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Can periodontal infection induce genotoxic effects?

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Abstract

Objective. This study aimed to evaluate the occurrence of chromosomal abnormalities, through micronuclei, and apoptosis by the sum of karyorrhexis, pyknosis and condensed chromatin in individuals with chronic periodontitis, gingivitis associated with biofilm and no periodontal disease. ***Materials and methods.*** This study included 72 individuals divided into three groups: gingivitis ($n = 21$), periodontitis ($n = 24$) and control ($n = 27$). Information on sociodemographic characteristics, health and lifestyle was obtained. Full mouth clinical examination was performed to define the periodontal condition. Exfoliated cells from gingival mucosa were collected for computation of micronuclei and nuclear changes indicative of apoptosis. The differences in the occurrence of endpoints (micronucleus, karyorrhexis, pyknosis and condensed chromatin) were evaluated using the conditional test to compare proportions in a rare events situation. ***Results.*** There was no statistically significant difference in the occurrence of micronucleus ($p > 0.1$) between gingivitis, periodontitis and control groups. The occurrence of apoptosis was significantly higher among individuals with periodontitis compared to individuals with gingivitis ($p < 0.05$) and controls ($p < 0.025$). ***Conclusions.*** The findings showed that the inflammatory process generated by gingivitis and periodontitis is not related to a higher occurrence of chromosomal damage. However, the higher occurrence of apoptosis in individuals with periodontitis points to genotoxic effects induced by periodontal infection.

Key Words: *periodontitis, gingivitis, micronucleus, apoptosis*

Introduction

Gingivitis and periodontitis are diseases highly prevalent, cosmopolitan [1,2] and in recent years have been associated with various systemic conditions such as diabetes mellitus, respiratory infections and osteoporosis [3-5]. Periodontal diseases have an inflammatory character induced by bacteria and affecting periodontal tissues, blood vessels and nerves.

It is known that inflammatory processes are associated with repeated cell divisions, thus enabling the occurrence of errors in the process of mitosis and consequent chromosomal alterations that, in turn, stimulate apoptosis [6-8].

By eliminating cells with genetic alterations, which may include genes engaged in DNA repair and/or in the control of cell proliferation and differentiation (proto-oncogenes and tumor suppressors), apoptosis constitutes a protective mechanism against cancer resulting from genetic and/or chromosomal mutations involving such genes [9]. By constantly renewing tissues, apoptosis ensures tissue homeostasis; however, its occurrence in increased frequencies can be indicative of genotoxic effects that are related to the initiation of a malignant transformation process [10-13].

Few studies have been conducted aiming to investigate the association between periodontal disease and occurrence of micronuclei and nuclear changes indicative of apoptosis [14-17]. In a recent study, Zamora-Perez et al. [16] observed higher incidence of micronucleus, karyorrhexis and condensed chromatin in both individuals with aggressive periodontitis

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and in individuals with chronic periodontitis when compared with those without periodontal disease.

Considering the association between lesions in the genetic material induced by inflammatory processes that may contribute to cancer development [18,19], the aim of this study was to evaluate the occurrence of chromosomal damage (through micronuclei) and apoptosis (inferred by the occurrence of karyorrhexis, condensed chromatin and pyknosis) in individuals with and without periodontal disease.

Materials and methods

Sample

This is a cross-sectional study. The sample was composed of adults of both genders, who sought the dental service of the school clinics of the dentistry course at a public university (Feira de Santana State University, Bahia, Brazil) between March–December 2011. Criteria of eligibility to participate in this study were: (1) aged 18 years or over; (2) not presenting oral alterations (controls) or presenting periodontal disease (chronic periodontitis or gingivitis associated with biofilm) and (3) agreed to participate in the study and signed a consent form. Individuals using dental prosthesis, fixed orthodontic appliances and/or who reported smoking, drug using and recent exposure to X-radiation were not included in the study. After full periodontal clinical examination, individuals were divided into three groups according to periodontal status: Group A, composed of individuals with gingivitis associated with biofilm; Group B, formed by individuals with chronic periodontitis; and Group C (control), composed of individuals without periodontal disease.

Interview and clinical examination

Each participant was invited to answer, through interviews, a questionnaire with the following sections: demographic data, medical history, lifestyle habits, aspects related to oral health and history of exposure to genotoxic agents.

