Cigarette Smoking, Intracellular Vitamin Deficiency, and Occurrence of Micronuclei in Epithelial Cells of the Buccal Mucosa

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Abstract

The study focuses on the assessment of chromosomal damage associated with folate and vitamin B₁₂ deficiencies, and with cigarette smoking in a tissue directly exposed to cigarette smoke (buccal mucosa) while controlling for potential confounding factors. A cross-sectional study was carried out among 39 current smokers (CSs) and 60 noncurrent smokers (NCSs). Buccal mucosal cells, saliva, and blood samples were collected from each subject. The Health Habits and History Questionnaire (Block et al., 1986) was modified to obtain dietary and other relevant information. Methods used to measure folate, vitamin B₁₂ levels, and the frequency of micronucleated cells in buccal mucosal cells gave reproducible results. The study results suggest that CSs have buccal mucosal folate and vitamin B₁₂ levels that are lower than those among NCSs. CSs were three times more likely to have micronucleated buccal mucosal cells compared to NCSs. There appeared to be no association between low buccal mucosal folate and vitamin B₁₂ levels and chromosomal damage. The salivary vitamin B₁₂ concentrations and plasma vitamin C and E concentrations, however, seem to be marginally protective against the occurrence of buccal mucosal micronuclei, whereas plasma β-carotene seems to increase the occurrence of micronuclei. Overall, the results do not support the concept that localized folate and vitamin B₁₂ deficiencies in the buccal mucosal cells of smokers are associated with chromosomal damage in those cells. The presence of vitamin B₁₂ in the immediate environment (saliva) and vitamin C and E in the plasma, however, appear to be marginally protective against chromosomal damage in buccal mucosal cells.

Introduction

Several chemical components of cigarette smoke have been shown to interact in vitro with folate and vitamin B₁₂ coenzymes transforming them into biologically inactive compounds (1, 2). On the basis of these observations, Krumdieck (3) hypothesized that repeated exposure of the respiratory epithelium to cigarette smoke might result in localized folate and vitamin B₁₂ deficiencies.

Chromosomal damage to human cells in vivo as a result of folate or of vitamin B₁₂ deficiency is extensive and commonly observed. Investigators have demonstrated increased frequencies of chromosomal breakage in direct preparations of bone marrow from individuals with megaloblastic anemia caused by folate or vitamin B₁₂ deficiency (4–7) and after use of methotrexate, a folate antagonist (8). In addition, use of folate-deficient media for lymphocyte cultures greatly increases frequencies of chromosomal breakage in in vitro assays for the fragile X syndrome, rare (9) and common (10) fragile sites, and total aberrations (11).

The finding that micronuclei appear more often in individuals with fragile chromosomal sites than in individuals without them suggests that chromosomal fragments resulting from breakage at such sites are expressed as micronucleus formation (12). A marked effect of mild folate deficiency on the frequency of micronucleated erythrocytes in splenectomized subjects was first reported by Everson et al. (13). They observed minimal spontaneous micronucleated cell frequencies only when plasma folate levels exceeded 15–20 ng/ml. Within the range of 5–10 ng/ml (well above the lower limit of normal value, i.e., 3 ng/ml), the frequency of micronucleated cells was significantly elevated. Schreinemachers and Everson (14) reported an inverse relation between serum folate and the frequency of micronucleated RNA-positive red cells in seven subjects with minimal spleen function. This study suggests an association between levels of folate and chromosomal damage in vivo among individuals with presumably normal plasma folate levels. The authors suggest the need to study the effect of supplementary vitamin B₁₂, because vitamin B₁₂ and folate deficiency may have similar effects on micronuclei formation. Virtually nothing is known about the relationship between the cellular folate or vitamin B₁₂ content and the frequency of micronucleated cells of a tissue that are directly exposed to cigarette smoke and at “risk” for neoplastic transformation.

