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Monitoring genotoxic damage in tobacco chewers using buccal mucosal cells: A comparative study

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Abstract
Deleterious changes in the genetic material of an individual, caused by various genotoxic substances, leads to various severe anomalies and cancers. Cancer may develop either due to carcinogens (environmental factors) or due to cancer causing genes called oncogenes (genetic factors). Nitrosamines, polycyclic aromatic hydrocarbons, nicotine are some carcinogens found in tobacco which causes oral cancer. These carcinogens are more effective in increasing the risk of cancer in case of chewing tobacco, as they directly come in contact with vestibule. Present research was conducted on 10 tobacco chewers and 30 control subjects. Slides were prepared from their buccal mucosal cells by cell suspension technique given by Nersesyan et al, 2006. Slides shows Binucleated cells (BN), Micronucleated cells (MN) and Karyolytic cells. The frequencies of Binucleated cells (BN) and Micronucleated cells (MN) are higher in tobacco chewers than non-tobacco chewers indicating high prevalence of nuclear abnormalities in tobacco chewers in comparison to non-tobacco chewers.
Key words: Genotoxicity, Carcinogens, Oral Cancer, Tobacco chewing, MN cells, BN cells.

Introduction
Various unwanted deleterious changes that occur in the genetic material of an individual are called genetic abnormalities. These abnormalities are caused by various genotoxic substances (chemicals, mutagens, and radiations) which are toxic and poisonous to genetic material. Exposure to genotoxic agents can result from natural and environmental factors, non-specific contamination, occupational environment, or industrial accidents (**Anderson, 1999).** These genotoxic substances lead to various severe anomalies and cancers. Cancer may develop either due to cancer causing agents called carcinogens (environmental factors) or by transformation of proto-oncogenes into activated cancer producing oncogenes by point mutation, chromosomal translocation etc. (genetic factors). Nitrosamines, polycyclic aromatic hydrocarbons, N-nitrosonornicotine and N-nitroso pyrrolidine, nicotine are some carcinogens found in tobacco which causes oral cancer. Genotoxic effects of tobacco fall under three categories according to the form of its consumption i.e. chewing, snuffing and smoking. Among these the use of chewing tobacco increases the risk of oral cancer, as in this practice, its irritating juices left in contact with gums, cheeks and/or lips for prolonged periods of time and results in leukoplakia. It results in cancer in 3 percent to 5 percent of all cases. **Hecht (2003)** opines that the tobacco specific nitrosamines induce miscoding DNA adducts, that could initiate the tumorigenic process in the oral cavity of betel quid/tobacco and gutkha chewers. Similarly, **Subdo et al. (2004)** studied DNA aneuploidy in oral leukoplakia in cave Asian tobacco users and found a very high risk of development of oral squamous cells carcinomas and associated mortality. **Gupta et al. (1998)** also linked Mawa to oral submucous fibrosis (O.S.F.), oral cancer and esophageal cancer.

These substances subject users to increased cancer risk not only of the oral cavity, but also the pharynx, larynx and esophagus**.** Other than these tooth abrasion, gum recession, increased tooth decay, tooth discoloration and bad breath, nicotine dependence, unhealthy eating habits are some outcomes of tobacco chewing. It is therefore important to identify any genetic toxicity due to these agents to assess their biological impact on man **(Vainio 1980).** So, the present study has been conducted with a view to evaluate nuclear abnormalities in buccal mucosal cells of tobacco chewers and its comparison with control group i.e. non-tobacco chewers.

Material and methods

The study was conducted on 40 subjects comprising of 10 tobacco chewers (25-45 years) and 30 healthy non tobacco chewers (21-27years). Tobacco chewers were selected among rickshaw pullers from rickshaw stand, Patiala. The detailed information including name, age, sex, education status, marital status, amount of tobacco chewed per day, duration of tobacco chewing, medical history and any addiction other than tobacco chewing etc. were also recorded. Non tobacco chewers were selected among students of Punjabi University, Patiala. Their detailed history was also recorded. The subjects were asked to rinse their mouth thoroughly with plain water. After rinsing they were asked to scrap their buccal mucosa for cells with the help of spatula. First scraping was discarded to avoid any bacterial contamination. The scrapings were collected in the centrifuge tubes containing 10 ml of sample buffer. After getting sufficient scrapings, the sample was brought to laboratory for analysis. Slides were prepared by cell suspension method given by **Nersesyan et al, 2006.** Cells were washed with sample buffer and fixed in 80% methanol for overnight. Fixed cells were dropped on pre-cleaned chilled slide and were blown to spread cells. The slides were air dried and stained with May Grunwald stain and counter stained with Giemsa stain. Dried slides were examined under trinocular Zeiss microscope at 800 X- magnification. Distinct cells with clear boundaries and without overlapping were chosen for study. 500 cells per person were examined for micronuclei and other nuclear abnormalities including binucleated cells, karyolitic, pyknotic cells and data was subjected to the statistical analysis.

