Extended Abstract

Assessment of Genetic Damage Induced by Exposure to Ionizing Radiation Using Micronuclei Assay in Oral Mucosa Cells

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Abstract - Orthopantomography, using low doses of ionizing radiation, is a frequently employed radiological examination being essential for the detection and evaluation of oral pathologies. The evaluation of micronuclei formation in the oral mucosa exfoliated cells is a non-invasive procedure that can give an idea of the damage caused by ionizing radiation, since they are biomarkers of genotoxic events and chromosomal instability. To achieve this goal, we have implemented and validated the technique to evaluate micronuclei formation in oral mucosa exfoliated cells in laboratory. Micronuclei are originated from acentric chromosome fragments, acentric chromatid fragments or whole chromosomes that failed to be included in the daughter nuclei. This study focused on the analysis of the micronuclei frequency in oral mucosa cells of 60 individuals, from both genders, in which 20 individuals are controls (CG), 20 were submitted to orthopantomography (EG) exam at the Centro Hospitalar de Lisboa Central - Hospital São José in Lisbon and 20 are smokers (SG). Statistically significant differences were observed (p=0.028) between micronuclei yields obtained before (0.004 ± 0.003) and 10 days after (0.005 ± 0.002) the orthopantomography, indicating that the exposure to ionizing radiation due to the exam induce chromosomal damage. In SG there was an increase in micronuclei frequency (0.005 ± 0.004) compared with the CG (0.002 ± 0.001), being this difference statistically significant (p=0.001). The micronuclei assay was successfully implemented in our laboratory for oral mucosa cells, allowing the assessment of the damage induced by exposure to ionizing radiation in orthopantomographies.

Keywords: Ionizing radiation, Orthopantomography, Buccal mucosa cells, Micronuclei assay.

Introduction

Orthopantomography is one of the most valuable diagnostic tools used in dental care, planning and control of orthodontic treatment [1, 2, 3, 4]. Orthopantomography uses low doses of ionizing radiation and is a frequently employed radiological examination being essential for the detection and evaluation of oral pathologies.

Many chemical, physical and biological agents are able to interact with DNA inducing mutations. X-rays are a potent mutagenic agent, able to induce both gene mutations and chromosomal aberrations [3].

The carcinogenic effect of cigarette smoke is driven largely by the mutagenicity of various chemicals in the smoke [5]. Tobacco products contain diverse chemicals, including nicotine and carcinogens. The combination of nicotine and these carcinogens is responsible for millions of preventable deaths worldwide [6]. The oral evaluation of the genotoxic effects (DNA damage) in smokers, on buccal mucosa cells, can be assessed by micronuclei assay [7].

The evaluation of micronuclei formation in the oral mucosa exfoliated cells is a non-invasive

procedure that can give an idea of the genetic damage, since they are biomarkers of genotoxic events and chromosomal instability. Micronuclei are small round extranuclear bodies originated from chromosome fragments or whole chromosomes that lag behind during nuclear division and are not included in the daughter nuclei [8]. In oral mucosa cells, the damage that leads to the formation of micronuclei takes place in the basal layer of the epithelial tissue where cells undergo mitosis. The turnover of epithelial tissues brings the cells to the surface where they are exfoliated, being the maximal rate of micronuclei formation seen 1 to 3 weeks after the exposure to the genotoxic agent [1].

The present study was undertaken to investigate the frequency of micronuclei in mononucleated cells in oral mucosa from individuals submitted to orthopantomography and in smokers.

Material and Methods

Subjects

This study comprised a total of 60 individuals (29 men and 31 women) aged between 6 - 86 years, in which 20 individuals are control (CG), 20 were submitted to orthopantomography at the Hospital de São José in Lisbon (EG) and 20 are smokers (SG), as summarized in Table 1.

Table 1- Sample characterization

Group	Number		Age years (Mean ± SD)
	M	F	
CG	9	11	39.0 ± 21.7
EG	8	12	43.9 ± 18.6
SG	12	8	42.9 ± 11.4

CG - control group; EG - exposed group; SG - smokers group; M - male; F - female; SD - standard deviation.