The cells of the buccal mucosa are directly exposed to cigarette smoke and are accessible for sample collection. Moreover, buccal mucosal cells can be used to estimate the frequency of micronuclei that indicates damage to the chromosomes. Also, it is possible to obtain an adequate sample of exfoliated buccal mucosal cells from human subjects (without causing discomfort) to measure total folate and vitamin B₁₂, as well as the frequency of cells with micronuclei. In a previous study, we demonstrated that smokers have significantly lower folate levels in the buccal mucosal cells compared to nonsmokers (15). In this study we assessed the relationship of low folate and vitamin B₁₂ status in the buccal mucosal cells with the

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occurrence of micronuclei as an indicator of chromosomal damage.

Materials and Methods
A cross-sectional study was carried out among the employees of the Baptist Medical Center at Birmingham, AL. CSs3 and NCSs between 30 and 60 years of age were recruited.

A cigarette smoker is defined as a CS with 10 or more pack-years (number of packs of cigarettes smoked/day multiplied by the number of years of smoking) of smoking. A NCS is defined as an adult who abstains from cigarette smoking, as well as from cigar and pipe smoking, or from using smokeless tobacco products. Subjects with a history of recent viral infections, use of antibiotics within 2 months before sample collection, or with premalignant oral lesions (e.g., leukoplakia) were excluded from the study. Also, subjects with a recent history of exposure to potential genotoxic agents, including X-rays, chemotherapy, and potential occupational exposures, were excluded. The study was approved by the Institutional Review Boards of the Baptist Medical Center and the University of Alabama at Birmingham.

The following samples were collected from each subject who participated in the study: (a) buccal mucosal cells were collected in 40 ml of HBSS, from which 15 ml each were used for folate and vitamin B12 assays; the remaining 10 ml were used to prepare cell smears for the micronucleus test; (b) a 10-ml sample of blood was drawn from each subject into EDTA vacutainers and was used for analysis of red cell folate, plasma folate, vitamin B12, β-carotene, and vitamins C, E, and A; (c) a saliva sample was taken to measure folate and vitamin B12 concentrations; (d) the Health Habits and History Questionnaire, which was designed to obtain information about dietary habits, alcohol ingestion, health history, and other important factors known to be associated with cancer risk, was modified and self-administered by both groups to ensure that the data concerning confounding or explanatory variables were available for analysis.

Determination of Folate and Vitamin B12 Concentrations in Buccal Mucosal Cells. Methods developed for the extraction and determination of buccal mucosal cell and salivary folate and vitamin B12 concentrations and their reproducibility have been published previously (15, 18, 19).

Preparation of Buccal Mucosal Cell Smears for Micronucleus Assay. One ml of Carnoy’s fixative (3:1 ratio of methanol and glacial acetic acid) was added to the pellet, and cells were gently suspended in it. After 1 h, the cell suspension was centrifuged, and the supernatant was discarded. The pellet was then gently resuspended in 500 μl of 100% methanol. After counting the number of cells/ml by using a Coulter Counter, six aliquots of the cell suspension (20 μl each) were dropped into 6 wells on hydrophobic printed slides (ER-202; Erie Scientific). The smears were air dried for 24 h.

Feulgen Staining Procedure. The cell smears were stained with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h. with the Feulgen reaction in the following way: the smears were air dried for 24 h.

Criteria for Scoring Classical Micronucleated Cells. The criteria for inclusion in the cells to be scanned for micronuclei were the following: (a) cytoplasm intact and lying relatively flat; (b) little or no overlap with adjacent cells; (c) little or no debris; and (d) nucleus normal and intact, nuclear perimeter smooth and distinct. Buccal mucosal cells that fulfill these criteria are shown in Fig. 1A.

The criteria for identifying micronuclei in buccal mucosal cells were as follows: (a) Feulgen positive and staining intensity similar to that of the nucleus; (b) less than one-third of the diameter of the associated nucleus but large enough to discern shape and color; (c) rounded, smooth perimeter suggesting the presence of a membrane; (d) texture similar to that of nucleus; and (e) location within 3 or 4 nuclear diameters of the nucleus and not touching it (20–22).

Before initiating the study, the above criteria were validated by collecting multiple buccal mucosal cell samples from five patients undergoing radiotherapy treatments at the Comprehensive Cancer Center of the University of Alabama at Birmingham. Eligible subjects were patients who had received a cumulative dose of at least 1000 rad to an area including buccal epithelium in the previous 2–3 weeks and who had been diagnosed as having a condition other than oral squamous cell carcinoma. In addition, demonstration slides for micronucleated cells were obtained from the M. D. Anderson Cancer Center, The University of Texas (Houston, TX) and studied carefully.

