Research Article

Cytogenetic Biomonitoring: Micronucleus Test in Buccal Epithelial cells of Tobacco Smokers in Tiruchirappalli District (Tamilnadu, India)

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ABSTRACT

We assessed the incidence of Micronuclei (MN) formation from 30 male smokers and who had smoked a minimum of 1 pack year (i.e. no. of packs of cig. smoked/day X duration of cigarette smoking in years) and maximum of 76. The cigarette consumption year also ranges from 2 to 43 years in smokers group. Buccal epithelial cells were selected because for the direct exposure of tobacco smoke. The smokers groups were divided into two groups based on their age as <40 and >40. Number of smokers in group II (>40) was found high. Also Group II (>40) showed high frequency of Buccal Micronuclei due to their increased Smoke consumption year, pack years. Significantly ($P<0.05$) increased frequency of MN was observed in smokers group in particular group II (>40) smoker group than non smokers (male; n=30) control group. Smoking leads to cytogenetical changes/damages to the human Buccal epithelial cells.

Keywords: Micronuclei, Tobacco smoke, Buccal cells.

INTRODUCTION

Cigarette smoking is responsible for a substantial number of human health problems.[1,2] Tobacco cigarette smoking is one of the most important leading causes of death and essential public health challenge in world over.[3,4] There are more than 4000 chemicals including over 50 known carcinogens such as polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, aromatic amines, and trace metals were found in cigarette smoke.[5,6] Smoking is a major cause of cancer, cardiovascular diseases, and chronic obstructive pulmonary diseases.[7] Annual mortality ascribed to tobacco use in India, has been estimated to be 1 million.[8]

Many of the substances contained in cigarette smoke are genotoxic.[6] The buccal epithelium is composed of four strata including the basal cell layer, prickle cell layer, intermediate and superficial layers. The oral epithelium maintains itself by a system of continuous cell renewal in which new cells produced by mitosis in the basal layer migrate to the surface to replace those that are shed. Thus, the mucosa is composed of progenitor and maturing cell populations.[9] Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. Their presence in cells is a reaction of structural and/or numerical chromosomal aberrations arising during mitosis.[10-12]

One of the best techniques for studying the effects of environmental factors on genetic stability in human cells is the micronucleus (MN) test.[13] MN may be products of early events in carcinogenesis, especially in the oral cavity, which is directly exposed to cigarette smoke.[14] The measurement of micronuclei (MN) appears to be one of the most suitable. MN originates from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division. They reflect chromosome damage and may thus provide a marker of early-stage carcinogenesis. Smoking habit as an important factor that induces significant alterations in the genetic material[15] and the genotoxic effects in lymphocytes of smokers are most likely caused by cigarette smoke constitutions.[16]

MN frequencies represent both clastogenicity (chromosome breakage) and aneuploidy (chromosome loss) in cells studied and it has been extensively used to identify potential genotoxic exposures and also chromosomal instability. The MN assay, when compared with the CA assay, has been relatively easier, faster and did not require metaphase cells.[17] Smoking is reported to increase the MN frequency in buccal cells.[18-20] The present study focused the occurrence of Micronuclei in buccal epithelial cells of smokers due to exposure of tobacco smoke.

MATERIALS AND METHODS

Subject Selection

Subjects (n=30) were the local residents of Tiruchirappalli district (Tamilnadu, India). The foremost inclusion criteria in the present study embrace the analysis of pack years (no. of packs of cig. smoked/day X duration of cigarette smoking in years)[21], life style factors (alcohol intake and smoking) and age. The exclusion criteria included the elimination of subjects from viral infection, occupational history, exposure to radiation and chemicals, surgery, chemotherapy, autoimmune diseases, immunology, and genetic disorders. All the controls were physically and

mentally normal subjects who had no history of any genetic disorders.

Sample Collection

The study was conducted according to the Institutional Human Ethical clearance and Helsinki 1964 procedure.[22] Informed consent was obtained from both the exposed subjects and controls. The Smokers (exposed subjects) and the Non-Smokers (controls) were divided into two groups based on their Age as Group I (≤ 40 yrs and below), Group II (41 yrs and above).

