

The prevalence and quantitative analysis of the Epstein–Barr virus in healthy implants and implants affected by periimplantitis: A preliminary report

Abstract

Objective

Viruses, in particular the Epstein–Barr virus (EBV), appear to play a role in the genesis and progression of human periodontitis and periimplantitis. The aim of the present study was to compare the presence of EBV in healthy periimplant sites or those affected by periimplantitis.

Materials and methods

From January 2013 to December 2014, 50 consecutive subjects with implants affected by periimplantitis and 50 subjects with healthy implants attending for a routine check-up or spontaneous visits during the study period in three private clinics (Rome and Genoa, Italy, and Belgrade, Serbia) were enrolled in this clinical study.

Quantitative real-time polymerase chain reaction assays for EBV were performed on every patient. The internal connections and external surfaces of the implants were evaluated. Independent sample *t*-tests or nonparametric Mann–Whitney *U* tests were performed to check for any statistically significant difference in each continuous variable between the two groups of patients (healthy vs. periimplantitis).

Results

Eighty-three patients (40 with healthy implants and 43 with periimplantitis-affected implants) concluded the study. The study evaluated 103 dental implants affected by periimplantitis and 197 healthy implants (mean time of loading: 5.31 ± 2.6 years). Although 28.6% of the healthy patients and 37.2% of the patients affected by periimplantitis presented at least one site with EBV, the differences were not statistically significant ($p > 0.05$).

Conclusion

This study did not find a clear link between periimplantitis etiopathogenesis and viral infection.

Introduction

Periimplantitis can be defined as an inflammatory process of the periimplant soft and hard tissue with or without primary infection, associated with clinically significant, progressing crestal bone loss after the adaptive phase after prosthetic loading.¹ Numerous studies have analyzed the bacterial flora associated with diseased implants to possibly understand the role played by the bacterial infection in the genesis of periimplantitis.^{2,3} This research underlines a similar microbial profile between periimplantitis and periodontitis, with high numbers of periodontal pathogens in periimplant sites (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Aggregatibacter actinomycetemcomitans*) confirming data previously reported by Leonhardt et al.⁴

Viruses, in particular the Epstein–Barr virus (EBV) and other herpesviruses, appear to play a role in the genesis and progression of human periodontitis.⁵ Viruses infect periodontal B lymphocytes⁶ exerting diminished ability to defend against bacterial challenge and permitting the overgrowth of periodontopathic microorganisms.

Besides in periodontitis, recent studies have shown a correlation between periimplant infection and the presence of EBV. Jankovic et al. found a high prevalence of *Human cytomegalovirus* (HCMV) and EBV DNA in the subgingival plaque of periimplantitis sites, suggesting a possible active pathogenic role in periimplantitis.⁷ They showed a higher prevalence of EBV and HCMV in periimplantitis sites compared with healthy periimplant sites. In a split-mouth study, Verdugo et al. suggested that EBV may be a likely candidate in the etiopathogenesis of periimplant disease and that periimplantitis etiopathogenesis could be orchestrated and fueled

by a combination of EBV and Gram-negative anaerobic rods.⁸ The aim of the present study is to compare the presence of EBV in periimplantitis-affected and healthy periimplant sites.

Materials & methods

From January 2013 to December 2014, 50 consecutive subjects with implants affected by periimplantitis and 50 subjects with healthy implants attending for a routine check-up or spontaneous visits during the study period in three private clinics (Rome and Genoa, Italy, and Belgrade, Serbia) were enrolled in this clinical study.

The inclusion criteria were systemically healthy nonsmoker subjects treated with at least one implant that had been functioning for at least one year. Patients were periodontally healthy and had not taken any systemic antibiotics, anti-inflammatory drugs or oral antimicrobial agents within the preceding six months.

Periimplantitis is commonly defined as reported by the Estepona consensus meeting: an infection with suppuration associated with clinically significant progressing crestal bone loss after the adaptive phase.¹ However, in the present study, to find a clinically feasible threshold, according to Renvert et al., periimplantitis was defined when an implant presented radiographic presence of bone loss of > 3 mm after implant integration, with a pocket probing depth of \geq 4 mm, bleeding on probing and/or suppuration.⁹

This human case–control study was conducted in accordance with the Declaration of Helsinki and all subjects provided written informed consent prior to their entry into the study. It conformed with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.¹⁰

All clinical examinations were performed by the same operators (LC, PP and MR) and subgingival plaque samples were collected with the GUIDOR Perio-Implant Diagnostic Kit (Sunstar Iberia, Barcelona, Spain). The sampling kit is intended for the collection and transport of samples containing periodontal and periimplant pathogens. Briefly, prior to subgingival plaque sampling, each tooth was isolated with cotton rolls. Absorbent paper points were inserted into the periodontal pockets. After 15s, these paper points were removed and placed into a 2 ml tube. The tubes containing the sample were sent to the Institut Clinident laboratory (France) in the pro-

vided mailing envelopes. The internal connections and external surfaces of the implants were evaluated.