The EG was submitted to an orthopantomography at the Stomatology Service of the Centro Hospitalar de Lisboa Central – Hospital de São José in Lisbon. Only healthier individuals without any exposure to ionizing radiation for at least the last 6 months were included in the study. Oral mucosa exfoliated cells were collected immediately before the exam and approximately 10 days after. The orthopantomography was requested by the physician and were performed using Instrumentarium Dental, Orthopantomograph® OP200 with the following settings: 66-77 kV, 8.0-16 mA, 17.6 s (depending on the face patient structure) with an output dose between 7.2 - 35.4 μSv. The study was approved by the hospital ethics committee.

In the CG and SG, the oral mucosa cells were collected from individuals without any exposure to ionizing radiation for at least the last 6 months and the CG had no smoking habits.

All individuals signed an informed consent and filled up a questionnaire before the collection of samples. Individual characteristics of the subjects were recorded, including health status, sex, age, previous exposure to diagnostic X-ray, medication, alcohol consumption and exposure to genotoxic agents that might interfere in the results obtained.

Micronuclei test in oral mucosa cells

After the subjects rinsed the mouth with water, cells were obtained by scraping the mucosa of the right and left cheeks with a plastic spatula. The biological material was placed in 5 mL of phosphate buffer solution pH 7.4, washed twice, centrifuged (104x*g*) for 5 minutes, fixed twice in cold 3:1 methanol: acetic acid and dropped onto pre-cleaned slides. The air-dried slides were stained during 10 minutes with 10% Giemsa solution in phosphate buffer pH 6.8. The frequency of micronuclei in buccal cells was evaluated by scoring 2000 mononucleated cells per individual for each sampling time (in EG the samples were scored before and 10 days after X-ray exposure). The samples were examined under a light microscope at 400x magnification. The number of binucleated cells (BN) was also recorded for each sample.

Statistical analysis

For the statistical treatment of the results as dependent variables were considered the frequency of mononucleated cells with micronuclei and the frequency of binucleated cells (BN). The independent variables were the ionizing radiation exposure from the orthopantomography, age, gender and smoking habits. The comparison of micronuclei frequency in all groups studied was done using the parametric Student's t-test. The level of statistical significance was $p<0.05$.

Results

During this study were scored 160000 mononucleated cells in the different groups (Figures 1 (b) and 2). In EG were observed 40000 mononucleated cells before the orthopantomography and 40000 after. It was also observed 40000 mononucleated cells in SG and 40000 in the CG.

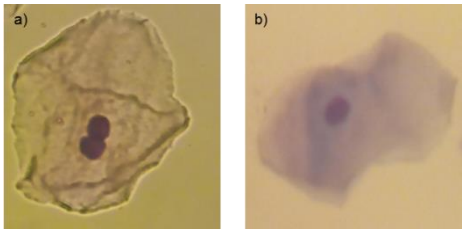


Figure 1 - Oral mucosal cells: a) binucleated cell; b) mononucleated cell.

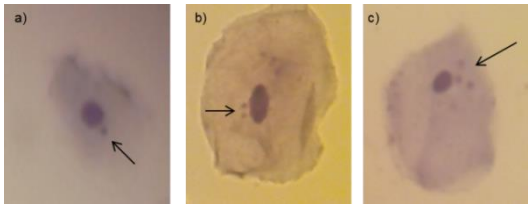


Figure 2 - Oral mucosa cells: a) cell with 1 MN; b) cell with 2 MN; c) cell with 3 MN. Micronuclei (MN) is identified by black arrows.

The number of micronuclei found in the CG were 77, in the EG to ionizing radiation before exposure were observed 159 micronuclei and 193 after exposure, in the SG were observed 213 micronuclei.

Exposure to ionizing radiation

The frequency of micronuclei in mononucleated cells in patients undergoing orthopantomography can be seen in Table 2. After X-ray exposure, the mean frequency of micronuclei in mononucleated cells was 0.09% higher when compared with the samples collected before the exam. The difference obtained was statistically significant (p=0.028).