Results

Table 1: General information about Tobacco Chewers

	Age	Sex	Duration of exposure (years)	Quantity of tobacco taken/day	Smoker/Non-smoker	Alcoholic /Non-alcoholic	Total no. of cells	No. of cells showing MN	No. of cells showing BN	No. of cells showing Karyolysis
1	42	Male	25	10 gm	S.	A	500	2(0.4%)	8(1.6%)	2 (0.4%)
2	30	Male	10	10 gm	N.S.	N.A.	500	1(0.2%)	6(1.4%)	-
3	26	Male	5	5 gm	N.S.	A	500	1(0.2%)	3(0.6%)	-
4	25	Male	7	8 gm	S.	A	500	-	4(0.8%)	-
5	45	Male	28	10 gm	S.	N.A.	500	2 (0.4%)	8(1.6%)	1(0.2%)
6	28	Male	7	8 gm	N.S.	A	500	-	4(0.8%)	-
7	40	Male	20	10 gm	N.S.	A	500	4(0.8%)	10(2%)	7 (1.4%)

8	27	Male	10	5 gm	S.	N.A.	500	-	2(0.4%)	-
9	35	Male	15	10 gm	N.S.	N.A.	500	-	4(0.8%)	-
10	31	Male	14	8 gm	N.S.	A	500	-	3(0.6%)	-

S=smokers, N.S.= nonsmokers, B.N.= binucleated, M.N.= micronucleated

Table 2: Compiled data of controls

S.No.	Age	Total no. of cells studied	No. of cells showing MN (%)	No. of cells showing BN (%)
1	24	1500	-	4(0.26%)
2	21	1500	-	-
3	22	1500	-	-
4	21	1500	2(0.13%)	4(0.26%)
5	23	1500	-	-
6	24	1500	-	-
7	24	1500	-	-
8	23	1500	-	1(0.06%)
9	27	1500	3(0.2%)	6(0.4%)
10	24	1500	-	1(0.06%)
11	22	1500	-	-
12	22	1500	-	-
13	23	1500	-	3(0.2%)
14	22	1500	-	-
15	21	1500	-	-
16	21	1500	-	-
17	22	1500	-	-
18	25	1500	-	-
19	23	1500	-	-
20	24	1500	2(0.13%)	4(0.26%)
21	22	1500	-	-
22	23	1500	-	-
23	22	1500	-	-
24	23	1500	-	-
25	22	1500	-	-
26	23	1500	-	-
27	24	1500	-	-
28	22	1500	-	-
29	22	1500	-	-
30	22	1500	-	-

Figures in parentheses are percentages

Table 3 (A): Comparison of tobacco chewers and total non tobacco chewers showing MN by t-test

Category	Number of individuals (N)	Mean frequencies of cells showing MN	Standard Deviation (S.D.)	t-value

Tobacco Chewers	10	0.04	± 0.084	
Control	30	0.015	± 0.048	1.151

Means are not different at CL 90%, 95% and 99% levels.
$p < 0.10$

Table 3 (B): Comparison of tobacco chewers and total non tobacco chewers showing BN by t-test

Category	Number of individuals (N)	Mean frequencies of cells showing BN	Standard Deviation (S.D.)	t-value
Tobacco Chewers	10	0.38	± 0.447	
Control	30	0.05	± 0.107	3.816

Means are different at 90%, 95% and 99% levels. $p < 0.001$

Discussion

Findings of the present study indicates that all the tobacco chewers show binucleated (BN) cells whereas micronucleated (MN) cells are seen in 5 subjects and Karyolitic cells in 3 subjects (Table: 1). Out of 30 controls, 7 shows binucleated cells (0.05%), 3 shows micronucleated cells (0.015%) and no Karyolitic cell is seen in anybody (zero %). The frequencies of MN and BN cells in 10 subjects are 0.04% and 0.38% respectively. The comparison of MN cells between controls and exposed subjects showed non-significant differences [t_{value}= 1.151, $p<0.10$]. But for BN cells the frequencies showed statistically significant differences [t_{value}= 3.816, $p<0.001$]. (Table 2, 3A and 3B). Similar finding has been reported by **Kayal et al. (1993)** who analyzed the frequency of MN in exfoliated buccal mucosal cells of healthy individuals and patients of oral submucosfibrosis who had the habit of chewing tobacco and shows statistically significant increase in MN frequency. **Stich et al. (1994)** applied MN test to buccal mucosal cells of two population groups at higher risk of oral cancer in Orissa. All the raw betel nut eaters or betel leaf with lime users had significantly high frequencies of MN over non chewing controls. Significantly elevated frequencies of MN in mucosal cells were observed in chewers of betel quid with tobacco (4.83/1000 cells) and of tobacco with lime (5.20/1000 cells) compared with control group (2.59/1000 cells) (**Nair et al. 1991).** Maras powder, a kind of smokeless tobacco used in South Eastern regions of Turkey. to assess possible DNA damage in exfoliated oral cells. The MN frequency in the inner lip mucosa site was 1.27 percent for Maras powder users, 0.88 percent for non-smoking control subjects and 0.82 percent for buccal site of Maras powder users (**Buirgazt et al. 2000)**

Conclusion

It is concluded from the study that person who is exposed to genotoxic substances like tobacco is more prone to oral diseases like oral cancer, leukoplakia, oral submucous fibrosis, oral squamous cell carcinoma. Irritating juices left in contact with cheeks, gums, lips or vestibular areas for prolonged periods resulted into the deterioration of oral mucous membrane and related structures and bring about various histopathological changes in the related tissues. Increased frequencies of micronucleated cell (MN Cells) in the tissue highlighted the prevalence and risk factors for developing nuclear abnormalities.

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