 year were considered as smokers and those who consumed  $>120$  gm of alcohol/day were considered as alcoholics<sup>[8]</sup>. Individual questionnaires were filled up to collect the details about their age, sex, dietary habits, smoking and drinking habits. The sampling was performed from October to December 2014.

Before sampling an informed consent was taken from each individual. Ethical clearance was obtained from Institutional Ethics Committee, Kurukshetra University, Kurukshetra.

The standard technique of Tolbert *et al.*<sup>[9]</sup> Was followed for micronucleus assay. Exfoliated buccal epithelial cells were scrapped gently from the inner cheek of the subjects with a moistened wooden spatula. Prior to buccal epithelial cells collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Then the cells were smeared on to the

pre-cleaned microscopic glass slides. Slides were stained with 2% Aceto-orcein (HIMEDIA, acetic acid RM5564, orcein RM277) for 20 minutes at 40°C and washed in ethanol and distilled water, respectively for two to three times.

Then slides were counter-stained with 0.1% fast green solution (HIMEDIA RM 4266) for 12 minutes and rinsed in ethanol and distilled water respectively and air dried.

For each individual 1000 cells were scored for MN and other nuclear anomalies under Olympus CX-41 trinocular microscope at 1000 X magnification. The criterion of Tolbert *et al.* [9] was followed for scanning cells for micronuclei and other nuclear anomalies. The suspected nucleus is required to meet the following criteria in order to be considered as micronucleus : (a) rounded, smooth perimeter suggestive of membrane; (b) less than third the diameter of the main nucleus, but large enough to discriminate shape and color; (c) staining intensity similar to that of nucleus; (d) same focal plane as nucleus. The following criteria for MN analysis were used in oral epithelial cells. A MN must be less than one third the diameter of the main nucleus and must have the same color, texture, and refraction as the main nucleus In addition to MN other nuclear anomalies were also studied like Binucleate (BN); the presence of two similar nuclei with in a cell, Broken egg (BE); nuclei that appear to be broken but still connected to main nuclei with a thin nucleoplasmic bridge, karyorrhexis

(KH); nuclear disintegration involving loss of integrity of the nucleus and Karyolysis (KL); complete nuclear dissolution, in which aceto-orcein negative, ghost-like image of the nucleus remains. Statistical analysis was done using Independent samples t test with the help of SPSS v16.