Cells with more than two micronuclei were classified as positive for micronuclei, provided that the main nucleus was more or less round rather than deeply lobed. Micronuclei attached to the main nucleus with a Feulgen-positive band were classified as positive for micronuclei if the size was less than one-third of the diameter of the main nucleus and if they were not in contact with the main nucleus and had all the other features of classical micronuclei. Some of the classical micronucleated cells observed in this study are illustrated in Fig. 2. Fig. 2, A–C, shows buccal mucosal cells with a single micronucleus but different sizes. Fig. 2, D and E, shows buccal mucosal cells with two micronuclei, and Fig. 2F shows a buccal mucosal cell with multiple micronuclei.

All the slides were read under fluorescent light at ×400 by a single investigator in a double-blind procedure. Each well (6 wells/subject) was scanned systematically until a high-certainty micronucleated cell could be found. At that point, the well was classified as positive for micronuclei, and scanning of the well was stopped. To classify a subject as negative for micronuclei, the cells in all six wells were read. Although infrequent, the following nuclear anomalies were also noted: pyknosis, or shrunk nuclei; condensed chromatin; and karyolysis, or nuclear dissolution, in which a Feulgen-negative, ghost-like image of the nucleus remains. Binucleated cells and cells with degenerated nuclei were fairly frequent (Fig. 1, B and C, respectively).

To determine the intra-examiner variability in scoring for
Feulgen staining with naphthal yellow cytoplasmic staining under fluorescence microscopy.

Fig. 1. Photographs of exfoliated human buccal mucosal cells. A, normal cells, × 400; B, a normal cell and a binuclear cell, × 400; C, a cell with degenerated nucleus, × 1000.

Data Analysis. Simple descriptive statistics (average, median, and percentage), scatterplots, and histograms were used to evaluate the distribution of the study subjects according to six sets of variables. Sociodemographic variables included race, gender, age, and education (years in school). Smoking variables...
Fig. 2. Photographs of exfoliated buccal mucosal cells with micronuclei. × 400: A-C, cell with single micronuclei, different sizes; D and E, cell with two micronuclei; F, cell with multiple micronuclei (Feulgen staining with naphthol yellow cytoplasmic staining under fluorescence microscopy).

included smoking status, number of pack-years of cigarettes smoked (i.e., the number of years as a smoker multiplied by the average number of packs smoked/day), and the number of cigarettes smoked/day (for CSs). Dietary intake variables, as estimated from the food frequency questionnaire, included the average daily intake of total calories; vitamins A, C, and E; total carotenoids; folate; calcium; intake of vitamin supplements, alcohol, and caffeine; and number of servings of food rich in vitamin B12. Systemic vitamin levels included plasma vitamins B12, C, E, and folate, β-carotene, and RBC folates. Local
vitamin levels included folate and B<sub>12</sub> levels in saliva and in buccal mucosa cells.

Summary statistics for all the variables listed above were computed separately for CSs and for NCSs. Simple correlation analyses were conducted to evaluate the association between pairs of variables. Nonparametric statistics (e.g., Spearman’s rank correlation coefficient and Wilcoxon’s rank-sum test) were used to evaluate the significance of the associations.

A binary indicator of the presence of positive wells (i.e., wells with at least one cell with micronuclei) was chosen as the dependent variable in the analyses. The measure of association used in this analysis was the OR. To compute the OR for exposure to categories of a risk factor, subjects with positive wells (“positive” subjects) and subjects with negative wells (“negative” subjects) were grouped by categories of the factor, one category of the factor was chosen as reference (“nonexposed”), and the positive/negative odds in each exposed category was divided by the corresponding odds in the nonexposed category. Each of the resulting ORs estimated the ratio of two prevalence rates.