Table 2 - Frequency of micronuclei in mononucleated cells in the individuals submitted to orthopantomography.

Exposed Group	Mean (MN/Cell) ± SD
Before X-ray exposure	0.004 ± 0.003
After X-ray exposure	0.005 ± 0.002*

MN - micronuclei; SD - standard deviation;
* - statistically significant difference.

Concerning genders the frequency of micronuclei in EG was higher for females (Figure 3), in both samples, collected before and after the orthopantomography. In both genders was observed an increase in micronuclei frequency after the exam.

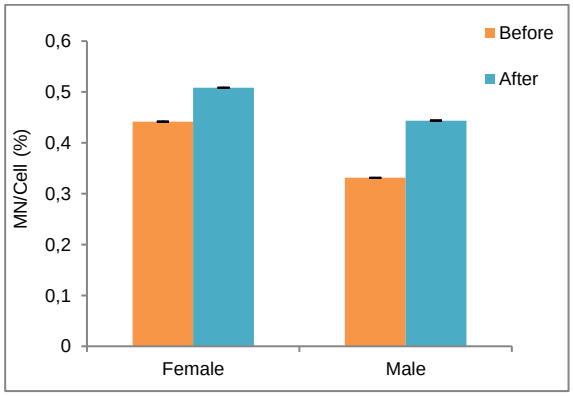


Figure 3 - Comparison between gender before and after the orthopantomography. MN - micronuclei.

Concerning age, all groups, with the exception for the 30 - 44 years group, have an increase in micronuclei frequency after the exam (Figure 4).

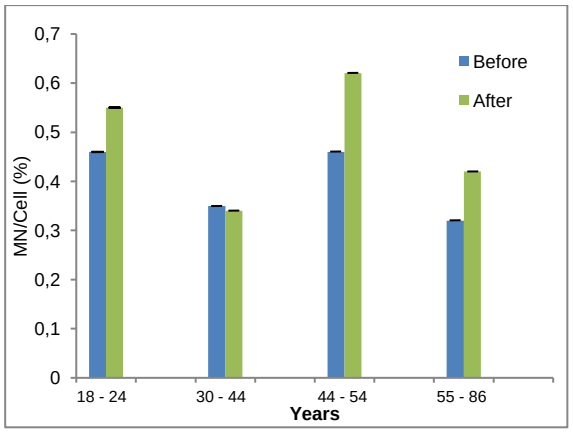


Figure 4 - Comparison between ages before and after the orthopanthomography. MN - micronuclei.

Smokers and control groups

Statistically significant differences were found between SG and CG (p=0.001). Table 3 shows the frequency of micronuclei in mononucleated cells in CG and SG group.

Table 3 - Frequency of micronuclei in mononucleated cells in the control group and in the smokers group.

Group	Mean (MN/Cell) ± SD
CG	0.002 ± 0.001
SG	0.005 ± 0.004*

CG - control group; SG - smokers group; MN - micronuclei; SD - standard deviation; * - statistically significant difference.

The males in CG have a micronuclei frequency higher than females, while in the SG the micronuclei frequency is identical between males and females (Figure 5).

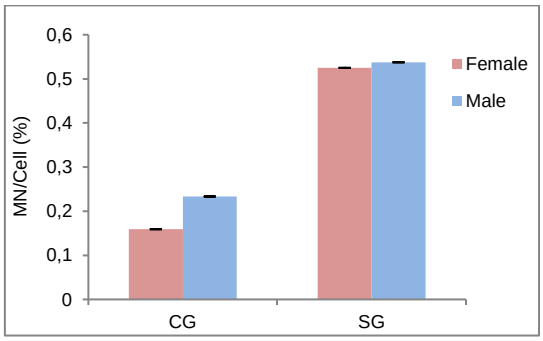
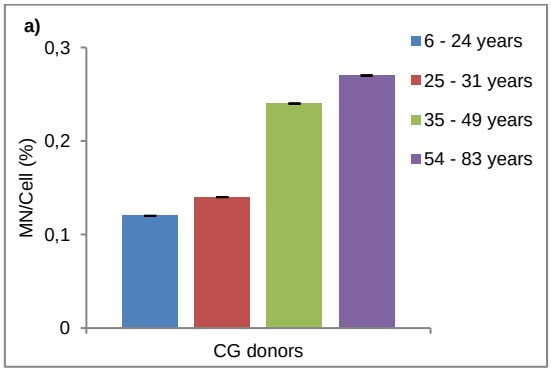


Figure 5 - Frequency of micronuclei by gender of the control group (CG) and smokers group (SG). MN - micronuclei.

