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# Evaluation of Buccal Cell Cytotoxicity in Gutkha/ Panmasala Chewers And Bidi Smokers

KEYWORDS: Cytotoxicity, Gutkha/Panmasala, Bidi, Buccal cells.

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**ABSTRACT** *Amongst Indian population Gutkha/panmasala and Bidi is a common source of addiction which contains some toxic and/or carcinogenic ingredients. Since, the buccal cells are directly exposed, a study was conducted in gutkha/panmasala chewers and bidi smokers to analyse the cytotoxicity by using Buccal Micronucleus Cytome assay (BMCyt). The observations were compared with healthy, age matched and strictly non-addicted control individuals. The results of this study showed that the mean frequencies of all the cell types including Micronucleus (MN) (p>0.05), condensed chromatin cells (CC), Karyorrhectic cells (KR), and Karyolytic cells (KL) (p>0.001) cells were significantly higher as compared to control subjects except BN, 2MN and 3MN which was found to be non-significant. Hence, the present study suggest careful monitoring and awareness of people addicted to bidi smoking and gutkha/panmasala chewing as it can lead to serious health problems.*

**Introduction**

Consumption of tobacco in different forms such as chewing gutkha/panmasala and smoking bidi are very common in India in almost all age groups, due to which many health related problems are arising rapidly. Addiction to tobacco (smoking/chewing) at an early stage is due to several reasons like ignorance, loneliness, curiosity, urbanization, unemployment, easy availability, inheriting habits from family elders who are already addicted and it is also a cheap source for relaxation and refreshment with instant stimulus. Aggressive advertising and marketing has greatly enhanced the sales of these products (Nitin et. al., 2010; Nayak, 2011). Gutkha contains granular white substance with areca nut, tobacco, lime, spices, cardamom, catechu, and colouring agents with flavours and panmasala is a balanced mixture of betel leaf with lime, areca nut, clove, cardamom, mint and essence (Nair et. al., 2004). According to IARC (2004), the main ingredients of gutkha/panmasala showed carcinogenic activity in many studies including *in vitro, in situ* oral cavity and also in experimental animals. Many studies have shown that chewing gutkha/panmasala can cause cancer in humans (Manikantan et. al., 2010). The chemicals present in these products causes formation of reactive oxygen species in buccal cavity of the chewers (Babu et. al., 1996), and can also affect the DNA repair pathways (Pershagen, 1996).

A bidi is a smoking stick 4–8 cm in length with 0.25 – 0.50g of coarse ground tobacco, made by rolling a dried piece of temburni leaf into a conical shape and securing it with a thread (Gupta, 1996). Bidi smokers have an increased risk of adverse cardiovascular and respiratory effects when compared to nonsmokers (John, 2005; Rahman and Fukui, 2000). The smoke from bidis contains many potentially harmful chemical constituents, including carcinogenic chemicals such as the tobacco-specific nitrosamines (TSNAs), polyaromatic hydrocarbons (PAHs), aromatic amines, phenols and metals (Mishra and Shaikh, 1983). Also, it has been shown that bidi smoke contains more carcinogenic chemicals as compared to the American cigarettes (Hoffman et. al., 1974) and tobacco smoke (particularly "bidi") can have carcinogenic effect due to prolonged use (IARC, 1986).

The Buccal Micronucleus Cytome (BMCyt) assay is a low cost, minimally invasive method for studying DNA damage, chromosomal instability, cell death and the regenerative potential of human buccal mucosal tissue. This method is increasingly used in epidemiological studies for investigating the impact of nutrition, lifestyle factors, genotoxin exposure, DNA damage, chromosome malsegregation and cell death (Thomas et. al., 2009). Different addictions (chewing/smoking) are also responsible in many cases for developing cancer of oral cavity, pharynx and esophagus (Zanoar et. al., 2003). Due to chewing and smoking habits the buccal cells are directly exposed to the harmful chemicals of gutkha/panmasala and bidi, some of which are having mutagenic or carcinogenic potential. Therefore, the objective of this study was to investigate the genotoxic effects of gutkha/panmasala chewing and bidi smoking on the buccal cells using the BMCyt assay.