Each risk factor was evaluated one at a time by computing unadjusted ORs. Unconditional logistic regression models were used to evaluate the effect of several risk factors simultaneously and to evaluate the interactions between factors. Nutritional indicators were evaluated both as continuous variables (e.g., plasma folate in ng/ml) and as categorical variables (e.g., tertiles of the distribution of plasma folate). To find an efficient categorization for a continuous variable, we examined its distribution in the micronuclei-negative group and identified cutpoints that would assign approximately equal numbers of micronuclei negative subjects to each category. Next, all subjects were categorized using the same cutpoints. Because of the limited study size, usually tertiles were used. To evaluate the overall strength of an association, we assigned equally spaced scores to the categories of the risk factor and tested the significance of the score considered as a continuous variable in a logistic regression model. CIs for an OR were computed assuming that the log-OR is a normal deviate with mean and SD equal to the logistic regression coefficient and its standard error estimate, respectively. To compute the 95% CI of category-specific ORs for variables with more than two categories, appropriate binary variables replaced the grouped continuous scores in the model (although keeping all other variables as grouped continuous scores).

A modified backward elimination procedure was used to select the most important predictors of outcome. Gender, race, age (categories), and sample cell counts (categories) were retained in all models regardless of the size and statistical significance of their effect, because they were a priori considered important. Other variables were excluded from the regression model if they made a clearly nonsignificant contribution to the model (P > 0.2) and did not appear to act as confounders. The Statistical Analysis System for Personal Computers was used in all data analyses.

Results

There were no significant differences in the distribution by age or years of education between the two subgroups. However, the majority (80%) of the NCSs were females. The proportion of blacks among CSs (1:3) was larger than among NCSs (1:6). CSs had an average of 20 pack-years of smoking (Table 1).

Plasma, RBC, and buccal mucosal folate levels were significantly lower among CSs compared to NCSs (Table 2). Univariate analysis of local and systemic vitamin B<sub>12</sub> levels showed significantly lower buccal mucosal vitamin B<sub>12</sub> levels among CSs (Table 3).

The distribution of CSs and NCSs according to the number of wells positive for micronucleated cells is given in Fig. 3. The frequency distribution of subjects with 0 and 1 well positive for micronucleated cells among CSs and NCSs is shown in Fig. 4. The difference was not statistically significant. The logistic regression model used to estimate the associations between selected variables and micronucleated cells is presented in Table 4. After adjusting for race, age, smoking status, cell count, and levels of selected vitamins, females were more likely to have micronuclei than males (95% CI = 0.8–9.4). The occurrence of micronucleated cells seems to be positively associated with age. Micronucleated cells occurred twice as often among whites compared to blacks. After adjusting for other variables, micronucleated cells were considerably more frequent among CSs than among NCSs (OR = 3.2; 95% CI = 0.9–11). Of the local vitamins, only salivary vitamin B<sub>12</sub> showed a marginally significant protective effect (OR = 0.6; 95% CI = 0.3–1.1). Among systemic vitamins, plasma vitamins C and E showed marginally significant protective effects (OR for one category increase in vitamin C = 0.6; 95% CI = 0.3–1.1; OR for one category increase in vitamin E = 0.5, 95% CI = 0.2–1.0). Plasma β-carotene seems to be positively associated with the occurrence of micronucleated buccal mucosal cells (OR for one category increase = 2.0, 95% CI = 1.0–4.1). None of the dietary intake variables were associated with the presence of micronuclei in buccal mucosal cells (results not presented in detail).

Discussion

The purpose of the present study was to investigate the relationship among low levels of folate and vitamin B<sub>12</sub>, cigarette smoking, and chromosomal damage as indicated by the presence of micronuclei in buccal mucosal cells. The methods used for all three measurements, folate/vitamin B<sub>12</sub> levels, and the occurrence of micronuclei in buccal mucosal cells gave reproducible results. Because buccal mucosal cell turnover is rela-

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**Table 1** Characteristics of the study group according to smoking status

<table>
<thead>
<tr>
<th>Variable</th>
<th>CSs (n = 39)</th>
<th>NCSs (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33-39</td>
<td>12 20</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>20 12</td>
</tr>
<tr>
<td>Race</td>
<td>Black</td>
<td>13 11</td>
</tr>
<tr>
<td>Pack-yr</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.8</td>
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</tbody>
</table>
Table 2  Plasma, RBC, buccal mucosal, and salivary levels of folate among CSs and NCSs

