THE EFFECTS OF SMOKING ON MICRONUCLEUS FREQUENCIES IN BUCCAL CELLS OF HEALTHY IRAQI INDIVIDUALS

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ABSTRACT

The genotoxic damage that Tobacco smoke produces in smoker individuals was evaluated using the micronucleus assay in exfoliated buccal cells. The study comprises 90 individuals of which 46 were smokers and 44 were nonsmokers. We found the mean of the micronuclei frequency in exfoliated buccal cells was \((10.18 \pm 1.07; 12.89 \pm 1.85)\) in nonsmoker and smoker individuals respectively, which is substantially higher than the baseline of the micronuclei expression in the general healthy population. There was a statistically significant difference in the micronuclei expression between smokers and non-smokers \((P<0.05)\). The mean value of micronuclei frequency in youngest age group \((20-30)\)years was \((14.27 \pm 1.63)\); in oldest age group \((31-40)\) it was \((9.75 \pm 0.87)\), the difference was significant \((P<0.01)\). We concluded that the higher frequency of micronuclei directly associated with the decrease of efficiency of DNA repair and increase of genomic instability. Micronucleus assay in the buccal cell is sensitive, practical, inexpensive method for monitoring genetic damage in human. Further researches are needed not only for count micronuclei but also to capture the frequency of apoptosis and necrosis cells to detect the further evaluation of cellular and tissue level biological damage.

KEYWORDS: buccal cells, healthy, Iraqi, Micronucleus assay, smoking.

1-INTRODUCTION

Tobacco smoking is considered the main public health problem that has negative impacts all over the world.\(^1\) The international agency for research on cancer recognized tobacco smoking as the cause of cancer at more organ sites than any other human carcinogen. In addition to the lung cancer, cigarette smoking is an important cause of esophageal, oral,
oropharyngeal, hypopharyngeal, and laryngeal cancer as well as pancreatic cancer, bladder cancer and cancer of the renal pelvis.\[^{[2]}\] There are over 60 carcinogens in cigarette smoke belong to various classes of chemicals. The carcinogenic effects of cigarette smoke are driven largely by the mutagenicity of various chemicals in the smoke.\[^{[3]}\] Many studies demonstrate that tobacco smoke induced DNA strand breaks, oxidative DNA adducts, gene mutation, sister cremated exchange, chromosomal aberration and micronuclei in the variety of systems.\[^{[4]}\]

Micronuclei (MN) are chromatin-staining structures in the cytoplasm surrounded by a membrane without any detectable link to the cell nucleus visualized using different staining techniques (Giemsa or fluorescent) and their frequency is quantified microscopically.\[^{[5]}\] In humans, MN can be easily assessed in erythrocytes, lymphocytes, and exfoliated epithelial cells (e.g. Oral, urothelial, nasal) to obtain a measure of genome damage induced in vivo.\[^{[6]}\]

The micronucleus assay was first adapted to exfoliated human cells in 1982 by Stich and coworkers.\[^{[7,8]}\] Since then, the MN assay has been applied to evaluate the chromosomal damage of human populations exposed to genotoxic agents in various occupational settings in the environment, or as a consequence of lifestyle.\[^{[9,10]}\] This is because of the ease of sampling, the possibility of obtaining large numbers of scorable cells, MN assay is relatively easy to score micronuclei as compared to chromosome analysis of chromosomal aberration moreover the essential advantage of micronuclei analysis in buccal cells is that neither cultivation nor cell division in vitro are needed.\[^{[11]}\]

Buccal epithelial cells are the direct route of exposure to the different types of genotoxic agents. micronucleus assay most frequently used for tobacco-associated buccal cell abnormalities. Several studies indicate that smoking habit elevated the micronuclei frequencies\[^{[12,13]}\] but some others have not found any effect with respect to the occurrence of micronuclei in buccal mucosa cells.\[^{[14,15]}\] The present study was designed to assess the frequency of micronuclei in buccal mucosa cells of smokers and its comparison with nonsmokers.