**Materials and Methods**

The samples were collected as per the ethical guidelines and with prior consent of subjects. A detailed questionnaire was filled up with information including age, gender, blood group, height, weight, occupation, addiction, exposure time, frequency, quantity, diet, medicines, physical condition, disease (if any), other health problems etc. A total of 80 subjects were studied, of which exposed individuals (n=40) between age group of 30 to 50 years were further divided into the type of addiction such as gutkha/

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panmasala and bidi smoking and the control (n=40) subjects were healthy, age matched and strictly without any addiction.

Samples were collected using a sterile, small headed plastic toothbrush from the inner walls of cheeks and then transferred to the tube containing chilled fresh fixative (1:3, Acetomethanol). The standard protocol of BMCyt Assay was followed to assess DNA damage and cell death biomarkers (Thomas et. al., 2009). Slides were stained with Giemsa, air dried and observed under the microscope. 1000 cells were scored per subject to find the frequency of various cell types observed in BMCyt assay. The observed cells included Binucleated cells (BN), Micronucleated cells (MN), Condensed chromatin cells (CC), Karyorrhectic cells (KR), and Karyolytic cells (KL).

All data was expressed as the mean ± standard error of the mean. The significance of the differences between control and exposed groups were analyzed using Student's t-test.

**Results**

The demographic characters of exposed male individuals are given in Table-1 which showed that the exposed subjects belonged to the poor financial and educational background. No significant increase was found in frequencies of BN, 2MN and 3MN for both addictions. The mean frequencies of cells with MN were found to be significant ($p>0.05$). Moreover, the frequencies of CC, KR, and KL cells were significantly higher ($p>0.001$) as compared to control subjects in both addiction groups (gutkha/panmasala and bidi) as shown in Table: 2. While scanning slides of most chewers, the cells showed lot of bacterial infection and were contaminated with particles of the ingredients, although they had properly gargled their oral cavity prior to sample collection. Such infection was absent in control subjects and bidi smokers.

**Discussion**

There is a possibility of increased risk for Oral Submucus fibrosis (OSMF) in individuals addicted to gutkha as suggested by Anila et. al. (2011), who showed an increase in MN in OSMF patients as compared to healthy individuals and emphasized that gutkha chewing habit in the younger age, increased the chances of malignant transformations. Chemopreventive effect of betel leaf on the genotoxicity of panmasala has been reported by Trivedi et. al. (1994), which is not used in gutkha/panmasala sold in packets and sachets. Positive associations between oral cancer and the habit of chewing areca quid which is main ingredient of gutkha/panmasala (Gupta, 1991) and increase in frequencies of MN in buccal cells in gutkha/panmasala chewers have also been reported (Siddique et al., 2008; Gandhi and Kour, 2000). MN formation has been observed in precancerous lesions of the oral cavity of chewers (Nair et al., 1991).

Many, Indian studies have reported risk of oral cancer in bidi smokers (Subapriya et. al., 2007; Muwonge et. al., 2008). Bidi smoke contains high concentrations of tar and carcinogenic polyaromatic hydrocarbons (PAHs). One notable PAH, benzo[a]pyrene, is classified as a probable human carcinogen by the U.S Environmental Protection Agency and by the International Agency for Research on Cancer as probably carcinogenic to humans (NCI, 1998). Tobacco smoke induces genetic aberrations, including gene mutations, CAs, MN, sister chromatid exchanges, DNA strand breaks, and oxidative DNA adduct in various experimental systems including humans (De Marini, 2004).