<table>
<thead>
<tr>
<th>Folate level/unit</th>
<th>CSs (n = 39)</th>
<th>NCSs (n = 60)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>Plasma (ng/ml)</td>
<td>5.12</td>
<td>4.06</td>
<td>8.21</td>
</tr>
<tr>
<td>RBC (ng/ml)</td>
<td>314.95</td>
<td>261.59</td>
<td>449.30</td>
</tr>
<tr>
<td>Buccal mucosa (ng/mg protein)</td>
<td>305.76</td>
<td>279.87</td>
<td>445.83</td>
</tr>
<tr>
<td>Saliva (ng/mg protein)</td>
<td>36.93</td>
<td>24.00</td>
<td>43.79</td>
</tr>
</tbody>
</table>

* Wilcoxon rank-sum test.

Table 3  Plasma, buccal mucosal, and salivary levels of vitamin B12 among CSs and NCSs

<table>
<thead>
<tr>
<th>Variable/unit</th>
<th>CSs (n = 39)</th>
<th>NCSs (n = 60)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>Plasma (pg/ml)</td>
<td>569.2</td>
<td>525.5</td>
<td>547.8</td>
</tr>
<tr>
<td>Saliva (pg/mg protein)</td>
<td>3220.4</td>
<td>2912.8</td>
<td>2778.7</td>
</tr>
<tr>
<td>Buccal mucosa (pg/mg protein)</td>
<td>1090.1</td>
<td>599.4</td>
<td>1155.2</td>
</tr>
</tbody>
</table>

* Wilcoxon rank-sum test.

Although the buccal mucosal micronucleus test has been applied by other investigators to assess the genotoxic action of several compounds in cigarette smoke and alcohol (20), betel quid (23–25), and curried food (26), none of these studies have investigated the association between the concentration of a chemopreventive agent and the prevalence of micronuclei in the same tissue. Considering that initiation and promotion events in carcinogenesis occur at the cellular level, the availability of chemopreventive agents within cells or in the microenvironment of cells, as opposed to their presence in the serum, should play a dominant role. Also, it seemed important to know the protective functions of the physiological levels of chemopreventive agents found in cells as opposed to cellular vitamin concentrations after vitamin supplementation. To answer these questions, it is necessary to measure the level of chemopreventive agent and the frequency of micronucleated cells in the same tissue. We attempted to answer these questions by measuring folate and vitamin B12 levels and the frequency of micronucleated cells in buccal mucosal tissue.

We have identified age, gender, and race to be important variables affecting the frequency of micronuclei. The effect of age and gender has been confirmed by other laboratories (27–29). Our results showed that smokers are three times more likely to have micronucleated buccal mucosal cells. Smokers also showed lower buccal mucosal folate and vitamin B12 levels compared to nonsmokers. The study did not show, however, any significant independent association between low buccal mucosal folate and vitamin B12 levels and a higher frequency of micronucleated buccal mucosal cells. Therefore, our results suggest that cellular folate and vitamin B12 levels are not associated with chromosomal damage in a tissue directly exposed to cigarette smoke. However, one could speculate several possible reasons for this study to have produced "false-negative" observations: (a) the systemic folate and vitamin B12 status observed in our population are within normal range and are not suggestive of folate or vitamin B12 deficiency in tissues.

Fig. 1  Distribution of 0–5 positive wells for micronuclei separately among CSs and NCSs.

tively rapid, this tissue was particularly well suited for studying the relationship among the parameters we were interested in. Buccal mucosa is directly exposed to cigarette smoke. In addition, an adequate number of cells for all three assays can be collected by using a noninvasive, inexpensive method.
which consists of basal cells. Existing methods do not allow for detecting when comparing pools of cells, a small proportion of difference would become insignificant on would be difficult to measure vitamin levels in cells from specific layers and to estimating chromosomal abnormalities and subjects who don't, such a technique to measure folate/vitamin B12 levels at the cellular level may require the development of immunohistochemical techniques to measure folate/vitamin B12 levels at the cellular level.

