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Influence of glycemic control on Epstein-Bar and Cytomegalovirus infection in periodontal pocket of type 2 diabetic subjects

Renato Corrêa Viana Casarin [a], *Poliana Mendes Duarte* [b], *Vanessa Renata Santos* [b], *Jadson Almeida Lima* [b], *Guy Gagnon* [c], *Márcio Zaffalon Casati* [a,d], *Reginaldo Bruno Gonçalves* [c,e,*]

[a] *Department of Prosthodontics and Periodontics, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil*
[b] *Department of Periodontics, Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil*
[c] *Groupe de Recherche en Ecologie Bucale, Université Laval, Quebec, Canada*
[d] *Department of Periodontics, University Paulista, São Paulo, Brazil*
[e] *Department of Oral Diagnosis, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil*

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ABSTRACT

Objective: The aim of the present study was to evaluate the influence of glycemic control on the frequency of Epstein-Bar (EBV) and Cytomegalovirus (CMV) in periodontal pockets of type 2 diabetic subjects with chronic periodontitis.

Design: Forty-six subjects presenting generalized chronic periodontitis and type 2 diabetes mellitus (DM) were selected for this study. Polymerase chain reaction (PCR) was used to determine the presence of EBV and CMV in shallow [Probing Depth (PD) $\leq$ 3 mm], moderate (PD = 4–6 mm) and deep (PD $>$ 7 mm) pockets. HbA1c levels $\leq$7%, $>$7 to $<$10%, and $\geq$10% defined good, moderate and poor glycemic control, respectively.

Results: Higher frequency of EBV was found in the shallow pockets of the subjects with poor glycemic control ($p < 0.05$; chi-square test). Moreover, EBV-free subjects presented moderate or good glycemic control. Glycemic control did not influence the frequency of CMV in all pocket categories.

Conclusion: Poor glycemic control in type 2 diabetic subjects can increase the occurrence of EBV in shallow periodontal pockets.

1. Introduction

Type 2 diabetes mellitus (DM) is a well-established risk factor for periodontitis.[1] At present, a bidirectional pathway has been suggested between DM and periodontitis, since patients with diabetes mellitus are more predisposed to periodontal diseases, the established periodontitis may simultaneously impair adequate glycemic control.[2] Moreover, poorly controlled diabetic subjects, presenting elevated levels of glycosylated haemoglobin (HbA1c), show higher attachment and alveolar bone loss and local inflammatory cytokines than well-controlled patients.[3,4] Exacerbated local inflammation in diabetic subjects with inadequate glycemic control could modify the subgingival environment, and consequently, the subgingival microbial profile. Makiura et al.[5] demonstrated a positive correlation between the presence of specific periodontal pathogens and the HbA1c levels in type 2 diabetic individuals. Therefore, particularly in a group susceptible to

* *Corresponding author at*: 2420 rue de la Terrasse, Quebec, Canada G1V0A6.
E-mail address: Reginaldo.Goncalves@fmd.ulaval.ca (R.B. Gonçalves).
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infection, such as diabetic subjects, identification of the microorganisms related to the subgingival biofilm seems to have critical local and systemic implications.

In the last decade, viruses have emerged as possible putative pathogens in periodontal diseases. Herpesviruses, alone or in association with other pathogens, have been related to the development and progression of periodontitis.[6–8] Epstein-Bar (EBV) and Cytomegalovirus (CMV) are the most common herpesviruses detected in periodontal pockets.[6–9] Previous investigations have reported a wide variation in the occurrence of herpesviruses in aggressive and chronic periodontitis.[7–9] A recent review reported a prevalence of EBV ranging from 3–89% and an occurrence of CMV ranging from 0.3% to 83% in periodontal sites of subjects with chronic or aggressive periodontitis.[8]

However, to date, no study has evaluated the relationship between the glycemic status and the occurrence of herpesvirus infection in periodontal pockets of type 2 diabetic subjects with chronic periodontitis. The possible involvement of viruses in the aetiology of periodontitis in diabetic subjects is certainly important, since previous studies were almost exclusively centred on a bacterial contribution. Thus, the aim of this study was to evaluate the influence of glycemic control on the frequency of Epstein-Bar (EBV) and Cytomegalovirus (CMV) in periodontal pockets of type 2 diabetic subjects with chronic periodontitis.