Complete clinical periodontal examination included the following measurements: bleeding on probing index, probing depth, gingival recession and plaque index [20,21]. In addition, clinical attachment loss level was obtained for all teeth. These observations were performed in six different sites for each tooth (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual, mesio-lingual), except for the visible plaque index. All measurements were performed using a William periodontal probe.

The evaluation of the principal examiner's reproducibility measurements was made by replicated periodontal clinical measurements, confirmed by another periodontist as a reference, in ~10% of the sample. The inter-examiner Kappa index (±1 mm) for probing depth and recession measurements were, respectively, 0.82 and 0.87. The index Kappa intra-examiner agreement (±1 mm) was 0.80 and 0.86 for these measurements, respectively.

Clinical parameters obtained made it possible to classify participants in accordance with the following periodontal conditions [5,22]: Individuals who had four or more teeth with one or more sites with probing depth greater than or equal to 4 mm, clinical attachment loss greater than or equal to 3 mm at the same site and the presence of bleeding after probing were considered to have periodontitis. With respect to the presence of gingivitis, the participants were diagnosed when they showed at least 25% of bleeding sites after periodontal probing, but without presenting the above criteria for periodontitis. Periodontal status was considered within the normal limit when the individual did not fit any of the above criteria, after periodontal examination.

Evaluation of cytological abnormalities

Cells were collected by gentle scraping of gingival mucosa, using brush type cytobrush. In individuals in Groups A and B, cells were collected from areas with lesions in the sites that had the worst periodontal condition, but far from root fragments and/or removable dentures. For Group C, cell collection was made at the sites of smallest probing depth following the same precautions mentioned above.

The collected material was transferred by smear to a microscopy slide containing two drops of sterile saline (0.9% NaCl). After drying at room temperature the preparations were immersed in a solution of methanol/acetic acid (3:1) for fixation. Twenty-four hours later the material was dyed using Schiff's reactive and counter-stained with fast green (1% in ethanol).

Every analysis was performed using a blind test, without reference to the data from the questionnaire and periodontal diagnosis, by a single examiner. For each individual, a minimum of 2000 cells with intact cytoplasm was analyzed. The criteria adopted for identification of micronuclei and nuclear degenerative changes indicative of apoptosis (sum of karyorrhexis, condensed chromatin and pyknosis) were described by Sarto et al. [23], Tolbert et al. [12] and Thomas et al. [24]. Micronuclei were considered to be the distinctively individualized structures within the cytoplasm of interphasic cells that measured between one-fifth and one-third of the size of the main nucleus, were observed in the same plane as the nucleus and presented similar staining and chromatin distribution (Figure 1A). Karyorrhexis, chromatin condensation and pyknosis are shown, respectively, in Figures 1B, C and D. Only the cells with full cytoplasm were counted.

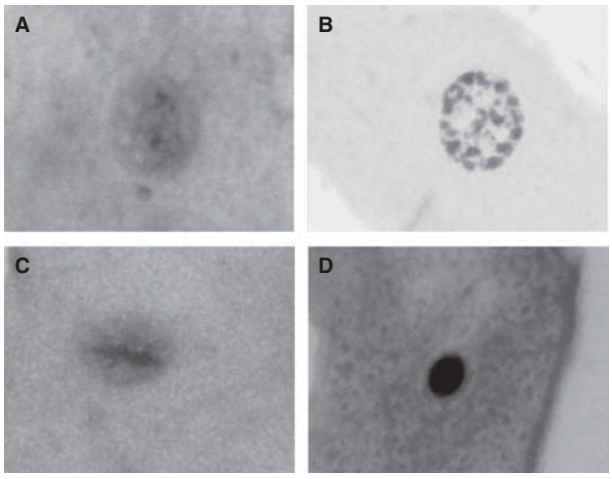

Figure 1. Photomicrographs of cells presenting Micronucleus (A), Karyorrhexis (B), Condensed Chromatin (C) and Pyknosis (D).

Statistical analysis

The data obtained from the questionnaires and cytological analyses were used to create a database in Excel and SPSS version 10.01.1. Chi-square test was applied to assess statistical differences between the frequencies of categorical covariables and Student's *t*-test for the continuous covariables, with a significance level of 5%. The categorization of continuous covariables, when required, was based on their distribution or cut-off points identified in previous studies.