In the present study, micronuclei were used as an indicator of chromosomal damage. Micronuclei are small, round, and often binucleate structures that result from cell division in which DNA is not replicated. They are considered an indicator of chromosomal damage because they are formed during mitosis, when DNA is not replicated, and are released from the nucleus during cell division. The presence of micronuclei has been associated with a variety of genetic and environmental factors, including smoking, alcohol consumption, and exposure to environmental toxins.

A limitation in this study is its cross-sectional design, in which outcomes and risk factors are ascertained concurrently. Stronger evidence would be provided by a prospective design in which information on the risk factors is gathered before the outcomes and, therefore, cannot be influenced by the outcomes. The information collected retrospectively in this study, however, reflects the desired temporal sequence. Information on dietary intake collected by using the food-frequency questionnaire is expected to reflect intake over a time period that is relevant to changes in current vitamin intake. Information on sociodemographic variables and smoking habits also reflects a prolonged experience in the past, and it is likely to be relevant for the associations of interest. The turnover of buccal mucosa is relatively fast, and the outcome observed is likely to reflect recent exposure to genotoxic agents. Insofar as systemic and local vitamin concentrations reflect the relevant recent history of vitamin levels, it also is reasonable to consider the cross-sectional analysis a valid assessment of the desired short-term antecedent-consequent sequence. Thus, we conclude that there are no serious threats to the validity of the study results derived from its cross-sectional design, although precision is limited by the small size of the study group and by the imbalance in the distribution of subjects by categories of important risk factors.

Acknowledgments

The technical assistance of Edward Phillips is greatly appreciated.

**Table 4** Association between micronucleated buccal mucosal cells and selected characteristics in multiple regression models

<table>
<thead>
<tr>
<th>Variable</th>
<th>unit</th>
<th>OR (OR)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: female vs. male</td>
<td></td>
<td>2.8 (1.6)</td>
<td>0.8-9.4</td>
</tr>
<tr>
<td>Race: white vs. black</td>
<td></td>
<td>2.1 (1.1)</td>
<td>0.6-4.2</td>
</tr>
<tr>
<td>Age: 1 category increase</td>
<td></td>
<td>1.6 (1.2)</td>
<td>0.8-3.3</td>
</tr>
<tr>
<td>Cell count (no/mL)</td>
<td></td>
<td>1.4 (1.2)</td>
<td>1.1-1.8</td>
</tr>
<tr>
<td>Cigarette smoking: CS vs. NC</td>
<td></td>
<td>3.2 (1.2)</td>
<td>0.9-11.0</td>
</tr>
<tr>
<td>BMfolate: 1 category increase</td>
<td></td>
<td>1.3 (1.2)</td>
<td>0.7-2.6</td>
</tr>
<tr>
<td>Salivary vitamin B12</td>
<td></td>
<td>0.6 (0.6)</td>
<td>0.3-1.1</td>
</tr>
<tr>
<td>Plasma vitamin C: (1 category increase)</td>
<td></td>
<td>0.6 (0.8)</td>
<td>0.3-1.1</td>
</tr>
<tr>
<td>Plasma β-carotene</td>
<td></td>
<td>2.0 (1.5)</td>
<td>1.0-4.1</td>
</tr>
<tr>
<td>Plasma vitamin E</td>
<td></td>
<td>0.5 (0.8)</td>
<td>0.2-1.0</td>
</tr>
</tbody>
</table>

* ≥1 well positive for micronuclei vs. 0 well positive for micronuclei.

**Fig. 4.** Distribution of 0 and ≥1 wells positive for micronuclei separately among CSs and NCSs.

effects of systemic vitamins C and E with the occurrence of micronuclei in buccal mucosal cells. Finally, our data suggest that an important consideration should be given to the role of β-carotene in facilitating spontaneous genetic damage in humans.

The protective effect of salivary vitamin B12 is comparable with the results of other investigators who reported a significant negative correlation of plasma vitamin B12 with the presence of micronuclei in human lymphocytes (30, 31). In contrast to their results, however, we report borderline significant protective
References
Cigarette smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial cells of the buccal mucosa.

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