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Incidence Of Micronuclei In Buccal Mucosa Of Tobacco Smokers And Alcoholics

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Abstract: ***Tobacco is a harmful genotoxic natural compound. Tobacco smoke is harmful to smokers and to those exposed to Tobacco smoke .The chemicals found in bidi smoke are known for their toxicity. Nicotine,the principal pharmacologic agent common to all forms of Tobacco .is a powerfully addicting drug.(PHS.DHHS publication no(CDC) 88-8406,1988).Thus nicotine in bidi smoke puts smokers at risk for addiction..Nicotine also has adverse effect on cardiovascular health. Tobacco is widely used in cigarette, cigar, chewing tobacco, gutkahs, etc.,. Exposure showed a high risk of human health and development of several types of oral cancers. A total of 50male daily wage workers in R.K. Puram area, Hyderabad, Telangana were investigated for genetic damage in buccal cells of exposed and control subjects. A questionnaire based survey was conducted and buccal smears were collected from oral cavity and analysed for nuclear damage. A high frequency of karyolysis and micronucleated cells were observed among males. The higher percentage of nuclear damage was observed in workers who were smokers. The habit of smoking along with consumption of alcohol enhanced the frequency of micronuclei in buccal cells when compared with controls. The present study clearly reveals the mutagenic and genotoxic nature of tobacco and its products along with alcohol.***

Keywords: ***Micronuclei, Buccal Mucosa, Smokers, Alcoholics.***

I. INTRODUCTION

Tobacco is a harmful genotoxic and mutagenic natural compound. It is genotoxic due to the chemical components contained in it like Nicotine ,phenols, benzene, carbon monoxide etc.,. Tobacco smokers are at risk of oral cancers due to the genotoxic nature of nicotine present in tobacco, which has the capacity to damage the DNA and alter its structure, finally resulting in the mutagenic and genotoxic effects. Tobacco smoke is harmful to smokers and to those exposed to Tobacco smoke in the surrounding. Genomic damage is probably the most important fundamental cause of developmental and degenerative diseases. It is also well established that genomic damage is produced by environmental exposure to genotoxins, medical procedures (e.g.: Chemicals and Radiations) Life style factors (e.g.: alcohol, smoking, drugs, stress, etc.) (Chenet.al 2006 Gabriel et.al 2006, V. Martinez et.al 2005. Majer et.al 2001, Kassie et.al 2001). The MN assay in exfoliated buccal cells is potentially an excellent method to serve as a biomarker to biomonitor the genetic damage in human population.

Tobacco is widely used in cigarettes, cigar, bidis, gutkahs etc,. The chewing of tobacco has a physically powerful association with the risk of oral leukoplakia, oral submucuous fibrosis, oral squamous cell carcinoma and squamous cell carcinoma of the head and neck. Oral squamous cell carcinoma arise through the accumulation of genetic alterations including chromosomal alterations. These events are further influenced by exposure to environmental agents including tobacco consumption ,smoking, alcoholic bevareges and viruses (Mork et al 2001).Chewing tobacco contains considerable nicotine, much more than contained in cigarette tobacco (Benowitz et al 2000).

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II. MATERIALS AND METHODS

CHEMICALS

Phosphate Buffer Saline, Fixative (Methanol and Acetic acid in the ratio 3:1), Giemsa Stain (HIMEDIA).

METHOD

a. STUDY POPULATION

The study was carried out in 50 daily wage workers who were smokers and alcoholics. The control group consists of 45 healthy individuals with no exposure to any toxicants or any other chemicals. Participants are informed about the study asked to sign the consent form and complete the questionnaire to obtain necessary information on their life style, personal habits (age, working duration, smoking habits, health etc).

b. COLLECTION OF BUCCAL CELLS

Exfoliated Buccal mucosa cells can be collected using a wooden tongue depressor, a metal spatula or a cytobrush moistened with water or buffer to swab or gently scrape the mucosa of the inner lining of one or both cheeks a few studies have collected from the inner side of the lower lip and from the palate, variability in MN frequency between these areas was minimal for control subjects as reported in earlier studies.(Korkmaz et.al 2007, Ayyad et.al 2006, Nersesyan et.al 2006, Bonassi et.al 2003, Ramirez et.al 2002).