The analysis of the occurrence of micronuclei and degenerative nuclear alterations indicative of apoptosis between groups was performed using the conditional test for comparing proportions in situations in which events are rare. This is an alternative significance test to the χ^2 test. It is similar to Fisher's exact test and appropriate for evaluating cytogenetic events when a great number of cells are required for detecting the occurrence of chromosomal aberrations [25].

Ethical aspects

The study was done in accordance with Brazilian legislation of the National Health Council (CNS 196/96), that is based in the Declaration of Helsinki/ Hong Kong. It was approved by the Ethics Committee of Feira de Santana State University (Protocol 121/ 2009). All participants signed an informed consent form.

Results

The final sample consisted of 72 individuals (19 men and 53 women). The mean age (± SD) was 41 years (±8.48). Age and gender of the participants in the different groups were similar. In accordance with the periodontal diagnostic criteria employed, 24 individuals were classified with chronic periodontitis, 21 were identified as having gingivitis associated with biofilm and 27 showed no changes in the periodontium.

Table I shows the general characteristics of the individuals from groups A, B and C in accordance with the diagnosis of periodontal condition. The comparison between groups showed that they are homogeneous for most of the features except for family income ($p = 0.02$), household density ($p = 0.02$) and flossing ($p = 0.03$).

Participants in Group C had a higher frequency of family income less than or equal to the minimum wage than those in Group A (55.56 vs 23.81%). On the other hand, in relation to household density, participants of Group A showed greater frequency of more than four people per household than those in Group C (38.10 vs 11.11%). The frequency of not flossing in participants of Group A (47.62%) and Group B (45.83%) was more than twice that observed in Group C (18.52%).

In relation to clinical periodontal parameters, only the number of present teeth did not show a statistical difference among the groups (Table II).

Thirteen individuals were excluded from cytological analysis due to the fact that their cytological preparations showed an insufficient number of cells: three from Group C, three from Group A and seven from Group B. The final cytological analysis included: 24 individuals from Group C; 18 individuals with gingivitis associated with biofilm (Group A) and 17 with chronic periodontitis (Group B).

Table III shows the distribution of the occurrence of micronuclei among groups. There was no statistically significant difference between groups ($p > 0.10$).

The statistical evaluation of the occurrence of apoptosis, inferred by the sum of karyorrhexis, condensed chromatin and pyknosis showed a significant difference among the groups: $\chi^2 = 6.48$; df = 2, $p < 0.05$. Partitions of chi-square test showed that this occurrence is significantly higher among individuals from Group B compared to both individuals from Group C and individuals from Group A: $\chi^2 = 5.94$, $p < 0.025$ and $\chi^2 = 4.36$, $p < 0.05$, respectively (df = 1). However, a statistically significant difference was not detected when comparing individuals from Group C with those from Group A: $\chi^2 = 0.04$, df = 1, $p > 0.9$ (Tables IV and V).

Discussion

Participants were individuals who sought dental treatment at the clinics of a public university, the majority of whom are from low-income families (less or equal to one minimum wage per month). It is known that poverty has an influence on the health condition of a population [26-28] and periodontal disease has been frequently associated with this factor [29,30]. The results of the present study, however, showed that

Table I. General characteristics of the groups (n = 72).

