IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Occurrence Of Nuclear Anomalies In Exfoliated Buccal Cells Of Tobacco Smokers

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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, cigarette, and cigar etc,. smoke are known for their toxicity. Nicotine, the principal pharmacologic agent common to all forms of Tobacco is a powerfully addicting drug. 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 56 male daily wage workers in Chandanagar 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 resulted in the frequency of formation of micronuclei in buccal cells when compared with controls. The present study clearly reveals the mutagenic and genotoxic nature of tobacco and its products. It is necessary to educate the public about the adverse effects of tobacco consumption and prevent nicotine addiction, to decrease genetic damage and risk of serious diseases.

Keywords: Micronuclei, Buccal Mucosa, Smokers.

Introduction:

Tobacco use, mainly active or passive smoking and smokeless consumption is the most common cause for head and neck cancers (HNCs) [1, 2, 3]. According to the WHO, globally 21% of people aged ≥15 years smoked tobacco in 2012 [4]. In India, prevalence of use of tobacco products is greater than that of global rates with 34.6% of people using some form of tobacco products during their lifetime. Of these, smokeless tobacco users accounted for 25.9% [5]

Oral cancer, a contemporary epidemic among the non-communicable diseases, is a main problem in the Indian subcontinent where it ranks among the top three types of cancer in the country. 20 per 100,000 individuals are affected by oral cancer accounting for about 30% of all types of cancer in the country. The global load of cancer continues to increase mostly because of increase in practices of tobacco, particularly smoke and smokeless forms[6]. Oral and spit tobacco increase the hazard for leukoplakia, a forerunner to oral cancer. Chewing tobacco has been well-known to cause cancer, particularly of the mouth and throat[7].

Micronucleus (MN) is a newly promoted subject, particularly in the arena of oral cancer. Micronuclei originate from chromosome fragments or complete chromosomes, which pause behind at anaphase during nuclear division. It could be debated that micronuclei in exfoliated oral epithelial cells signify a chosen target site for early genotoxic events brought by carcinogenic agents. Numerous studies has revealed the correlation of frequency of micronuclei and severity of this genotoxic injury [8]. Micronuclei are symptomatically seen in exfoliated epithelial cells such as buccal mucosa and urinary bladder wall during precancerous and cancerous conditions [9]

Micronucleus assay in the exfoliated buccal epithelia can be used to demonstrate the effect of smoking in human population [10, 11] and is a great biomarker for early detection of malignancy [12].The cells are observed for micronucleus(Mn) and other cytogenetic anomalies like binucleated cells , karyolysis and karyorrhexis . The present study clearly reveals the mutagenic and genotoxic nature of tobacco and its products.

2. Materials and Methods :

2.1. Study Population:

The study was carried out in 56 daily wage workers who were smokers of tobacco along with 30 non-users of tobacco that is control group. The control group consists of 30 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).

2.2. Collection of Buccal cells:

Workers were requested to wash their mouth gently with water. The buccal cells were obtained by rubbing the Exfoliated buccal mucosa of individuals with a wooden 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 of mouth, a few studies have collected from the inner side of the lower lip and from the palate, variability in micronuclei (MN) frequency between these areas was minimal for control subjects as reported in earlier studies [11, 13, 14,].

2.3. Preparation of Buccal cell sample:

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 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.

2.4. 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* [15]. These criteria are proposed to classify buccal cells into categories that differentiate between normal and abnormal established on their abnormal nuclear morphology. The anomalous morphologies are due to the DNA injury and cell death.

2.5. Statistical Analysis.

To determine the frequency of various cell types, about 1000 cells were scored for the presence of micronucleated cells, binucleated cells, karyorrhexis and Karyolytic cells. All the data were stated as the Mean Standard Deviation. Data were subjected to statistical analysis using unpaired t-test and Karl Pearson's correlation coefficient. Comparison of mean percentage of micronucleated cells in different groups was made using unpaired t-test and using the Graph pad. A value of P = 0.05 or less was considered for statistical significance and percentage of micronucleated cells in different groups.