c. PREPARATION OF BUCCAL CELL SAMPLE

Prior to the collection of buccal cell the smokers and alcoholics are advised to rinse their mouth thoroughly with water to remove the unwanted debris. Sterile wooden spatula was used to obtain the buccal cell sample. The buccal cell was transferred to centrifuge tube with PBS (Phosphate Buffer saline) solution with P^H 7.0 and centrifuged for 10 mins at 1500 Rpm. Supernatant was removed and replaced with fresh PBS solution. This process was repeated 2 to 3 times and the pellet was smeared on the clean slides. Smeared slides were air dried and fixed in 1:3 Acetic Acid and Methanol fixative for 10 min slides are air dried and stained with 2% Giemsa for 10 min and rinsed the slides with distill water and air dried and observed under microscope.

d. SCORING METHOD

Scoring criteria for Buccal cytome assay from each sample three slides were scored and nuclear abnormalities were classified according to the Tolbert et.al (1992) These criteria are intended to classify buccal cells into categories that distinguish between normal and abnormal based on their aberrant nuclear morphology. The abnormal morphologies are due to the DNA damage and cell death.

e. STATISTICAL ANALYSIS

To determine the frequency of various cell types, about 1000 cells were scored for the presence of micronuclei cell, binucleated cells, Karyorrhectic and Karyolytic cells. All the data were expressed as the Mean Standard Deviation. The synergistic effect between smoking and exposure were tested with a two way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student + test.

Micronuclei are identified with the presence of main nucleus and one or more smaller nuclei (MN) in the cells. The MN are usually round or oval in shape and their diameter may range between 1/3 to $1/16^{th}$ the diameter of the main nucleus. Binucleated cells have two nuclei very close to each other, it is due to the failed cytokinesis. Karyolytic cells devoid of nucleus indicate the very late stage in cell death process. Karyohectic cells have dense network of nucleochromatin leading fragmentation of DNA

III. RESULTS AND DISCUSSION

The control group data was compared with the smokers and alcoholic group. There was an increase in the micronuclei frequency in the smoker (9.91%) and alcoholics group (7.05%) when compared with the control group (nonsmokers) (3.73%). The difference in the total percentage of the micronuclei between smokers and non smokers (control group) was found to be statistically significant ($p< 0.05$).

There was an increase in the incidence of micronuclei with increased duration of smoking and increased frequency of smoking. The increase in th micronuclei at all time intervals were significant when compared to the control subjects. The differences for micronuclei between the time intervals were also significant. There was a significant increase in the frequency of micronuclei in smokers when compared to control group.

Totally a significant increase was observed in the micronuclei frequency at the exposure duration intervals when compared to the control subjects. Further the frequency of micronuclei was directly proportional to the duration of exposure and also the intensity or frequency of smoking and alcohol consumption.

The results were also analyzed according to the age exposure duration, smoking frequency of the smokers group to evaluate the effects of these factors on the incidence of micronuclei frequency.

The frequency of Micronuclei in the control group (nonsmokers) is shown in the Table-2. The incidence of micronuclei in smokers and alcoholics is shown in the Table-3.

Individuals(No. of samples (Age b/w 30-45 yrs)	**89**	**MNC**	**BNC**	**KRC**	**KLC**
Controls (Non-smokers & Non-Alcoholics)	25	3.73±0.02	2.10±1.20	1.01±0.20	3.02±1.01
Smokers	21	9.91	7.85	2.79	7.01
Alcoholics	23	7.05	6.72	1.85	6.05

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Smokers +Alcoholics	20	12.01*	10.89*	2.79	7.51*

Table 1: Demographic and cytological observations of individuals

The harmful effect of dust in various forms of human health have already been demonstrated (Guthrie 1992, Dong et.al. 2006). The MN Test, that is scientifically approved, is important in demonstrating the genotoxic effects of harmful substances on health (Nerseyan,

2005, Fenech et.al. 2007) such as measuring genotoxicity in petrol station employees (Celik 2003, Benities et.al. 2006) agricultural workers (Pastor et.al) Cigarette smokers and tobacco users (Prioita et.al. 2006) workers exposed to pesticides (Pastor et.al. 2002) Polycyclic Hydrocarbons (Karahalil et.al.1999) timber dust (Celik and Kanik 2006) Ozone and Cancer patients (Chen et.al. 2006, Bloching et.al. 2000).

The Micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factors that may influence the rate of DNA damage such as incidence of micronuclei in humans (Celik et.al. 2003). It is reported that cigarette smoking significantly increased the frequencies of nuclear abnormalities in both controls and exposed subjects. Increase in exposure to toxic chemicals such as formaldehyde and benzene induces a significant increase in the buccal cell micronuclei (Titenko Holland et.al. 1996) .

Nicotine an important component of tobacco is a widely distributed contaminant most of the percent of the nicotine is derived from the tobacco and tobacco containing compounds like bidi, cigarette, cigar, tobacco dust, chewing tobacco, gutkahs etc.,. The occupational exposure to nicotine generally takes place in bidi industry and bidi rollers. Epidemiological studies showed a clear relationship between the increase in micronucleus frequency and exposure to tobacco and tobacco containing compounds.

In the present study, individuals have been exposed either by nasal or oral inhalation of the volatile organic compounds present in the tobacco smoke. Exposure to tobacco smoke may occur directly or indirectly. Increase in the frequency of micronuclei in the exfoliated buccal mucosa cells in smokers was observed. In this study 28 subjects were smokers, 29 subjects were alcoholics and 24 subjects were both smokers and alcoholic. Predominantly all the male workers included in this study were habitual smokers. A higher frequency of karyolysis was observed in smokers than the nonsmokers. Rural workers without formal education reported habitual chewing of tobacco. Smokers (smoking for more than 5 years) have higher degree of nuclear anomalies probably due to their excessive smoking habit (15cigarettes/day). Alcoholics including smokers also revealed higher degree of nuclear anomalies. These factors are to be considered as an implicative parameter for high degree nuclear anomalies in buccal smears of men.

The micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure of genotoxic chemicals. Cigarette smoking is one of the factor that may influence the rate of cytogenetic damage, such as micronuclei in humans. (Celik et.al, 2003) reported that cigarette smoking significantly increase the frequencies of micronucleus and other nuclear abnormalities in both controls and smokers. Increase in micronuclei frequency was reported in bidi smokers (Suhas et .al, 2004) (Ozkul.Y. et.al, 1997) reported that the micronuclei frequency is around 8.40/1000 cells in cigarette smokers. In our present study we observed an increase in the frequency of micronuclei and nuclear anomalies in smokers when compared to the controls.

A higher frequency buccal cells with MN, binucleate, karyolysis and karyohexis was observed in the study subjects, probably due to the genotoxic effect of the nicotine .Only heavy smoking and other forms of tobacco consumption have been associated with oral malignancies. It is necessary to educate the working population and the smokers about the genotoxic effects of tobacco and its compounds. The present results are comparable with our earlier studies such as increased frequencies of micronuclei in industrial painters (Madhavi 2008) and in shoe factory workers (Jitender Naik et al.2005). In summary, this study shows a clear genotoxic effect associated with occupational exposure to lead .These data are relevant and permit an estimate of genetic risk of lead by using biomarkers of exposure.

The micronucleus assay in human exfoliated cells is one of the most sensitive methods used for measuring DNA damage rates in human populations; because it is relatively easier to score micronucleus compared to other methods, such as chromosome aberrations. This assay can be used to identify not only groups that are at risk for developing cancer, but also specific individuals who are susceptible to cancer development.