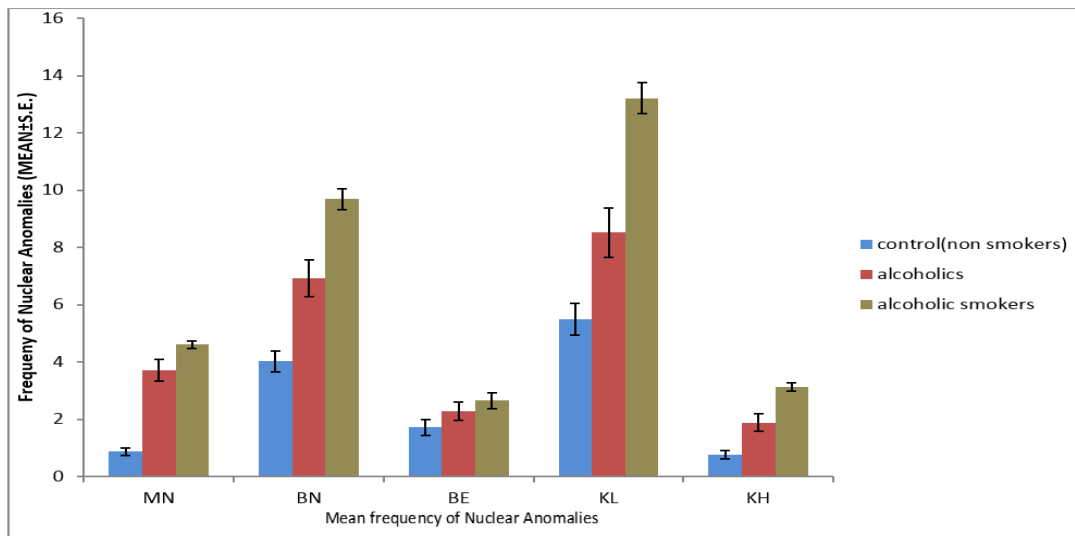
**Results**

Nuclear anomalies were assessed using micronuclei assay in exfoliated buccal epithelial cells. The result of micronucleus assay showed an increase in the frequency of micronuclei (MN). The mean frequency of micronuclei and other nuclear anomalies such as binucleate (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH) in alcoholic, alcoholic smokers and control subjects are shown in Table 1. In alcoholics the frequency of MN was found to be higher (3.707±0.379) as compared to healthy controls (0.869±0.141) the difference was significant at (p<0.001). Similarly the frequency of MN in alcoholic smokers subjects was found to be increased 4.608±0.585 as compared to healthy controls (0.869±0.141).The significant difference was observed at p<0.001, like MN other nuclear anomalies BN, KL and KH also showed a significant difference (p>0.001) in alcoholic smoker subjects. Figure.1 depicts the Comparison of the mean frequency of nuclear anomalies in control (non smokers), alcoholics and alcoholic smoker subjects.

**Table 1:** Mean frequency of nuclear anomalies in alcoholics, contol (non smokers) and alcoholic smoker subjects.

Characteristics	MN	BN	BE	KL	KH
ALCOHOLICS	3.707±0.379*	6.925±0.642*	2.29±0.317	8.518±0.868*	1.888±0.313*
CONTROLS (NON SMOKERS)	0.869±0.141*	4.021±0.361*	1.717±0.277	5.500±0.545*	0.760±0.143*
ALCOHOLIC SMOKERS	4.608±0.585*	9.695±0.964*	2.652±0.571	13.217±1.063*	3.130±0.573*

Values for MN, BN, KL and KH are expressed in Mean ± S.E and \*significant at p>0.001. MN- micronucleus, BN- binucleate, BE- broken egg, KL- karyolysis, KH- karyorrhexis



**Fig 1:** Comparison of the mean frequency of nuclear anomalies in control (non smokers), alcoholics and alcoholic smoker subjects.

**Discussion**

Alcohol consumption and smoking are the two most common risk factors which are responsible for the development of oral cancer [10]. Recent epidemiological studies have reported chronic alcohol consumption as well as smoking habits to be associated with the occurrence of different kinds of oral carcinoma [11-15]. Gattás *et al.* [16] studied the mutagenic effects of alcohol on human chromosomes in vitro.

Micronucleus Test on exfoliated buccal epithelium cells undertaking biomonitoring on human populations which are

exposed to genotoxic agents was first proposed by Stich *et al.* [17]. One study examined the type of MN formation by alcohol consumption using centromere probes and concluded that the increase in MN frequency in mainly due to MN containing whole chromosomes [18]. In the present study we have found an increase in the frequency of micronuclei, binucleate, karyolysis and karyorrhexis in alcoholics and alcoholic smoker subjects as compared to healthy controls. Chadha *et al.* [19] have also reported the increased frequency of micronucleated (MN) and binucleated (BN) in alcoholics as compared to non

alcoholics. Stich and Rosin [20] observed an increased MN frequency related to the mutagenic effects of alcohol in buccal mucosal cells of alcoholic smokers. Khan *et al.* [21] have reported enhanced frequency of micronuclei in smokers and tobacco chewers with oral carcinomas. Recent studies have also reported an association between alcohol consumption and higher MN frequency in peripheral blood lymphocytes [22-26, 18]. The findings of the present study thus corroborate with the previous studies. From the present study it can be concluded that due to alcohol consumption there was increase in the frequency of micronuclei and other nuclear anomalies which lead to the DNA damage.

#### Conflict of interest

There are no conflicts of interest.

#### Acknowledgement

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