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The Micronucleus Test to Evaluate Cytogenetic Damage in Patients with Periodontitis

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Abstract

Background and aims. The role of genetic factors and oxidative damage in the etiopathogenesis of periodontal disease is well documented in the literature. The micronucleus test is a sensitive method that indicates DNA damage. The aim of the present study was to investigate the micronuclei frequency in different forms of periodontitis in comparison with healthy controls.

Materials and methods. Micronuclei frequency was analyzed in the peripheral lymphocytes of 10 patients with chronic periodontitis (CP), 10 patients with generalized aggressive periodontitis (GAP) and 10 healthy controls. Various clinical parameters like the probing depth, clinical attachment level, and percentages of sites with bleeding and plaque were recorded. After the slides were processed, they were stained with 5% Geimsa solution and 1000 cells per sample were counted for the presence of micronuclei.

Results. The mean micronuclei observed in the control group, CP group, and GAP group were 9.8, 10.1 and 9.9, respectively. The differences between the groups were not significant (P=0.978).

Conclusion. The results indicated that the cytogenetic damages in the periodontitis groups were not different from those in the control group. The role of cytogenetic damage in peripheral blood cells may have a limited prognostic value in the etiopathogenesis of periodontal tissues and further studies are necessary to assess cytogenetic damage in periodontal tissues to clarify local tissue destruction in periodontal disease.

Key words: **Micronucleus test, aggressive periodontitis, chronic periodontitis, cytogenetic damage.**

Introduction

Periodontitis is a multifactorial disease in which disease expression involves complex interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis.[1-3] While microbial and other environmental factors play a major role in the initiation and modulation of periodontal disease, individuals respond differently to common environmental challenges and this differential response is influenced by the individual's genetic profile.[4] Genetic susceptibility of periodontal disease has been shown by studies on twins, linkage studies and segregation analyses in families with aggressive (early onset) forms of periodontitis and association studies.[5-10]

The importance of genetic variations in determining the development and severity of periodontal disease is well established, with genetic influences accounting for as much as 30% to 60% of the variability in the clinical severity of periodontitis.[5,6] Aggressive periodontitis (AP) comprises a group of periodontal diseases characterized by rapid loss of periodontal tissues in otherwise clinically healthy subjects whereas chronic periodontitis (CP), is a more common form seen in older individuals, characterized by slow progression of periodontal attachment loss.[11-13]

It has been demonstrated that cases of AP cluster within the families and this familial aggregation suggests a role of genetic factors in the pathogenesis of the disease.[14-16] Various modes of inheritance have been suggested, including autosomal dominant, autosomal recessive and X-linked dominant.[17-20] It is very important to understand the genetic basis of periodontal disease susceptibility so as to unravel some unanswered questions like why various clinical forms of periodontitis exist and such information will have diagnostic and therapeutic values in clinical practice.

The role of reactive oxygen species (ROS) in the pathogenesis of periodontal disease has gained popularity with various mechanisms such as DNA damage, lipid peroxidation, protein disruption and inflammatory cytokines contributing to periodontal tissue destruction.[21-23] Oxidative damage due to excess production of ROS, leading to structural alterations in the mitochondria, has recently been proposed.[24] It is suggested that genetic damage has an important role in various chronic inflammatory and degenerative diseases like Behcet's disease, Parkinson's disease, chronic obstructive pulmonary disease, coronary artery disease, inflammatory bowel disease and Raynaud's phenomenon.[25-30] The role of chromosomal instability in the predisposition of these diseases has been evaluated with the help of cytogenetic tests.

The micronucleus (MN) test is a well established standard assay for genotoxicity assessment at a chromosomal level and is one of the most widely applied short-term tests in genetic toxicology to evaluate mutagenecity of and sensitivity to xenobiotics.[31-33] The test is based on the formation of 'micronuclei' which, due to chromosome breakage or spindle dysfunction, do not migrate to the poles during anaphase and are not incorporated into the telophase nuclei of the dividing cell. Such chromatin condensations result in the formation of one or more satellite nuclei in the cytoplasm of the daughter cells.[34] Although this test does not provide a measure of subtle changes like balanced translocations, it provides a readily measurable index of chromosome breakage and loss.[35]

The aim of the present study was to assess DNA damage if any, in the peripheral lymphocytes, in patients with generalized aggressive periodontitis (GAP), chronic periodontitis (CP) and healthy control subjects. A further aim of the study was to determine if micronuclei frequency could be used as a genetic marker in the pathogenesis of periodontitis.