3. Results:

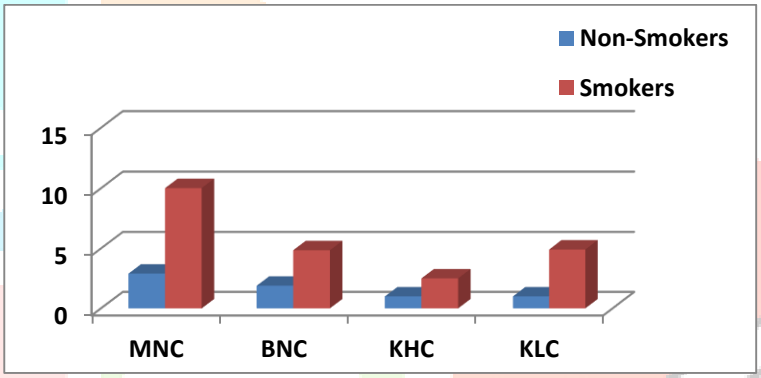
Micronuclei are identified with the presence of main nucleus and one or more smaller nuclei (MN) in the cells. The MN are usually round or oval in shape and their diameter may range between 1/3 to 1/16th the diameter of the main nucleus. Binucleated cells have two nuclei very close to each other, it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

Table 1: Cytological observations in Control group (Non-Smokers) and Tobacco Smokers is shown in the Table-1.

Individuals		MNC (%)±SD	BNC (%)±SD	KHC (%)±SD	KLC (%)±SD
Non-smokers (20)		2.90 ± 0.02	1.89±0.02	1±0..20	1.0±0.03
Smokers (56)		10.01(±1.7)*	4.85(±0.0.2)*	2.50(±0.05)*	4.91(±0.9)*
Duration of exposure	<5 years	8.51± 0.02	5.19± 0.03	2.9 ± 0.03	4.51± 0.03
	>5 years	11.26± 0.03*	6.86 ± 0.03*	4.01± 0.03*	6.09 ± 0.03*

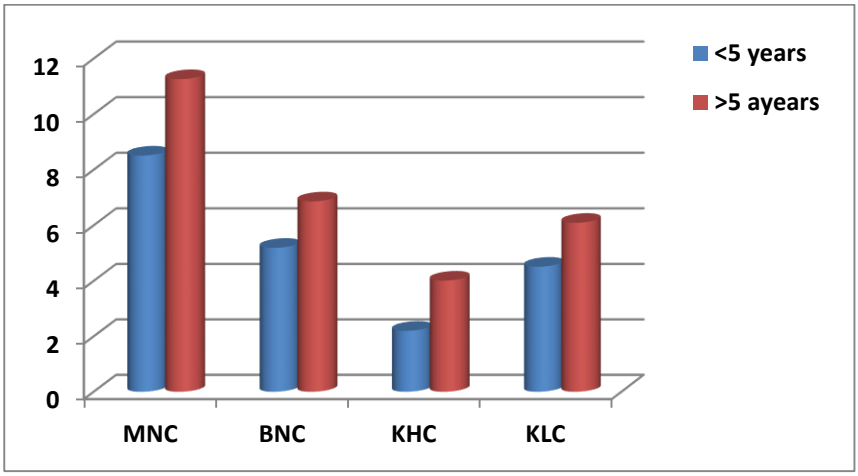
p< 0.05*

The control group that is Non-Smokers data was compared with the smokers group. There was an increase in the micronuclei frequency in the smoker (10.01%) when compared with the control group (non-tobacco-users) (2.90%). The difference in the total percentage of the micronuclei between smokers, non- tobacco users (control group) was found to be statistically significant (p< 0.05).



Graph:1 Cytological observations of different types of buccal cells in Non-Smokers and Tobacco Smokers

There was a rise in the incidence of micronuclei with increased duration of smoking and increased frequency of tobacco usage (Table 1, Graph 2). The rise in the micronuclei at all time intervals was significant when compared to the control subjects. The differences for micronuclei between the time intervals was also significant. There was a significant rise in the frequency of micronuclei in tobacco smokers when compared to control group.



Graph: 2 Cytological observations of different types of buccal cells in Non-Smokers and Tobacco Smokers based on Exposure duration.

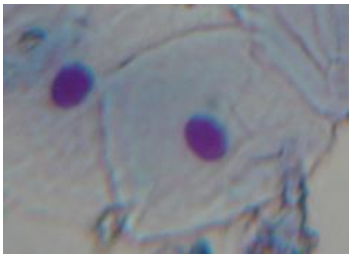
Totally a significant increase was observed in the micronuclei frequency at the exposure duration intervals when compared to the control subjects. Further the frequency of micronuclei was directly proportional to the duration of exposure and also the intensity or frequency of smoking tobacco.