Since, the prolonged use of the chewing panmasala/gutkha or bidi smoking can result into risk of developing health consequences including oral cancer, it becomes important to analyse the possible risk in the exposed population and identify certain biomarkers which can help in the early presymptomtic counseling and management of such exposed individuals before the onset of OSMF, precancerous lesions or oral cancer. Hence, the individuals addicted to gutkha/panmasala chewing and bidi smoking were recruited for evaluation of genotoxicity in buccal epithelial cells in the present study. Our results showed significant increase in some cell anomalies (MN, CC, KR and KL) as shown in Table: 2 while non-significant frequencies were found in the remaining cell types (BN, 2MN, 3MN). The observation of our study showed that the buccal cells of the gutkha/panmasala chewers were infected with some particles and/or bacteria, which can be a matter of concern since, it has been previously reported that high frequency of Human Papilloma virus (HPV) in gutkha/panmasala chewers can cause oral lesions which may be a risk factor for squamous cell carcinoma of the oral cavity (Baig et. al., 2012). Another study on oral mucosal lesions in the oral cavity showed that chewing gutkha elevates the risk of Oral Submucous Fibrosis (OSMF), Leukoplakia, cancer and oral precancers (Ramlal et. al., 2011). The demographic details given in Table : 1, predict that most of the exposed subjects belonged to labour class, poor financial as well as educational background, which suggests that they may be facing problems like malnutrition and also they are ignorant towards all the future health epidemics.

Many individuals start such addiction habits under the influence of advertisement by film celebrities, thinking that it makes them look more fashionable. This suggests that false images of tobacco users promoted by the tobacco industry, easy access to tobacco products (gutkha/panmasala and bidi) through public stores and availability even in cheaper small packets, may lead to initiation of such widespread addiction. Also, the awareness of the hazards of chewing gutkha/panmasala and smoking bidi remains very low in rural population in India. The involvement of educated and younger individuals in chewing habits suggest that there is a need to educate them about these substances which can lead to various health hazards including oral cancer. This could be done not only through law and order but also by creating awareness among the population regarding the consequences of such habits.

**Conclusion**

The observations of this study showed that both chewing of gutkha/panmasala and smoking bidi resulted in increased nuclear anomalies in buccal cells which reflects cell injury, cell death and mitotic errors which can further lead to consequences such as OSMF and oral cancer. Hence, this study reemphasizes the efficacy of BMCyt assay biomarkers for studying the risk assessment and early diagnosis of tobacco related health hazards including oral carcinogenesis.

**Table: 1 Demographic character recorded in Exposed Subjects.**

| Sr. No. | Age (Years) | Exposure Time (Years) | Frequency (every_ Hours) | Health | Addiction | Occupation |
|---|---|---|---|---|---|---|
| 1 | 45 | 30 | 2 | Obese | Gutkha | Grocer |
| 2 | 30 | 8 | 2 | Obese | Gutkha | Painter |
| 3 | 36 | 15 | ½ | Obese | Gutkha | Painter |