Materials and Methods

Patient selection

Ten patients with generalized aggressive periodontitis (GAP), 10 patients with chronic periodontitis (CP), and 10 control subjects participated in this study after informed consent. The subjects were selected based on the recruitment criteria from the Department of Periodontics of Sri Saicollege College of Dental Surgery, Vikarabad. The controls were recruited from the faculty and students of the same institute. Ethical clearance was obtained from the Ethics Committee of Sri Sai College of Dental Surgery.

Patients were excluded from the study if they were suffering from any chronic inflammatory diseases like diabetes mellitus, arthritis, history of viral infection or pyrexia over the past one month, history of receiving antibiotics, corticosteroids, cytotoxic agents, NSAIDS, radiation therapy or periodontal treatment over the past 3 months. Pregnant or lactating mothers, smokers and alcoholic subjects were also excluded from the study. Care was also taken so that none of the patients had any dental

restorations as some dental restorations are known to increase micronuclei frequency.[36]

The patients were classified as follows:[12]

Chronic periodontitis group (CP): Systemically healthy individuals with moderate to severe alveolar bone loss and attachment loss of 5 mm or more in multiple sites of all four quadrants of the mouth.

Generalized aggressive periodontitis group (G-AP): Generalized pattern of severe destruction and attachment loss of at least 5 mm on at least three permanent teeth other than central incisors or first molars.

Control group: The control group consisted of 10 subjects with no clinical evidence of periodontal disease.

Clinical examination

Periodontal parameters included assessment of probing pocket depth (PPD) and clinical attachment level (CAL), which were recorded clinically at six sites per tooth for the whole mouth and bleeding on probing (BOP) and plaque assessment was recorded by dichotomous measurement. The extent of bone loss was assessed radiographically using panoramic views.

The micronucleus test procedure

Five mL of venous blood was collected from each subject in sodium heparinized vacutainers. After the samples were collected, they were coded to ensure an unbiased assay of the samples. One of the investigators maintained the code in a sealed envelope. The samples were then transported at room temperature on the same day to the genetic institute (GeneTech, Hyderabad, India). A thin blood smear was prepared on the same day of sample collection. The slides were air-dried for at least 30 minutes before fixing at room temperature. Once the slides were prepared, fixation was carried out using freshly prepared cold 3:1 methanol and acetic acid solution for 10– 15 minutes. The slides were removed from the fixative and washed twice in PBS (phosphate buffer solution). The slides were then stained immediately in 5% Geimsa solution (GS500-Sigma Aldrich Lot #090M4340) and dried on a hot plate for 5 minutes. The slides were stored in slide boxes before examining under a microscope. Three slides were analyzed using ×100 objective and ×10 eyepiece magnifications with oil and 1,000 cells were counted per sample.

Only nucleated cells that were separate without overlapping or folds were analyzed. Micronuclei (MN) were counted if the structures had a regular border and were located inside the cytoplasm, with an intensity of staining less than or equal to that of the main nucleus and a size less than two-thirds of the size of the main nucleus (Figure 1).

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package (version 17.0, IBM, Chicago, IL), after breaking the code. The clinical parameters as well as the mean value of micronuclei present were calculated as subject means with standard deviation. One-way ANOVA was used for inter-group comparisons and pair-wise comparisonss were carried out by post hoc LSD tests. Statistical significance was defined at $P \leq 0.05$.

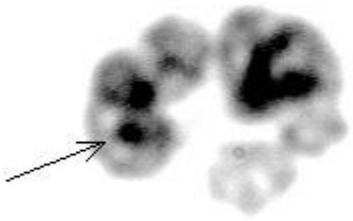

Figure 1. Lymphocyte showing micronucleus (depicted with an arrow) and the adjacent cell without any micronuclei.