4. Discussion:

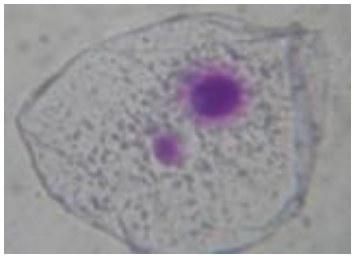
Cancer is the another most common cause of mortality in developed countries and tenth most mutual cause of mortality in developing countries like India. The world wide occurrence of cancers of larynx, pharynx and oral cavity is approximately 500,000 cases per year with mortality of approximately 270,000 cases per year [16]. The incidence of oral cancer in the world is mostly related with the design of tobacco products intake. There exists a dose-response relationship between occurrence of oral cancer and the level of tobacco products intake [17].

Chief risk factors for oral cavity cancers are chewing of tobacco and cigarette smoking[18]. Lips, palate, tongue and almost all other parts of the oral cavity are susceptible to cancer from tobacco smoking or chewing[19]. Several carcinogens are present in cigarette. Cigarette ignition creates smoke which comprises free radicals and other combustion byproducts which are carcinogenic. These free radicals can react with additional additives or additional combustion byproducts or living cells and cause DNA injury [20].

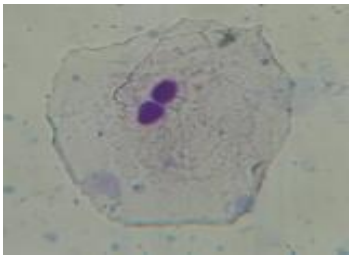
Different types of Buccal Cells



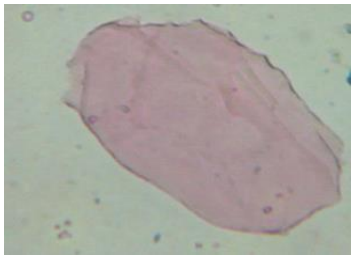
Normal Cell



Micronucleated Cell



Binucleated Cell



Karyolytic Cell

Micronucleus assay with exfoliated buccal epithelial cells is a cost effective and a less invasive technique introduced by stich *et al* and it has been assumed that the number of micronucleus is related to growing effects of carcinogens[21]. Micronucleus (MN), is an oval or round chromatin mass in the cytoplasm which is microscopically evident in the extra nuclear area. It is made from abnormal mitosis and contains of chromatin fragments, eccentric chromosomes or whole chromosomes, which do not reach the spindle poles during mitosis. For the assessment of DNA injury, MN has been frequently used as a biomarker[22]. The cells are viewed for micronucleus and other cytogenetic anomalies like Pyknosis, karyolysis and karyorrhexis[23]. Hence, the present investigation was done to evaluate the DNA injury and cellular death in exfoliated buccal mucosal cells of tobacco users and non-smokers.

Cells with micronuclei are highest in both cases than the controls, but the percentage of MN increases in tobacco users. The studies of Nersesyan *et al.* [11]. revealed condensed chromatin, karyorrhexis, karyolysis and binucleation were higher in smokers than in non-smokers. In our study, though fragmentation, karyorrhexis, karyolysis,and binucleation have increased frequency, only micronucleated cells increased significantly. Condensed nuclei, karyorrhexis, karyolysis, pyknosis are also seen in cells experiencing necrosis and cannot be viewed as reliable markers for increased DNA injury and cancer risk. Nuclear anomalies reflect the consequences of any form of cell injury. However, micronuclei (chromosomal breakage or loss), nuclear budding (gene amplification) do not occur as part of normal cell death or in apoptosis. Hence these anomalies indicate some sort of DNA injury[11].

A higher frequency buccal cells with micronuclei, 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 [24].The present study outcomes were comparable with that of Baxi *et al* [25]. who revealed that the nuclear anomalies and Mean MN frequency are increased in any form of tobacco users.

5. Conclusion:

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. The present study revealed that the habit of smoking tobacco enhanced the frequency of micronuclei in buccal cells when compared control group. The present study clearly reveals the carcinogenic nature of tobacco and its products. It is necessary to educate the public about the adverse effects of tobacco consumption and prevent nicotine addiction, to decrease genetic damage and risk of serious diseases.

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