Concerning the age in CG, there is an increase of the micronuclei frequency with age, while in the SG we cannot observe a relationship between age and the increase of micronuclei frequency (Figure 6 (a) and (b)).



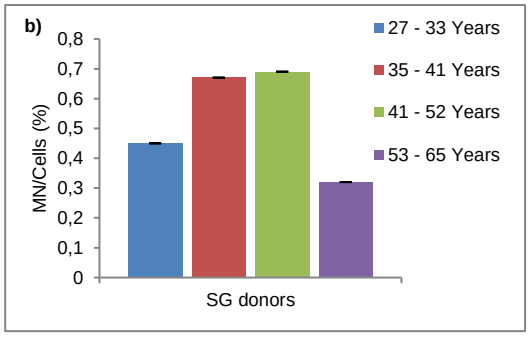


Figure 6 - Micronuclei frequency in the age group of the control group (CG) (a) and in the smokers group (SG) (b). MN - micronuclei.

Binucleated cells

The frequency of BN is different between the three groups studied (Figure 7). In the EG during the analysis of the 80000 mononucleated cells, were scored 512 and 572 BN in the samples taken before and after the orthopantomography, respectively. In the CG and SG were observed 553 and 443 BN, respectively.

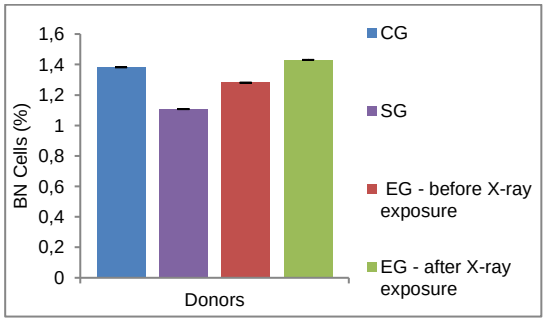


Figure 7 - Frequency of binucleated cells (BN) in the 3 groups studied.

In the group of BN cells any micronucleus was observed. Also, no statistically significant differences were detected in the frequency of BN cells.

Discussion and Conclusions

The micronuclei assay in exfoliated buccal cells was used in this study as it is a useful and minimally invasive method for monitoring genetic damage in humans. This assay was successfully implemented in our laboratory.

Regarding the samples studied statistically significant differences were observed in mean between micronuclei yields, indicating that orthopantomography and tobacco smoke induce genetic damage.

In the CG, men showed a micronuclei frequency higher than women (Figure 5). Concerning the age study, an increase in micronuclei frequency was observed to progress along with the ageing (Figure 6 (a)).