Micronuclei assay in buccal epithelial cells

The Subjects were goggled and washed their mouth with sterile water. Buccal cells were collected by gentle scrapping of wooden spatula on their cheek. The spatula was stored in saline and centrifuged at 8000rpm for 5min. The cell pellet were collected and fixed in Methanol:Acetic acid (3:1) solution. The fixed cells onto a slide were air dried and stained with Felugen: Fast Green stain and observed under Leica Microscope for Micronulcei. For each sample 1000 cells were scored according to the criteria described by Sarto.[18]

Statistical Analysis

The statistical significance of the differences in the frequencies-genotypes between groups was calculated. All the analyses were performed with IBM-SPSS software 20.0 version. Mean and standard deviation were calculated to assess the difference between the smokers and non-smokers and the level of significance was calculated by ANOVA.

Table1: General Characteristics of the smoker group (n=30)

S. No	Age	Groups		Pack year	No. of. cig/day	No. of Pack/day	Consumption of yr	Buccal MN/1000 cells
		≤ 40	≥ 41					
01	20	*		1	5	0.5	2	1
02	24	*		1.5	5	0.5	3	1
03	22	*		1.5	5	0.5	3	2
04	21	*		1	5	0.5	3	2
05	55		*	33	15	1.5	22	2
06	65		*	42	15	1.5	28	3
07	56		*	50	20	2	25	3
08	65		*	76	20	2	38	6
09	41		*	14.4	8	0.8	18	03
10	31	*		18	20	2	09	4
11	35	*		11	10	1	11	2
12	40	*		18	10	1	18	4
13	25	*		1.2	3	0.3	4	3
14	45		*	42	20	2	21	4
15	47		*	37.5	15	1.5	25	5
16	68		*	43	10	1	43	6
17	39	*		15.2	8	0.8	19	5
18	34	*		12	10	1	12	3
19	38	*		18	10	1	18	3
20	52		*	54	20	2	27	4
21	62		*	15	10	1	15	5
22	23	*		3.2	8	0.8	4	2
23	61		*	45.6	12	1.2	38	5
24	49		*	37.5	15	1.5	25	3
25	43		*	18	10	1	18	4
26	52		*	37.5	15	1.5	25	6
27	48		*	25	10	1	25	5
28	41		*	20	10	1	20	4
29	49		*	39	15	1.5	26	4
30	62		*	50	14	1.4	40	7

Table 2: General Characteristics of the nonsmoker group (n=30)

S. No	Age	Groups		Pack years	Buccal MN 1000 Cells
		≤40	≥41		
01	18	*		NA	0
02	22	*		NA	0
03	20	*		NA	0
04	19	*		NA	0
05	53		*	NA	1
06	63		*	NA	2
07	54		*	NA	1
08	63		*	NA	2
09	41		*	NA	1
10	29	*		NA	0
11	33	*		NA	1
12	38	*		NA	1
13	23	*		NA	0
14	45		*	NA	1
15	47		*	NA	1
16	66		*	NA	2
17	37	*		NA	2
18	32	*		NA	1
19	36	*		NA	1
20	50		*	NA	1
21	60		*	NA	2
22	21	*		NA	0
23	59		*	NA	2
24	47		*	NA	1
25	41		*	NA	1
26	50		*	NA	2
27	46		*	NA	1
28	41		*	NA	0
29	47		*	NA	1
30	60		*	NA	2

Table 3: Overall information about smokers group.

