Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells

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In the present work the frequency of micronuclei (MN) in exfoliated buccal cells in 120 healthy individuals with relation to sex, age and smoking was investigated. Neither age nor sex showed any effect on the level of micronuclei. Smoking has shown a significant effect upon basal DNA damage. In the present study the calculated background frequency of micronuclei (‰) in oral epithelial cells of 50 smokers and 70 non-smokers were 1.50 ($\pm$0.47) and 0.55 ($\pm$0.32), respectively.

Key words: Micronuclei, exfoliated buccal cells, smoking, aging.

The micronucleus test is the most frequent technique used to investigate effects of different chemical agents in relation to DNA damage in epithelial cells. Buccal mucosa is a good site for use in monitoring human exposure to environmental and occupational agents because its cells are in direct route of exposure. Analysis of micronuclei is a simple, fast and sensitive method for monitoring genetic damage in human population. Micronuclei are small round bodies within cytoplasm having the same staining properties as main nucleus. They arise from chromosomal acentric fragments or whole chromosomes that are not included in the daughter nuclei during mitosis [4]. Some of the basic parameters of micronucleus assay in exfoliated buccal cells were first established by Stich and coworkers [14]. This report includes the Feulgen DNA staining procedure. This test has been adapted to exfoliated cells from various target tissues such as bronchi or urinary bladder [6, 12]. Increases in the frequency in exfoliated clls were observed as a result of exposure to environmental agents such as PAHs [7], pesticides [11] and formaldehyde [2]. The average reported healthy population MN frequency is 1–3 per 1000 cells, with no significant variation between different types of exfoliated cell [5]. Data from different studies indicate that the normal background frequency of micronuclei in human oral epithelial cells varied between 0.04% [7] to 0.16% [15]. The difference can be due to lifestyle habits like smoking, consumption of spicy food and genetic factors of various ethnic groups. It is of interest to know the effect of different lifestyle agents on the spontaneous micronucleus frequency in buccal cells. The aging and smoking seems to be the most important. The compounds identified in tobacco, in both the gaseous phase and condense particles interact with cellular DNA resulting in strand breaks and base lesion [10]. Several studies have indicated that smoking habits influence the micronucleus frequencies [6, 9, 13] but some others have not found any effect of smoking with respect to incidence of micronuclei in oral epithelial cells [1, 7, 16].

The present work was performed to compare the influence of age and sex on the micronucleus frequencies in exfoliated buccal cells between smokers and non-smokers.

Material and methods

Subjects. Buccal mucosa cells were obtained from 120 healthy persons ranging from 18 to 68 years of age (60 females and 60 males; 50 smokers and 70 non-smokers). Smokers used at least 10 cigarettes per day. All individuals were of similar dietary habits. They were not occupationally exposed to mutagens.

Micronuclei in exfoliated buccal cells. Exfoliated epithelial cells from the buccal mucosa were collected by scraping

the cheeks with a wooden spatula. The cells were next smeared on the slides, dried in the air and fixed with a cold solution of 1% glutaraldehyde (Serva) in 1/15 M fosphate buffer (pH=7.5) for 20 min. Then the slides were stained by the Feulgen reaction and then contrasted for 30s by Fast Green. Two thousand cells were analyzed in each person and the number of micronuclei was noted. The scoring was done according to the established criterion [14, 15].

Statistical analysis. All the data were expressed as the mean and standard deviation of the mean. Comparison of mean values in subgroups were examined using the Student's t-test. The relationship between frequency of micronuclei and age was determined using linear regression analysis.

Results

Table 1 summarizes general characteristics of the subjects. The average age of the subjects in both female and male sex was 38.42 ± 13.01 and 35.16 ± 12.88, respectively. There were 70 non-smokers and 50 smokers.

Table 1. General characteristics of analyzed groups

Parameter	Female	Male	Total
Number	60	60	120
Age mean ± SD	38.42 ± 13.01	35.16 ± 12.88	36.79 ± 12.40
Smoking habits			
non-smokers	35	35	70
smokers	25	25	50

Table 2. The frequency of micronuclei (MN) in exfoliated buccal cells of 120 healthy subjects

Groups	N	MN (‰)
Female	60	0.94 ± 0.66
Male	60	0.85 ± 0.60
Total	120	0.90 ± 0.62

Table 3. Effect of smoking on micronuclei frequencies in human exfoliated buccal cells of 120 healthy subjects

Smoking habits	Groups	N	MN (‰)
Non-smokers	Female	35	0.52 ± 0.33
	Male	35	0.50 ± 0.21
	Total	70	0.55 ± 0.32
Smokers	Female	25	1.54 ± 0.42
	Male	25	1.31 ± 0.56
	Total	50	1.50 ± 0.47*

Data represent means ± SD; *shows significantly different from non-smokers ($p>0.05$).