Damage that leads to the formation of micronuclei takes place in the basal layer of the epithelial tissue, where cells undergo mitosis. The cells takes 7 - 21 days to emerge to the surface, so, oral mucosa exfoliated cells were collected immediately before ionizing radiation exposure and 10 days after [8, 9]. Results can be affected by confounder factors such as lifestyle, drinking habits, cigarette smoke and occupational exposure. From EG each subject has as its own control, the collected cells before the exposure to ionizing radiation, so differences between the first and the second study are attributed to exposure to radiation in EG [10]. The EG revealed the existence of statistically significant differences, in the frequency of micronuclei in buccal mucosa cells before (0.004 ± 0.003) (mean ± standard deviation) and after (0.005 ± 0.002) orthopantomography (p=0.028). A similar study was conducted by Arora *et al*. [11], the micronuclei frequency before (0.10 ± 0.09) and after X-ray exposure (0.13 ± 0.08), showed an increase statistically significant (p=0.004) in micronuclei frequency after orthopantomography. Also in the studies of Cerqueira *et al*. [12] and Lorenzoni *et al*. [10] were observed an increased frequency of micronuclei after exposure to X-ray. On the other hand, in the study of Angelieri *et al.* [1] there was no difference before and after exposure to X-ray. However, these differences were not statistically significant. Females presented higher micronuclei frequency than males, before (0.0044 ± 0.0024 vs 0.0033 ± 0.0028, respectively) and after the orthopantomography (0.0051 ± 0.0024 vs 0.0044 ± 0.0023, respectively). There is an increased frequency of micronuclei in both genders after the exam (Figure 3). These results were opposite to the CG. In the EG, age didn´t show any relation to the micronuclei frequency along the ageing of this group. The group of 30-44 years presented frequencies lower (both before and after the exam) (0.0035 ± 0.0019 vs 0.0034 ± 0.0005, respectively) than the group of 18-24 years (0.0046 ± 0.0030 vs 0.0055 ± 0.0031, respectively). The same happened with the group of 55-86 years (0.0032 ± 0.0019 vs 0.0042 ± 0.0027, respectively) with the group of 44-54 years (0.0046 ± 0.0035 vs 0.0062 ± 0.0017, respectively) (Figure 4).

Consumption of tobacco is a global public health problem, as it contains a mixture of nicotine, carcinogens and toxicants. Nicotine is not a direct chemical carcinogen, but it causes addiction leading to the chronic exposure to tobacco smoke that increases cancer risk for smokers [6]. The micronuclei frequency in buccal mucosa cells of SG was higher (0.005 ± 0.004) than the frequency of micronuclei found in CG (0.002 ± 0.001), being this difference statistically significant (p=0.001). Bansal *et al.* [13] and Naderi *et al.* [14] also found a relationship statistically significant between SG and CG, having the SG a higher frequency of micronuclei than the CG. Regarding to gender the results were identical in both genders in the SG, being in agree with the study of Dórea *et al.* [15] that refers any type of association. In contrast, Bloching *et al.* [16] observed that average micronuclei in men is higher (2.12 ± 0.98) than in women (1.73 ± 0.96), being the differences statistically significant. In the CG of this study we observed the same trend. Concerning age the SG didn´t show the same tendency of micronuclei frequency in comparison with the CG (Figure 6 (a) and (b)). The age group 53 - 65 years had a decrease on the micronuclei frequency in comparison with the others age groups (Figure 6 (b)). It was observed that in the SG with increase age there was an increase in tobacco consumption, which should result in an increase in micronuclei frequency along ageing. These results were not observed in the SG. The study of Thomas *et al.* [17] refers that exist a clear association between ageing and a significant increase in the micronuclei frequency in the CG.

Concerning the results from BN cells, the mean was higher in the samples collected after orthopantomography (28.60 ± 12.36) than before the exam (25.60 ± 11.79). The SG showed the lowest mean of BN (22.15 ± 8.21) compared with the CG (27.65 ± 12.60) and EG. Also, from a comparison between a non-smokers group and a smokers group, Khlifi *et al.* [18] noted that BN frequency in the smokers group was lower (2.83 ± 1.73) than in the non-smokers group (3.95 ± 1.90) and these results were statistically significant (p=0.046). Raj and Mahajan [19] observed that with increasing dose of ionizing radiation there is an increase of BN. In this study was observed the same trend of results, in the EG. There were not observed micronuclei during the scoring of BN cells.

The study of more individuals with more stringent selection criteria is necessary to clarify these findings. A further study in different stages of cell death in the oral mucosa cells after exposure to ionizing radiation in orthopantomography exams

and in smokers, would be an interesting approach to understand the mechanisms of buccal mucosa cells in response to these genotoxic agents.

Considering the results obtained the orthopantomography should be used when essential to treatment planning following the ALARA principle (As Low As Reasonably Achievable), every possible precaution should be taken to limit radiation levels when exposing patient's and the health staff to radiation.

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