2-SUBJECTS, MATERIALS AND METHODS
The study involves a total of 90 apparently healthy male with an age range (20-40) years. They were a mixed of the social class of students and administrative employees. Information on age, smoking habit, marital status, alcohol consumption, diagnostic X-rays, chemical
exposure during the occupation, family history of cancer, medical and residential history were collected by questionnaire. All analyses were carried out on anonymous, coded samples. Individuals having potentials confiding factors other than smoking, including (major illness, recent radio diagnostic exposure, alcohol and drug consumption) were excluded from this study. A smoker was defined as one who had reported to smoke, no fewer than 10 cigarettes per day.

2-1 Collection of exfoliated buccal mucosa cells
Prior to buccal cell sampling, the subject participants were advised to rinse their mouth thoroughly with water to remove unwanted debris. buccal cells were collected from each subject using a soft toothbrush gently from the oral mucosa of cheeks. The cells were smeared on clean glass slides, slide were air dried for 10 to 15 minutes and then fixed with cold methanol for 15 minutes, slide were air-dried and stained in 2% Giemsa stain for 15 minutes, rinsed with double distal water. A total of 1000 cells per individuals was analyzed and the number of micronuclei were noted. Scoring criteria for micronuclei in buccal cells were, according to Sarto et al.\cite{16}

2-2 Statistical analysis
All statical analysis was conducted using Statistical Analysis System- SAS.\cite{17} Least significant difference –LSD test was used to test the difference in the mean count of micronuclei between study groups and subgroups. The value of \( P < 0.05 \) was considered statistically significant.

3-RESULTS
All individuals of study sample smokers and nonsmokers were with a positive expression of the micronuclei in different numbers (Fig.1) The distribution of the sample study with respect to age, smoking habits is given in the table (1). 90 individual participants in this study are divided into two groups according to their smoking habits and age, there were 46 smokers and 44 non smokers. The mean value of MN frequency in the youngest age group (20-30)years was \( (14.27±1.63) \), it was higher than the mean value of MN frequency in the oldest age group \( (9.75 ± 0.87) \), so the difference was statistically significant (\( P< 0.01 \)).

MN Frequency (mean ± SE) of smokers were averaged and found to be higher \( (12.89±1.85) \)as compared with nonsmokers \( (10.18 ± 1.07)( P<0.05) \)
Table (1): General characteristics of analyzing group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>MN/1000 cells Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 20-30</td>
<td>46</td>
<td>14.27±1.63</td>
</tr>
<tr>
<td>31-40</td>
<td>44</td>
<td>9.75±0.87**</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>46</td>
<td>12.89±1.85</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>44</td>
<td>10.18±1.07*</td>
</tr>
</tbody>
</table>

* P<0.05, **P<0.01

Figure (1) Photomicrographs of exfoliated buccal cells stained using Giemsa stain in micronucleus assay , A: cell with three micro nuclei ; B,C  cells with two micronuclei . All photos were taken at 400X magnification.

Table (2) provides data on the mean frequency of MN of smokers and nonsmokers according to age group . Smokers within the age group(20-30) showed a significant increase in the mean count of micronuclei (16.27 ± 2.73) compared with nonsmokers (12.36 ± 1.74) and when compared with smokers within the oldest age group (31-40). In contrast, the difference in MN frequency between smokers and nonsmokers was not statistically significant within the older age group (31-40).

Table (2): Comparison of micronuclei frequencies by stratification of sex and smoking status. All values are given as mean±SE.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N.</th>
<th>MN/1000 cells Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>Smoker 25</td>
<td>16.27±2.73</td>
</tr>
<tr>
<td></td>
<td>Nonsmoker 21</td>
<td>12.36±1.74</td>
</tr>
<tr>
<td>31-40</td>
<td>Smoker 21</td>
<td>9.50±1.16</td>
</tr>
<tr>
<td></td>
<td>Nonsmoker 23</td>
<td>8.00±0.96</td>
</tr>
</tbody>
</table>
4-DISCUSSION

The micronuclei were round or ovoid with the same color as the main nuclear (Fig. 1). Micronuclei arise from chromosome breakage due to unrepaired or mis-repaired DNA lesion, or chromosome malsegregation due to mitotic malfunction. Many events induced formation of micronuclei such as exposure to clastogenic or aneugenic, oxidative stress more over genetic defect in DNA repair gene and/or a cell cycle checkpoint and deficiencies in nutrient required as cofactor in chromosome segregation and DNA metabolism machinery.