Characteristics	Group C (n = 27) n (%)	Group A (n = 21) n (%)	p-value	Group B (n = 24) n (%)	p-value
Age (years)					
≤41 years	12 (44.44)	12 (57.14)	0.38	13 (54.17)	0.48
>41 years	15 (55.56)	9 (42.86)		11 (45.83)	
Mean ± SE	40.59 ± 5.20	39.14 ± 4.58		43.08 ± 4.90	
Sex					
Female	22 (81.48)	15 (71.43)	0.41	16 (66.67)	0.23
Male	5 (18.52)	6 (28.57)		8 (33.33)	
Schooling level (years of study)[a]					
≤8 years	8 (30.77)	10 (47.62)	0.23	11 (47.83)	0.22
>8 years	18 (69.23)	11 (52.38)		12 (52.17)	
Family income (in minimum wage/month)[b]					
≤1 Minimum wage	15 (55.56)	5 (23.81)	0.02	9 (37.50)	0.19
>1 Minimum wage	12 (44.44)	16 (76.19)		15 (62.50)	
Household density					
≤4 Persons	24 (88.89)	13 (61.90)	0.02	17 (70.83)	0.10
>4 Persons	3 (11.11)	8 (38.10)		7 (29.17)	
Alcoholic beverage consumption					
No	15 (55.16)	14 (66.67)	0.43	14 (58.33)	0.84
Yes	12 (44.44)	7 (33.33)		10 (41.67)	
Frequency of tooth brushing per day					
≤ One time	0 (0.00%)	1 (4.76%)		1 (4.17%)	
Twice or more	27 (100%)	20 (95.24%)	0.25	23 (95.83%)	0.28
Dental flossing					
No	5 (18.52%)	10 (47.62%)		11 (45.83%)	
Yes	22 (81.48%)	11 (52.38%)	0.03	13 (54.17%)	0.03
Use of mouthwash					
No	21 (77.78%)	15 (71.43%)		20 (83.33%)	
Yes	6 (22.22%)	6 (28.57%)	0.61	4 (16.67%)	0.61
Regular visits to dentist					
No	18 (66.67%)	13 (61.90%)		13 (54.17%)	
Yes	9 (33.33%)	8 (38.10%)	0.73	11 (45.83%)	0.36

Statistical significance: ≤0.05.
[a]One lost information for control and periodontitis groups.
[b]Minimum wage at the time of data collection: R$545.00 (equivalent to US$302.78).

controls had the family income significantly higher than the gingivitis group and statistically similar to periodontitis groups.

As expected, individuals in the control group had better oral clinical conditions, while those from periodontitis and gingivitis groups consistently showed the worst values of periodontal clinical parameters, with statistical significance difference. The only factor related to the prevention of periodontal disease that differed between controls and individuals with gingivitis or periodontitis was flossing, pointing to the importance of this factor in prevention of these diseases.

The inflammatory process in the periodontium, similar to that which occurs in other tissues, triggers a proliferative response that, in turn, facilitates the occurrence of genetic damage by increasing the likelihood of errors in DNA replication [6-8,31].

The relationship between the loss of cellular capacity to develop to death by apoptosis and the development of neoplastic transformation process is due to the survival of genetically modified cells that exhibit adaptive advantage compared to their normal counterparts [9].

There are few studies in which the occurrence of genetic damage associated with periodontal disease has

Table II. Distribution of the variables related to periodontal condition in groups (n = 72).

Clinical parameters	Group C (n = 27)	Group A (n = 21)	p–value	Group B (n = 24)	p–value
Bleeding on probing (%)					
Mean ± SE	7.71 ± 1.44	50.36 ± 2.76	0.00	47.93 ± 5.06	0.00
Median	5.7	50		52.9	
Variation	0–22.2	25.00–75.00		7.5–100	
Visible plaque index (%)					
Mean ± SE	10.98 ± 2.69	58.14 ± 5.53	0.00	68.76 ± 6.77	0.00
Median	5.68	63.00		80.7	
Variation	0–59.37	10.18–100		3.84–100	
Probing depth (mm)					
Mean ± SE	1.83 ± 0.05	2.21 ± 0.06	0.00	3.00 ± 0.18	0.00
Median	1.84	2.26		2.86	
Variation	1.27–2.77	1.53–2.79		1.87–5.48	
Clinical attachment loss (mm)					
Mean ± SE	2.01 ± 0.09	2.52 ± 0.18	0.01	3.52 ± 0.31	0.00
Median	1.92	2.41		2.95	
Variation	1.39–2.69	1.56–5.69		1.83–7.51	
Number of present teeth (n)					
Mean ± SE	19.33 ± 1.32	18.76 ± 1.56	0.78	20.20 ± 1.16	0.62
Median	22	19		20.5	
Variation	6.00–28.00	6–28		6–27	
Number of sites with clinical attachment loss 1–2 mm (n)					
Mean ± SE	9.25 ± 1.38	3.23 ± 0.83	0.00	2.08 ± 0.65	0.00
Median	8	2		0	
Variation	0–35	0–14		0–12	
Number of sites with clinical attachment loss 3–4 mm (n)					
Mean ± SE	9.33 ± 1.05	13.42 ± 1.44	0.02	9.45 ± 1.27	0.93
Median	8	14		11	
Variation	1–20	0–25		0–19	
Number of sites with clinical attachment loss ≥5 mm (n)					
Mean ± SE	1.11 ± 0.25	2.09 ± 0.44	0.04	8.7 ± 1.31	0.00
Median	1	2		6.5	
Variation	0–4	0–6		1–21	
Number of sites with probing depth ≥4 mm (n)					
Mean ± SE	0.88 ± 0.21	1.57 ± 0.24	0.04	12.5 ± 1.39	0.00
Median	0	1		9.00	
Variation	0–3	0–4		4–25	

Statistical significance ≤0.05.

