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Evaluation of micronuclei and other nuclear abnormalities in buccal cells of tobacco chewers

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ABSTRACT
Genotoxic substances i.e. chemicals, mutagens and radiations are those substances which are toxic to the genetic material. Damage to the genetic material can be assessed in number of ways by studying chromosomal aberrations, sister chromatid exchanges and other nuclear aberrations like micronuclei, binucleated cells etc. Present research was conducted on 10 tobacco chewers. Slides were prepared from their buccal mucosal cells by cell suspension technique. Cells were spread on slide and stained with May Grunwald stain followed by Giemsa stain. Standard slides were examined under trinocular Zeiss microscope at 800 X-magnification. 500 cells were examined for each 10 tobacco chewers. Slides shows Binucleated cells (BN), Micronucleated cells (MN) and Karyolytic cells. Study concludes that age, duration of exposure and other addictions like smoking and intake of alcohol affects the frequencies of nuclear abnormalities. Duration of exposure significantly affects BN frequency but not MN frequency. The mean values for MN cells were not different but for BN cells they were different among two age groups taken.

Key Words
Micronucleated (MN) cells , Binucleated (BN) cells, Oral cancer, Chromosomal Aberrations, Karyolytic cells, Genotoxic, Mutagens

INTRODUCTION

Oral cancer is one of the 10th most common cancer as stated by WHO and each year 5, 75,000 new cases and 3, 20,000 deaths occurs worldwide. In India, oral cancer is major health problem which accounts for 50-70% of all cancers diagnosed and are correlated with tobacco chewing (Jayant et al.1997).Cancer is an example of one of the environmental diseases. Nearly 85% of all cancers have an environmental component and

stressors and is therefore it is imperative and important to identify any potential genetic toxicity due to these environmental agents and to assess their biological impact on man (Vainio et al.1980).Genotoxic substances (chemicals, mutagens and radiations) are those substances which are toxic and poisonous to the genetic material. There are numerous methods to judge the genetic damage which may include sister chromatid exchange, chromosomal aberrations, binucleated cells, presence of micronuclei, telomere lengths, etc. The later half of twentieth century has mainly focused on the analyses of chromosomal aberrations in order to judge the genetic damage to a person (Littlefield and Lushbaugh 1990, Lloyd 1990). An alternative method which is faster and simpler is the micronucleus assay (Hall and Wells,1988; Fenech,1993).The occurrence of micronucleus has been used as an indicator of the clastogenic effects of both chemical and physical agents (Wakata and Sasaki,1987; Odagiri et al.,1990).Micronuclei are the small round bodies found in the cytoplasm outside the main nucleus. Structurally, micronuclei appears to be similar to the main nucleus as they are encapsulated by a nuclear envelope with nuclear pores (Schiffmann and De Boni,1991). Since micronuclei contain DNA, they may represent a detrimental change of genetic information to the cell. According to Major et al. (2001) the analysis of micronuclei using Micronucleus test is not only a non-invasive method but also is a good way of judging the genetic damage. It has a great advantage in mass screening as it is highly cost effective (Titenko-Holland et al. 1994). It does not require the culturing of the cells. The present study has been conducted with a view to evaluating the micronuclei and other nuclear abnormalities of tobacco chewers.

MATERIAL AND METHODS

The study has been conducted on 10 tobacco chewers (25-45 years of age). Tobacco chewers were selected from amongst the rickshaw pullers of rickshaw stand, Patiala. The detailed information including name, age, sex, educational 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. The samples were collected in the month of February 2008. The subjects were asked to rinse their mouths thoroughly with plain water. After rinsing they were asked to scrap their buccal mucosa for cells with the help of a spatula. First scrapings were discarded to avoid any bacterial

contamination. The sample of scrapings were collected in centrifugal tubes containing 10 ml of sample buffer. The cell suspension method as given by Nersesyan et al. (2006) was used for preparing the slides. The cells were washed and then fixed with 80% methanol for overnight. The fixated cells were put on the pre-cleaned chilled slide and then were evenly spread on the slide. The slides were then dried and thereafter stained with May Grunwald stain and again counter stained with Giesma stain. Observations were made using Zeis microscope at 800 X-magnification. Only non overlapping and distinct cells with clear boundaries were included in the study. In all, 500 cells per person were observed for various nuclear abnormalities, binucleated cells, mcronuclei, piknotic cells, karyolysis, etc.

RESULTS

After the complete analysis of samples, observations indicate that Binucleated cells (BN) are present in all the tobacco chewers where as micronucleated cells (MN) and karyolitic cells are seen in 5 and 3 subjects, respectively. Duration of tobacco exposure ranged from 5-10 gm/day. General information of tobacco chewers is given in Table 1.

Relationship between age and frequencies of BN and MN indicates that older subjects show higher frequencies of BN (3.2%) and MN(0.8%) than younger subjects (0.8% and 0.4%, respectively) (Table: 2).

