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Cytogenetic analysis of gingival epithelial cells, as related to smoking habits and occurrence of periodontal disease

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ABSTRACT

Periodontal disease, progressing from gingivitis to periodontitis, affects the majority of the world population. Its pathogenesis is related to a complex interaction between environmental, microbial, genetic and other host factors, tobacco smoking being the most important environmental risk factor. Conflicting results are reported in the literature regarding the effects of smoking habits on cytogenetic damage in exfoliated oral cells. We report herein the results of a study evaluating, for the first time, the frequency of micronucleated and binucleated cells in the gingival epithelium. There was no significant elevation of these cytogenetic end-points in 43 subjects as related to smoking habits (never-smokers, ex-smokers, and current smokers) and periodontal disease (mild, moderate, or severe forms of gingivitis and periodontitis). Therefore, the overall data emerging from the present study do not support the evidence for an association between smoking habits, periodontal disease and genotoxic damage in gingival epithelial cells.

Introduction

Periodontal disease is a chronic, destructive condition, characterized by a bacterial infection which results in inflammation of the gums leading to the gradual destruction of periodontal tissues and alveolar bone supporting the teeth. Therefore, it is one of the major causes of tooth loss in adults (Papapanou, 1996). The term periodontal disease usually refers to the common inflammatory disorders of gingivitis (the mildest form, readily reversible), which may progress to periodontitis (the most severe form). The prevalence of periodontal disease varies worldwide, but it is estimated that it affects the 50–90% of the adult world population (Albandar and Rams, 2002). One large survey estimated that about 22% of US adults had mild disease and 13% had moderate or severe disease (Albandar et al., 1999). Thus, periodontal disease represents a significant public health problem.

The etiopathogenesis of periodontal disease could be described as a complex interaction of environmental, microbial, genetic and other host factors, which are responsible for the interindividual variability in susceptibility (Baldi et al., 2009). Tobacco smoking is recognized as the most important environmental risk factor for periodontal disease. It exerts several detrimental effects on the oral environment, the gingival tissues and vasculature, and it affects the inflammatory and immune responses, homeostasis, and healing potential of the periodontal connective tissues (Palmer et al., 2005).

Cytogenetic analyses are widely used to detect early biological damage in many pathological conditions. Among all cytogenetic assays, the micronucleus (MN) test, due to its reliability and simplicity, has become the most popular method for detecting both structural and numerical chromosome aberrations (Fenech et al., 2011). This test has extensively been used for biomonitoring of subjects exposed to environmental risk factors, also including smoking habits. It is noteworthy, however, that negative results were reported in the majority of population studies that used the MN test by using peripheral blood lymphocytes of smokers (Bonassi et al., 2003). Exfoliated mucosal cells of the oral cavity have been tested for MN frequency as related to smoking, with conflicting results (Stich and Rosin, 1983; Sarto et al., 1987; Piyathilake et al., 1995; Wu et al., 2004; Liou et al., 2005; Bohrer et al., 2005; Thomas et al., 2009; Nersesyan et al., 2011).

The aim of the present study was to investigate, for the first time, the induction of micronucleated cells (MNC) and binucleated cells (BNC) in the gingival epithelium. Gingival mucosal cells are nonkeratinized epithelial cells, characterized by a high turnover rate, which have previously been used for evaluating molecular and

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biochemical alterations occurring in gingivitis (Davis and Gibbons, 1990) and periodontitis (Ansai et al., 2002; Çanakçi et al., 2006; Matsuyama et al., 2008). Our results do not show any significant association between cytogenetical damage in these cells, smoking habits, and occurrence of gingivitis or periodontal disease.

Materials and methods

Selection and clinical examination of subjects

Forty-three subjects were recruited amongst adult patients attending a dental examination. All subjects voluntarily agreed to participate in this study, and all procedures were performed in compliance with institutional guidelines. A questionnaire was submitted to every subject in order to collect information about age, gender, smoking, drinking and dietary habits, as well as therapeutic drug intake. The privacy of participating subjects was warranted by their identification with a code number.

A complete inspection of the oral cavity was performed by a dentist and the presence of oral pathologies was recorded. According to their clinical features, periodontitis and gingivitis were classified as mild, moderate, and severe.

Cell collection, preparation of slides and cytogenetic analyses

Gingival epithelial cells were collected through a noninvasive approach, by scraping the gingival sulcus with a Heidemann spatula. Cells were immediately smeared onto cleaned microscope slides. Within 24 h after sampling, all slides were fixed in methanol/glacial acetic acid (3:1) and then air-dried. For cytogenetic analysis, cell smears were stained with the Feulgen reaction according to the following procedure: slides were pretreated in 1 N HCl for 2 min at room temperature, placed for 6 min into 1 N HCL at 60 °C, transferred into 1 N HCl for 2 min at room temperature, rinsed twice in distilled water, placed into Schiff reagent for 90 min and washed with three changes (2 min each) of freshly prepared sulfite solution followed by two rinses in running water. The slides were counterstained for 10 s with 2.5% fast green dissolved in 95% ethanol and then mounted with Eukitt. Gingival epithelial cells were scored through a microscope at a final magnification of 1000×. MNC were evaluated according to the criteria described by Stich et al. (1982). One thousand cells from each subject were examined and the frequencies of MNC and BNC were recorded.