Results

The clinical parameters of the control, CP and GAP groups are presented in Table 1. The mean age of the subjects in the CP group was higher in comparison with the G-AP and the control groups, which had younger subjects ($P<0.001$). As expected, the mean probing depths and clinical attachment loss of both the G-AP and the CP groups were higher than those in the control group ($P<0.001$). Percentages of bleeding sites and plaque were significantly higher in the G-AP and the CP groups than the control group ($P<0.001$) (Table 1).

The results are expressed as MN observed per 1000 cells examined. The means of MN in the control, CP, and GAP groups were 9.8, 10.1 and 9.9, respectively (Table 2), with no significant differences between the groups ($P=0.978$).

Table 1. Clinical parameters of control, CP and G-AP groups (Mean ± SD)

	CONTROL	CP	G-AP
AGE (years)	28 ±7.27§	40.1±4.6	26.8±4.42§
PD (mm)	1.5±0.42	4.23±0.82*	4.92±0.70*
CAL (mm)	0.23±1.2	4.56±0.53*	4.85±0.63*
Percentages of sites with bleeding	8.71±2.51	57.58±12.9*	52.63±8.74*
Percentages of sites with plaque	8.0±2.4	55.61±13.9*	53.28±8.53*

§ Statistically significantly lower than CP group (P< 0.001).
*Statistically significantly higher than control group (P< 0.001)

Table 2. Micronuclei frequency of control, CP and G-AP groups

	n=30	Mean	SD	SE	Min	Max	F value	P value
Control	10	9.8	3.04	0.96	4.0	15.0		
CP	10	10.10	2.99	0.94	6.0	15.0		
GAP	10	9.9	3.63	1.14	5.0	16.0	0.022	0.978

Discussion

Recent research has focused on the role of cytogenetic damage in the etiology of various inflammatory systemic diseases and the role of ROS in causing oxidative damage at a cytogenetic level has also been highlighted. Periodontal disease is an inflammatory disease of complex etiology, leading to loss of the supporting structures of the tooth.

Previous studies have evaluated cytogenetic damage in periodontitis patients using the sister chromatid exchange (SCE) analysis,[37,38] revealing no differences in cytogenetic damage in the peripheral blood lymphocytes of periodontitis patients and healthy controls. The aim of the present study was to identify cytogenetic damage, if any, in periodontitis patients compared to healthy controls using another sensitive method, the micronucleus test. To our knowledge, this is the first paper in the literature that evaluates cytogenetic damage in subjects with periodontitis using the micronucleus test. The assessment of cytogenetic damage by the micronucleus test is a sensitive tool which measures the effect of cytogenetic agents on various cells. In the present study, the end points of micronucleus assay were chosen as the primary measure of cytogenetic damage as this method has proven to be informative in relation to genetic damage in blood and exfoliated buccal cells in humans.[39]

The present study was undertaken considering two possibilities in the pathogenesis of periodontitis; the first was the role of genetic susceptibility in the expression of different forms of periodontal disease and whether cytogenetic damage could play a role and secondly, whether increased oxidative damage seen in periodontal disease due to an exaggerated production of ROS could manifest as cytogenetic damage in the peripheral blood cells.

In the present study, there were no significant differences in the micronuclei frequency in the periodontitis groups compared to healthy controls and indicating a possible lack of cytogenetic damage in peripheral blood cells. The findings are consistent with other studies reporting a lack of such association.[37,38] A recent study on mitochondrial DNA damage in chronic periodontitis showed that most of the mitochondrial mutations seen in the gingival tissues were absent in blood.[24] This further questions the role of cytogenetic damage as observed in the peripheral blood cells, as a marker of periodontal disease. Further studies are required assessing DNA damage at the periodontal tissues and as suggested by Emingil, periodontal tissue cultures may be more valuable in clarifying the role of local tissue damage seen in periodontal disease. However, although our study does not support the role of cytogenetic damage, further studies with more sensitive assays are necessary to confirm these findings; most importantly, the findings of this study do not preclude the role of genetics and oxidative damage in the pathogenesis of periodontal disease.

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