Table 1. General information about Tobacco Chewers

Sr. no	Age (years)	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, NS- Non-Smokers, A - Alcoholic, NA - Non-Alcoholic

Table 2: Age wise distribution of exposed subjects

Age of group	No. of Individuals	No. of cells showing BN	No. of cells showing MN	No. of cells showing Karyolysis
20-25 years	1 (10%)	4 (0.81%)	-	-
26-30 years	4 (40%)	15 (3%)	2 (0.4%)	-
31-35 years	2 (20%)	7 (1.4%)	-	-
36-40 years	1 (10%)	10 (2%)	4 (0.8%)	7 (1.4%)
41-45 years	2 (20%)	16 (3.2%)	4 (0.8%)	3 (0.6%)

Table 3 depicts the frequency of BN and MN as well as Karyolytic cells in relation to smoking and alcohol intake as well as non-smokers and non-alcoholics. The frequency of BN (1.1%) is higher in smokers than non-smokers (1%) where as the frequency of MN is the same in smokers and non-smokers . The frequency of BN in alcoholics is 1.06% and that of MN it is 0.23%, which is higher than non-alcoholics who have frequencies of 1% and 0.15%, respectively. The subjects who are smokers as well as alcoholics have BN frequency of 1.2%, MN frequency 0.2% and karyolitic cell frequency 0.2% where as these frequencies in non-smokers as well as non-alcoholics are 1%, 0.1% and zero%, respectively.

Table 3: Number of BN and MN cells in relation to smoking and alcohol intake

Category	No. of individuals	No. of cells showing BN	No. of cells showing MN	No. of cells showing Karyolysis
Smokers	4	22 (1.1%)	4 (0.2%)	3 (0.15%)
Non-smokers	6	30 (1%)	6(0.2%)	7 (0.23%)
Alcoholics	6	32 (1.06%)	7 (0.23%)	9 (0.3%)
Non-alcoholics	4	20 (1%)	3 (0.15%)	1 (0.05%)
Smokers and alcoholics	2	12 (1.2%)	2 (0.2%)	2 (0.2%)
Non-smokers & non-alcoholics	2	10 (1%)	1 (0.1%)	-
Non-smokers and alcoholics	4	20 (1%)	5 (0.25%)	7 (0.35%)
Smokers and non-alcoholics	2	10 (1%)	2 (0.2%)	1 (0.1%)

Tobacco chewers are distributed in two categories according to the duration of exposure (Table 4). They fall under categories of less than 13 years and more than 13 years of exposure. Incidence of MN and BN cells is less in subjects who have exposure

less than 13 years (MN=0.08% and BN=0.765) from the subjects who have exposure more than 13 years (MN=0.325 and BN=1.32%).

Table 4: Distribution of tobacco chewers showing MN and BN according to exposure of duration

Duration of exposure (years)	Total subjects	Total no. of cells examined	Cells showing MN (%)	Cells showing BN (%)
<13	5	2500	2 (0.08%)	19 (0.76%)
>13	5	2500	8 (0.32%)	33 (1.32%)

Comparisons of mean values of MN cells and BN cells among two age groups (Table 5A and 5B) shows that mean values of MN cells are not statistically different between the two age groups.

Table 5 (A) t-test applied on tobacco chewers showing MN below age of 30 years and above age of 30 years

Age (years)	Number of individuals (N)	Total no. of cells examined	Mean frequency of cells showing MN	Standard Deviation (S.D.)	t-value
≤ 30	5	2500	0.08	± 0.11	
> 30	5	2500	0.32	± 0.335	1.524

Table 5 (B) t-test applied on tobacco chewers showing BN below age of 30 years and above age of 30 years

Age (years)	Number of individuals (N)	Total no. of cells examined	Mean frequency of cells showing BN	Standard Deviation (S.D.)	t-value
≤ 30	5	2500	0.76	± 0.297	
> 30	5	2500	1.32	± 0.593	1.88

Table 6 (A): Comparison of tobacco chewers showing MN cells according to duration of exposure by t-test

Duration of exposures (years)	Number of individuals (N)	Mean frequencies of cells showing MN	Standard Deviation (S.D.)	t-values
≤ 13	5	0.08	± 0.11	
> 13	5	0.32	± 0.335	1.524

Table 6 (B): Comparison of tobacco chewers showing BN cells according to duration of exposure by t-test

Duration of exposures (years)	Number of individuals (N)	Mean frequencies of cells showing BN	Standard Deviation (S.D.)	t-values
≤ 13	5	0.76	± 0.297	
> 13	5	1.32	± 0.593	1.88

Comparisons of MN cells and BN cells among two groups made on the basis of duration of exposure in tobacco chewers shows statistically non significant differences (Table 6A and 6B).

DISCUSSION

Findings of the present study indicate that all the tobacco chewers shows binucleated (BN) cells whereas micronucleated(MN) cells are seen in 5 subjects and Karyolytic cells are seen in 3 subjects. The frequencies of MN and BN cells in 10 subjects are 0.04% and 0.38%, respectively.

Kayal et al. (1993) 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 show statistical significant increase in MN frequency. Similarly Stich et al. (1983) applied MN test to buccal mucosal cells of two population groups at higher risk of oral cancer in Orissa and observed significantly high frequency of MN in raw betel nut eaters or betel leaf with lime users. Though the frequency of MN cells was higher in the chewers who had the history of longer duration of tobacco chewing (more than 13 years) but it was not statistically significant. The results of the present study are well correlated with the findings of Das and Dash(1992), Mishra et al.(1998) and Kavita (2005)who emphasized that genotoxic effects are more in those who had consumed tobacco, pan masala, gutkha, areca nut alone or in combination of some or all of these for more than five years. The authors have found a significant correlation between the increased incidence of MN and duration of tobacco used. Patel et al. (1994) studied the clastogenic effect of ethanol and pan masala in different combinations on Chinese hamster ovary cells and reported that alcohol consumption may potentially increase the risk of oral cancer among pan masala chewers. Sankaranarayanan et al. (1997) also correlated the tobacco chewing and alcohol drinking with the risk factors for cancer.

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