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| Sr. No. | Age (Years) | Exposure Time (Years) | Frequency (every_ Hours) | Health | Addiction | Occupation |
|---|---|---|---|---|---|---|
| 4 | 30 | 12 | 1 | Weakness | Gutkha | Electrician |
| 5 | 29 | 15 | ½ | Oral Ulcer | Gutkha | Soap factory |
| 6 | 31 | 12 | 2 | Oral Pain | Gutkha | Tea Stall |
| 7 | 34 | 10 | 2 | Obese | Pan Masala | Merchant |
| 8 | 50 | 7 | 3 | Colic Pain | Pan Masala | Electrcian |
| 9 | 37 | 3 | ½ | Low weight | Pan Masala | Tea Stall |
| 10 | 49 | 30 | ½ | Piles | Gutkha | Painter |
| 11 | 32 | 10 | 2 | Oral Pain | Pan Masala | Driver |
| 12 | 31 | 7 | 1 | Weakness | Gutkha | Soap factory |
| 13 | 35 | 10 | 3 | Low weight | Gutkha | Clerk |
| 14 | 31 | 3 | 4 | Oral Ulcer | Pan Masala | Clerk |
| 15 | 40 | 15 | 2 | Obese | Pan Masala | Grocer |
| 16 | 30 | 8 | 3 | Low weight | Gutkha | Tea Shop |
| 17 | 35 | 10 | 2 | Low weight | Pan Masala | Metal Shop |
| 18 | 36 | 8 | 2 | Gums Pain | Pan Masala | Stainless Steel |
| 19 | 36 | 20 | 1 | Low weight | Gutkha | Labourer |
| 20 | 31 | 10 | 3 | Weakness | Pan Masala | Driver |
| 21 | 50 | 25 | 1 | Healthy | Bidi | Electrician |
| 22 | 52 | 25 | 1 | Thyroid | Bidi | Clerk |
| 23 | 51 | 40 | 1 | high BP | Bidi | Dyeing |
| 24 | 46 | 30 | 1 | Healthy | Bidi | Driver |
| 25 | 50 | 35 | ½ | Healthy | Bidi | Engineer |
| 26 | 48 | 30 | 2 | Healthy | Bidi | Labourer |
| 27 | 44 | 19 | 1 | Diabetes, Constipation | Bidi | Grocer |
| 28 | 50 | 25 | ½ | Eye Problem | Bidi | Grocer |
| 29 | 50 | 20 | 1 | Teeth Problem | Bidi | Grocer |
| 30 | 42 | 20 | 1 | Healthy | Bidi | Plastic business |
| 31 | 50 | 35 | ½ | Healthy | Bidi | Watchman |
| 32 | 49 | 10 | 1 | Healthy | Bidi | Mill worker |
| 33 | 47 | 22 | 1 | Healthy | Bidi | Printing Press |
| 34 | 50 | 27 | ½ | Healthy | Bidi | Cobbler |
| 35 | 42 | 16 | 1 | Healthy | Bidi | Labourer |
| 36 | 47 | 22 | ½ | Healthy | Bidi | Electrician |
| 37 | 45 | 30 | 1 | Healthy | Bidi | Labourer |
| 38 | 41 | 30 | ½ | Healthy | Bidi | Watchman |
| 39 | 51 | 25 | 1 | Healthy | Bidi | Watchman |
| 40 | 50 | 30 | ½ | Healthy | Bidi | Mill worker |

**Table: 2 Frequencies of various cell anomalies all groups.**

| Sr. No | Cell-type | Control (Mean ± S.E) | Bidi Smokers (Mean ± S.E) | Gutkha/Pan-masal chewers (Mean ± S.E) |
|---|---|---|---|---|
| 1 | MN | 1.1 ± 0.2982 | 4.1 ± 0.7287 ** | 1.85 ± 0.48** |
| 2 | 2MN | 0.3 ± 0.102 | 0.14 ± 0.231 NS | 0.35 ± 0.131 NS |
| 3 | 3MN | 0.3 ± 0.1468 | 0.25 ± 0.123 NS | 0.35 ± 0.05NS |
| 4 | BN | 5.3 ± 0.677 | 5.6 ± 0.5684 NS | 5.7 ± 0.729 NS |
| 5 | CC | 0.2 ± 0.0917 | 1.3 ± 0.1277*** | 3.05 ± 0.66*** |
| 6 | KR | 0 ± 0 | 5.75 ± 1.483*** | 3.65 ± 0.392*** |
| 7 | KL | 0 ± 0 | 20.6 ± 2.3378*** | 58.7 ± 5.67*** |

MN = Micronucleated cells, BN = Binucleated Cells, CC = Condensed chromatin cells,

KR = Karyorrhectic Cells, KL = Karyolytic Cells

NS = Non-Significant, ** = Significant (p>0.05), *** = Highly Significant (p>0.01)

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