2. Materials and methods

2.1. Study population

Forty-six subjects presenting generalized chronic periodontitis[10] and type 2 DM for at least the past 5 years were selected among the population referred to the Periodontal Clinic of Guarulhos University, from July 2007 until January 2008. All eligible subjects were thoroughly informed of the nature, potential risks and benefits of their participation in the study and signed their informed consent. This study protocol was previously approved by the Clinical Research Ethics Committee of the Guarulhos University. All subjects were >35 years old, had at least 15 teeth excluding third molars and teeth with advanced decay indicated for extraction, and over 30% of the sites with probing depth (PD) and clinical attachment level (CAL) ≥4 mm.

Exclusion criteria were pregnancy, lactation, current smoking and smoking within the past 5 years, periodontal or/and antibiotic therapies in the previous 6 months, use of mouthrinses containing antimicrobial agents in the preceding 3 months, any systemic condition (except DM) that could affect the progression of periodontal disease (e.g. immunological disorders) and long-term administration of anti-inflammatory and immunosuppressive medications. Subjects with periapical pathology, orthodontic appliances and multiple systemic complications of DM were also excluded from the study.

2.2. Glycemic status

Peripheral blood samples were collected to assess the HbA1c level by high-performance liquid chromatography and fasting plasma glucose (FPG) by the glucose oxidase method. According to HbA1c levels, subjects were allocated to one of the following groups:

- good glycemic control: HbA1c level ≤ 7%[11];
- moderate glycemic control: HbA1c >7–<10%;
- poor glycemic control: HbA1c levels ≥ 10%.

2.3. Clinical parameters

The following parameters were assessed at six sites of all teeth, excluding third molars (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual), using a manual periodontal probe (UNC15, Hu-Friedy, Chicago, IL, USA): plaque index (PI), bleeding on probing (BoP), suppuration (SUP), probing depth (PD; mm) and clinical attachment level (CAL; mm).

2.4. Herpesviruses detection

Subgingival biofilm samples were collected from one shallow (PD ≤ 3 mm), one moderate (PD = 4–6 mm) and one deep pocket (PD > 7 mm) per subject using a Gracey curette. Prior to sampling, supragingival plaque was removed with sterile cotton pellets and the sites were isolated with cotton rolls to avoid saliva contamination. DNA was extracted following the protocol previously described by Saito et al.[12] After DNA extraction, the presence of EBV-1 and CMV-1 were evaluated by a nested PCR. Detection of EBV was performed using outer primers (5′-AGGGATGCCTGGACACAAGA-3′ and 5′-TGGTGCTGCTGGTGGCAA-3′) and inner primers (5′-TCTTGATAGGGATCCGCTAGGATA-3′ and 5′-ACCGTGGTTCTGGACTATTCGGATC-3′). Outer primers used in CMV detection were 5′-CAGACACAGTGTCCTCCCGCTCCTC-3′ and 5′-CCTAGTGTGGATGACCTACGGGCCA-3′ and inner primers 5′-CAGACACAGTGTCCTCCGGCTCCTC-3′ and 5′-CCAGAGTCCCCTGTACCCGC-3′). For EBV and CMV outer amplification was 25 cycles at 60 °C and inner amplification was 30 cycles at 55 °C. This procedure was successfully used in previous study.[13] PCR products were then loaded onto a 1% agarose gel stained with ethidium bromide and amplicons were detected under UV light.

2.5. Statistical analysis

Demographical, clinical and metabolic comparisons among glycemic groups were performed by one-way ANOVA. When there were significant differences by one-way ANOVA, a pairwise comparison was performed by Tukey's test. Gender distribution and EBV and CMV frequencies within different PD categories among glycemic groups were compared using the chi-square test. The level of significance was set at 5% for all statistical analysis.

3. Results

3.1. Subject characteristics

Table 1 shows the demographical, clinical and metabolic characteristics of the study population according to glycemic