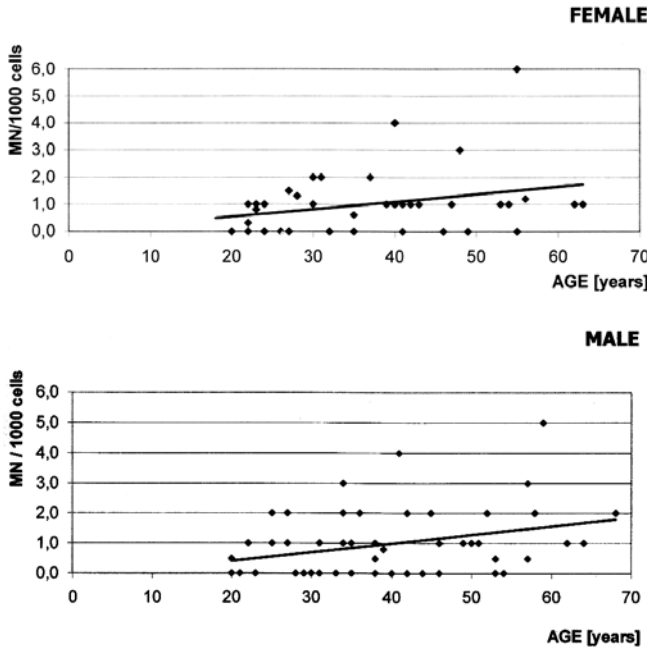

Figure 1. Effect of sex and age on micronucleated cell rates.

The mean values for MN frequencies in exfoliated buccal cells of 120 healthy persons are presented in Table 2. The base-line frequencies of micronuclei were calculated as 0.90‰ ± 0.62. Female and male did not differ significantly with respect to the incidence of micronuclei in oral epithelial cells and the micronucleus frequencies in female was 0.94‰ ± 0.66, similar to that found in male (0.85‰ ± 0.60).

Age dependence of MN formation was analyzed separately. The correlation between age and the spontaneous frequency of micronuclei in buccal epithelial cells is presented in Figure 1. Data show that there is no correlation between the analyzed parameters. The linear regression was:

Female: $Y=0.029\ X - 0.173$ ($r=0.342$)

Male: $Y=0.028\ X - 0.025$ ($r=0.298$),

Where Y = number of micronuclei, and X = age in years.

The results of micronucleus frequencies in exfoliated cells of smokers and non-smokers are summarized in Table 3. Statistically significant difference in the number of micronuclei was found between smokers (1.50‰ ± 0.47) and non-smokers (0.55‰ ± 0.32). We observed a higher level of micronuclei in cells collected from female smoker (1.54‰ ± 0.42) than from male smoker (1.31‰ ± 0.56).

Discussion

The background level of micronuclei in epithelial buccal cells of healthy persons tested in our study was 0.90‰ ± 0.62. It is consistent with the published data where micronucleated cells rates in oral mucosa varied from 0.04% to 0.16% [7, 15].

The possible influence of age on micronucleus frequencies in oral mucosa cells was tested in our study but we observed no significant effect. Our data are consistent with the published reports [3, 7, 11] that showed that micronucleus frequency did not vary with the age and sex of healthy persons. In the present study we also observed not significant difference in the level of spontaneous frequency of micronuclei between female ($0.94‰ \pm 0.66$) and male ($0.85‰ \pm 0.60$). In regard to smoking we found significantly elevated level of micronuclei in 50 smokers compared to 70 non-smokers. The data presented in Table 3 show that the level of micronuclei in oral epithelial cells in smokers are 3 times greater than those in non-smokers. Although there is a similar grand-beck level of micronuclei in both sexes, we observed higher level of micronuclei in female smokers ($1.54‰ \pm 0.42$) than in male smokers ($1.31‰ \pm 0.56$).

Several studies have previously compared the frequency of micronuclei in oral epithelial cells of smokers and non-smokers but the results are not clear. Smoking habits have been reported to influence the micronucleus level in exfoliated cells from oral mucosa. One study found a 3.4-fold increase of the number of micronuclei in epithelial cells in smokers compared to non-smokers [6]. A significant elevation of the frequency of micronuclei in tracheobronchial epithelial cells in 9 smokers as compared to 16 non-smokers has also been reported [9]. It has been demonstrated that cigarette smoking elevated the MN frequency in urothelial cells [8, 12]. Our data are consistent with the results of the authors cited above.

On the other hand smoking has been reported to have no significant effect with respect to the incidence of micronuclei in unexposed healthy persons [1, 7, 16].

The differences in the results of the published studies may be partly due to a small number of individuals in each group and different number of cigarettes smoked. Sarto and coworkers concluded that micronucleus test in exfoliated buccal cells is sensitive and able to detect the effect of smoking 20 cigarettes per day [13].

In conclusion, the results from the present study and from several others as well, have indicated that age and sex are not significant, but smoking appears to have a significant effect upon basal DNA damage in buccal cells.

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