Table III. Data related to the occurrence of micronuclei (MN) among groups (n = 59).

Group	Observed MN	Expected MN	# Cells	χ^2
Group C (n = 24)	10	11.41	50,112	
Group A (n = 18)	14	10.06	37,600	2.43
Group B (n = 17)	9	9.52	35,589	df = 2, $p > 0.10$

Cells, Total cells; df, degrees of freedom.

Table IV. Occurrence of nuclear alterations indicative of apoptosis ($n = 59$).

Group	Picnosis	Condensed chromatin	Karyorrhexis	Apoptosis*	Total cells
Group C ($n = 24$)	56	1034	708	1798	50,112
Group A ($n = 18$)	45	867	481	1359	37,600
Group B ($n = 17$)	53	779	561	1393	35,589

*Sum of karyorrhexis, condensed chromatin and pyknosis.

Table V. Data related to apoptosis among the groups ($n = 59$).

Group	Observed apoptosis	Expected apoptosis	# Cells	χ^2	Partitions of χ^2; p; df = 1
Group C ($n = 24$)	1798	1849.21	50,112	6.84	Group A × Group C = 0.04; $p > 0.75$
Group A ($n = 18$)	1359	1387.50	37,600	df = 2	Group A × Group B = 4.37; $p < 0.05$
Group B ($n = 17$)	1393	1313.29	35,589	$p < 0.05$	Group B × Group C = 5.94; $p < 0.025$

#Cells = Total cells; df, degrees of freedom.

been assessed. The frequency of exchanges between sister chromatid in individuals with different forms of periodontal disease was analyzed by Emingil et al. [32]. The authors found no significant differences in the frequency of these exchanges between individuals with aggressive periodontitis when compared with those with chronic periodontitis and without periodontal disease.

Genotoxic effects of the inflammatory process associated with periodontal disease were evaluated in this study using the micronucleus test, following the differentiated protocol suggested by Tolbert et al. [12,13] and Thomas et al. [24]. With this method, besides the computation of micronuclei, structures that reveal aneugenic and clastogenic chromosomal damage, the computation of degenerative nuclear phenomena, indicators of apoptosis occurrence (chromatin condensation and pyknosis, karyorrhexis), is also performed, thereby increasing the sensitivity of the test.

The results obtained in this study did not show a higher frequency of chromosomal damage, assessed by the occurrence of micronuclei, in consequence of the inflammatory process associated with periodontal disease. In a similar study, D'Agostini et al. [14] also did not observe increased frequency of micronuclei in individuals with gingivitis and periodontitis with varying degrees of severity. However, Bloching et al. [15] have described greater occurrence of these structures in individuals with severe periodontitis (2.16 ± 0.85) when compared to individuals with moderate periodontitis (1.91 ± 1.04) and without periodontal disease (1.50 ± 1.06).

In the present study, the greater occurrence of apoptosis in individuals with periodontitis points to genotoxic effects associated with the inflammatory process and may suggest that this process could have masked the occurrence of chromosomal damage by elimination of genetically-impaired cells.

Finally, it should be noted that, although the findings showed genotoxic effects of periodontitis, this study points to an important health area research line by simultaneously evaluating chromosomal damage and apoptosis and points to the need for additional investigations on the theme. Furthermore, it suggests that intervention studies should be conducted to test the effects of periodontal treatment on the occurrence of degenerative nuclear changes indicative of apoptosis. In conclusion, the findings indicate that the inflammatory process generated by gingivitis and periodontitis is not related to a higher frequency of chromosomal damage; however, in individuals with periodontitis, a higher occurrence of apoptosis is indicative of genotoxic effects of periodontal infection.

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