S. No	Particulars		Group-I	Group-II
1.	Age	Control	27.33±7.28	51.8±7.99
		Smoker	29.33±7.28	53.38±8.48
2.	Pack Years	Control	NA	NA
		smoker	8.46±7.22	37.75±15.10
3.	Number of cigarette smoking/Per day	Control	NA	NA
		Smoker	8.25±4.28	14.11±3.87
4.	Consumption of year	Control	NA	NA
		Smoker	8.83±6.33	26.6±7.82
5.	Buccal Micronuclei 1000/ Cells	Control	0.5±0.6	1.33±0.57
		Smoker	2.66±1.17	4.5±1.34

RESULTS AND DISCUSSION

The primary objective of our study was to study the primary cytogenetic abnormality i.e. micronuclei. A total of 60 male subjects, corresponding to 30 smokers (Table 1) and 30 non-smokers (Table 2) were recruited for this study. The exposed subjects were categorized into 2 groups, group I (≤ 40 yrs and below) group II (41 yrs and above) based on pack years. There were no change in age and gender (all males) distributions between the Smokers (Experimental subjects) and non-smokers (controls). The number of subjects in group I and group II were 12 (40%) and 18 (60%) respectively. The mean age of the group I (smokers) was 29.33±7.28 and 53.38±8.48. The mean age of the group I non-smokers was 27.33±7.28 and 51.8±7.99 yrs old.

In cells, the molecular and chromosomal changes lead to the formation of micronuclei.[23] Smokers Buccal epithelial cells showed higher frequency (P<0.05) of micronuclei in all the groups than non-smokers group due to the increased pack years and smoke consumption rate. Figure 1 showed the occurrence (mean value) of MN in control and smokers group (Group I and Group II).

Wu[24] reported that, prolonged smoking was associated with increased buccal micronuclei frequency. A significantly higher MN frequency was observed in the buccal cells of smokers than the nonsmokers by Konopacka[25]. MN and other nuclear anomalies such as nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) are biomarkers of genotoxic events and manifestations of chromosomal instability. Genetic damage events such as MN, NPB or NBUDs provide valid measures of misrepaired DNA breaks, dysfunctional telomeres or lack of telomeres as well as defective separation of sister chromatids at anaphase due to failure of decatenation, DNA amplification and formation of DNA repair complexes.[23]

MN frequency in exposed and controls are exhibited in Table 3. In smokers group, MN in group I and group II were 5.25±1.42 and 6.94±1.17 were found to be significant when compared to their non-smokers group I and II (1.25±0.92 and 2.22±0.53). The buccal MN frequency of group I and II exposed subject were 2.66±1.17 and 4.5±1.34 which were significant compared to their group I and II controls were 0.5±0.6 and 1.33±0.57 respectively.

Rosin and German[26] showed that a chromosome instability syndrome like Bloom syndrome heterozygotes was characterised by increased buccal MN frequencies. Benner[27] reported significantly increased (P<0.01) frequency of micronuclei in buccal cells of smokers mucosa layer than non smokers which contains normal appearing mucosa which is similar to our results.

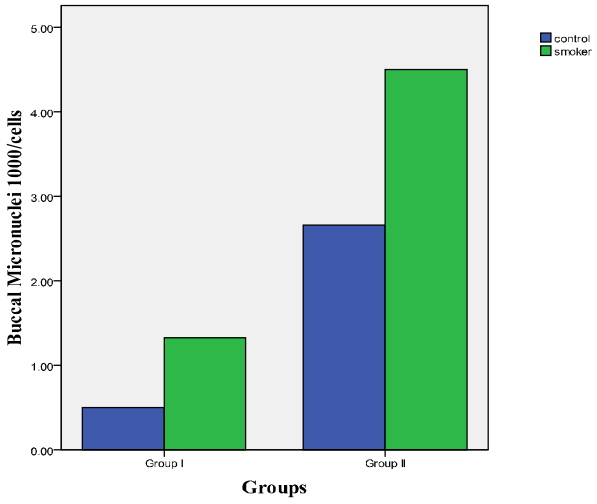

Figure 1: Bar diagram of Buccal Micronuclei cells in Control and smoker (Group I & Group II)

CONCLUSION

Smoke is a carcinogen which damages the genetical nature of the cell. Buccal epithelial cells of smokers were selected because for the direct exposure of tobacco smoke. The observation of high frequency of Buccal Micronuclei in smokers group due to their increased Smoke consumption year, pack years. It concluded that Smoking leads to cytogenetical changes/damages to the human buccal epithelial cells.

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