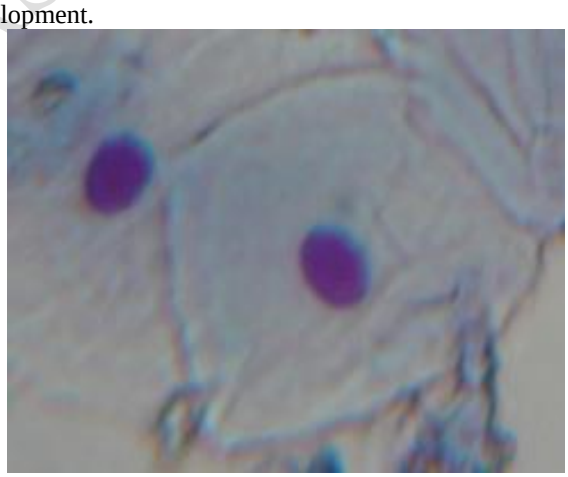

Figure 1: Cell without Micronuclei

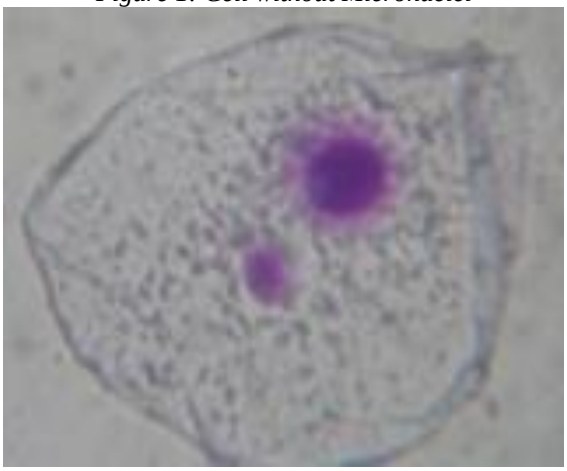

Figure 2: cell with Micronuclei

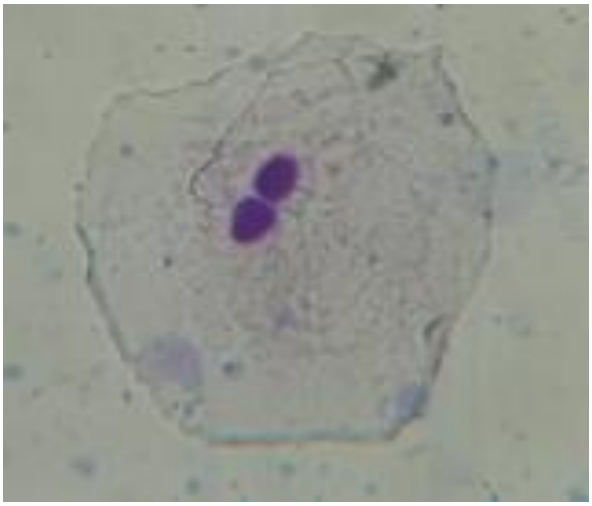

Figure 3: Binucleated cell

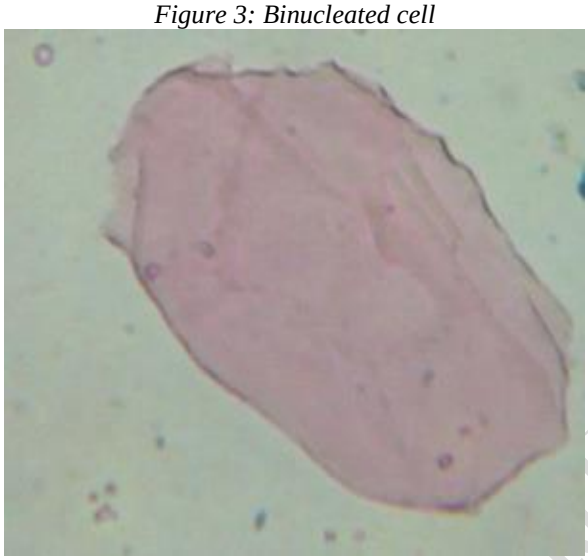

Figure 4: Karyolytic cell

Fenech [18] showed that, after adjustment for age and sex, individuals with high cigarette usage [17] had statistically greater MN compared to non-smokers. The evidence regarding an effect of drinking alcoholic beverages on increased MN is inconclusive. An increase in MN has been observed in alcoholics consuming alcoholic beverages but not in abstainers of a year or more (Castelli et.al, 1999, Maffei et.al 2002).

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