Tobacco is the most extreme example of a systematic human mutagen. Our study demonstrates increased frequency of MN in buccal cells in smokers compared to nonsmokers. These results are in full agreement with other studies. Many studies have reported the positive relation between elevated frequency of micronuclei in the buccal cells and different forms of smoking and related agents such as cigarette, Betel nut and Quid and reverse smoking.

Tobacco smoke has been associated with the formation of micronuclei in different types of human and animal cells. The higher MN frequency in cigarette smokers has been attributed to benzo(a)pyrene, a component of tobacco smoke that consistently induced MN in a linear fashion, using an in vitro system. Other studies demonstrated that smoking induces oxidative DNA damage, most likely as a result of the upregulation of the primary defense antioxidative system and/or complementary DNA repair system.

Other researchers have reported the positive relation between micronuclei frequency and smoking intensity as well as smoking duration. We found the mean of MN frequency in human buccal cells was (10.18 ± 1.07; 12.89±1.85) in nonsmoker and smoker individuals respectively, which is substantially higher than the baseline of the micronuclei expression in the healthy population(0.5-2.5/1000 cells), it is also higher than the mean count reported by Saeed and Younis on an Iraqi population which was (2.36) micronucleus per 1000. the mean count of MN reported in the present study markedly is higher than those reported by other studies, Naderi et al. estimated frequency of cells with micronuclei to be 0.94 ± 0.94 in buccal cells of a control group in Iran and 2.01 ± 0.93 in smokers.

A wide variation in the level of micronuclei in the buccal cell has been reported, Zamani et al. found that 0.26% buccal cell contained MN in a control Turki population and this level
increased to 1.20% in smokers. Jvoti et al. reported that the level of MN in buccal cells of healthy individuals in Aligarh was 4.53±0.33 and in gutha user 17.4±0.94.

The age and sex of the subjects are being reported as the major contributors to differences in micronuclei frequency, in the present study all the samples were men. A possible influence of age on the MN frequency in buccal cells was tested in our study. The mean value of MN frequency in the youngest age group (20-30) years was (14.27±1.63) was significantly higher than the mean value of MN frequency in the oldest age group (9.75±0.87). The present study detected that there is no relation between age the increase of micronuclei mean count. This conforms to the findings of other studies the age of the individuals did not affect the number of micronuclei of both exposed and control individuals.

There are many hypotheses to explain the low frequency of micronuclei in oldest age groups. Firstly, micronuclei in buccal cell original from genome damage events in the basal layer of the oral mucosa, the number of micronuclei in buccal cells depend on the proportion of the cells that have divided more than one, individual genotoxic and cytotoxic response led to reducing the dividing rate of basal cells thus hinder micronuclei expression and observation. Secondly, the cell with DNA damage, induce some degree of the blockade of cell cycle in order to repair the damage and avoid the fixation of mutation during replication. It is important to refer to that in the current study measurement of DNA damage is restricted to micronuclei, non-dividing cell and the some of the damaged cells on the other hand may tend to undergo apoptosis or necrosis instead of dividing. Haveric et al. studied the effects of cigarette consumption on micronucleus frequencies exfoliated buccal cells of smokers. They observed the elevated frequency of apoptotic buccal cells in smokers and the frequency of apoptotic cells in this group was significantly influenced by the age of participants and duration of smoking.

Effect of age on the frequency of micronuclei in buccal cells has been evaluated in numerous studies and the results are controversial. Some authors were able to establish a statistically significant effect by age. However, other authors did not find an association between age and elevated frequency of micronuclei.

Based on these results it can conclude that the frequency of micronuclei in exfoliated buccal cells in smokers was significantly higher compared to non-smokers, also the mean count of MN of nonsmokers markedly higher than those reported by other studies, higher frequency
of micronuclei directly associated with decrease efficiency of DNA repair and increase of genomic instability. Micronucleus assay in the buccal cells is sensitive, practical, inexpensive method for monitoring genetic damage in human. Further researches are needed not only for count micronuclei but also to capture the frequency of apoptosis and necrosis cells to detect the further evaluation of cellular and tissue level biological damage.

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