Statistical analysis

All data were expressed as mean values ± SE (standard error) and significant differences among groups were evaluated by ANOVA followed by Student's *t*-test for individual comparisons.

Results

Table 1 summarizes the frequencies of MNC and BNC in each one of the 43 examined subjects, together with their main anamnestic data. No significant relationship was detected between age, drinking and dietary habits, drug intake, and frequency of MNC and BNC in gingival epithelial cells (data not shown).

Tables 2 and 3 show the mean frequencies of MNC and BNC, respectively, in each group of subjects as related to gender, smoking habits and oral pathology. The mean frequency of MNC in all examined subjects was 0.61 ± 0.10. Slight increases were observed in the frequencies of MN cells of total current smokers, male current smokers and female current smokers, as compared with total never-smokers, total ex-smokers, male ex-smokers, female never-smokers, and female ex-smokers, respectively. A higher frequency of MN cells was recorded in periodontitis-positive females as compared with periodontitis-negative females, while a higher frequency of MN cells was observed in total and male gingivitis-positive subjects, as compared with total and male gingivitis-negative subjects, respectively. On the other hand, the males suffering from periodontitis had a lower frequency of MN cells as compared with healthy subjects. However, none of the observed differences was statistically significant.

The mean frequency of BNC in all examined subjects was 1.37 ± 0.13. No induction of BNC was observed in the gingival epithelium of current smokers, as compared with never-smokers or ex-smokers. A higher frequency of BN cells was observed in female and total periodontitis-positive subjects, as compared with female and total periodontitis-negative subjects. The frequency of BNC was higher in total, male and female gingivitis-positive subjects, as compared with total, male and female gingivitis-negative subjects. The mean frequency of MNC was higher in total periodontitis-positive current smokers (0.50 ± 0.19), as compared with total periodontitis-positive ex-smokers and never-smokers (0.33 ± 0.33), and in total gingivitis-positive current smokers (0.82 ± 0.23), as compared to total gingivitis-positive ex-smokers and never-smokers (0.50 ± 0.27). Likewise, the mean frequency of BNC was higher in total periodontitis-positive current smokers (1.63 ± 0.32), as compared to total periodontitis-positive ex-smokers and never-smokers (1.33 ± 0.33), and in total gingivitis-positive current smokers (1.70 ± 0.26), as compared to total gingivitis-positive ex-smokers and never-smokers (1.25 ± 0.36). Again, none of these differences was statistically significant.

Discussion

As extensively discussed in a recent review article (Baldi et al., 2009), genotoxic damage and gene–environment interactions play a key role in the pathogenesis of degenerative periodontal diseases. In particular, tobacco smoke, which is known to induce a variety of genetic alterations in different organs (Phillips, 2002; DeMarini, 2004), has been indicated as the major risk factor for periodontitis in a large number of studies (Haber et al., 1993; Barbour et al., 1997; Gelskey, 1999; Kinane and Cestnutt, 2000; Mullally, 2004; Palmer et al., 2005).

In the present study we investigated whether cytogenetic damage in gingival epithelial cells could be related to the pathogenesis of periodontal disease in association with tobacco smoke exposure. Oral mucosal cells have extensively been used for exploring the induction of micronuclei by genotoxic agents. In particular, several authors investigated the role of tobacco smoking on induction of MNC in the buccal mucosa. However, the results of these studies are rather controversial. Stich and Rosin (1983) demonstrated that the frequency of MN in the exfoliated buccal mucosal cells of heavy smokers was the same as in never-smokers. On the contrary, Sarto et al. (1987) and Piyathilake et al. (1995) reported that the frequency of MN was approximately twice as high in smokers compared with never-smokers. Another study (Konopka et al., 2006) showed that the frequency of MNC was three times greater in smokers than in never-smokers. Suhas et al. (2004) showed an increased MN frequency in smokers of bidi, an indigenous cigarette containing low-grade tobacco. Wu et al. (2004) found that cigarette smoking did not increase the frequency of MN in the whole group of smokers, as compared with nonsmoking persons. In another study, a statistically significant positive relationship was only found in heavy smokers (Liou et al., 2005), whereas Bohrer et al. (2005) reported that there was no significant increase of MNC in the oral mucosa of tobacco users, and tobacco/alcohol users as compared to controls. When comparing different types of cigarettes, the MN