Quantitative real-time polymerase chain reaction assays for the Epstein–Barr virus

Quantitative real-time polymerase chain reaction (PCR) assays were performed to detect the presence or absence of and quantify EBV DNA in the paper points. First, total DNA was isolated using the QIAextractor DNA Plasticware and QIAextractor DX Reagents (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. Then, real-time PCR was carried out for EBV using the Epstein–Barr virus quantitative Real Time PCR kit (Diagenode, Liège, Belgium) and the Rotor-Gene Q thermal cycling system (Qiagen, Hilden, Germany).

Briefly, quantitative real-time PCR assays were performed in a volume of 25 μ l, composed of 12.5 μ l of MasterMix Optima Multiplex 2X DNA, 2.5 μ l of EBV primers and double-dye probe (FAM, emission 520 nm), 2.5 μ l of internal control DNA, 2.5 μ l of internal control primers and double-dye probe (Yellow Dye, emission 548 nm), and 5 μ l of DNA extract or EBV-positive control or EBV-negative control or DNA Standard (for quantitative standard curve; all products by Diagenode, Liège, Belgium). Five EBV DNA dilutions were used for the standard curve (from 200 copies to 2,000,000 copies of EBV amplicon/PCR reaction).

Assays were carried out on the Rotor-Gene Q thermal cycling system with the following program: 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles of 15 s at 95 °C, and 60 s at 60 °C. Fluorescence signals (FAM, emission 520 nm; Yellow Dye, emission 548 nm) were measured every cycle at the end of the extension step. The resulting data were analyzed using Rotor-Gene Q Series Software (Qiagen, Hilden, Germany).

Statistical methods

Normality of variables was assessed by graphical methods (mean of histograms) and confirmed by the Shapiro–Wilk normality test and the Levene test of homogeneity of variance. All characteristics were summarized using mean (standard deviation) median (range) or frequencies (percentages).

Table 1

		N (%)	Mean ± S.D.	Median [min.–max.]
Group	Healthy	42 (49.4)		
	Periimplantitis	43 (50.6)		
Number of implants assessed per subject			3.5 ± 2.2	3.0 [1.0–13.0]
Number of EBV-positive implants			0.8 ± 1.4	0.0 [0.0–6.0]
Positivity %			25.1 ± 39.5	0.0 [0.0–100.0]
EBV-positive internal surfaces		6 (7.1)		
Internal surfaces, EBV copies (highest value recorded)			2941.7 ± 4433.1	100.0 [100.0–9450.0]
EBV-positive external surfaces		25 (29.4)		
External surfaces, EBV copies (highest value recorded)			396643.8 ± 144106 8.4	100.0 [100.0–6840000.0]

Table 1
Clinical characteristics of the sample.

Table 2
Differences between the two groups of patients.

Table 2

	Healthy			Periimplantitis			P-value
	Mean ± S.D.	Median [min.–max.]	N (%)	Mean ± S.D.	Median [min.–max.]	N (%)	
Number of implants assessed per subject	4.7 ± 1.8	5.0 [2.0–9.0]		2.4 ± 2.0	2.0 [1.0–13.0]		0.001*
Subjects with EBV-positive implants	Yes		30 (71.4)			27 (62.8)	0.54
	No		12 (28.6)			16 (37.2)	
Number of EBV-positive implants	1.0 ± 1.7	0.0 [0.0–6.0]		0.6 ± 0.9	0.0 [0.0–4.0]		0.21
Positivity %	21.9 ± 37.7	0.0 [0.0–100.0]		28.3 ± 41.4	0.0 [0.0–100.0]		0.46
EBV-negative internal surfaces			39 (92.9)			40 (93.0)	1.00
EBV-positive internal surfaces			3 (7.1)			3 (7.0)	
Internal surfaces, EBV copies (highest value recorded)	3216.7 ± 53 98.2	100.0 [100.0–9450.0]		2666.7 ± 4445.6	100.0 [100.0–7800.0]		1.00
EBV-negative external surfaces			30 (71.4)			30 (69.8)	1.00
EBV-positive external surfaces			12 (28.6)			13 (30.2)	
External surfaces, EBV copies (highest value recorded)	805152.8 ± 2044335.3	2600.0 [100.0–6840000.0]		19558.5 ± 37449.4	100.0 [100.0–109200.0]		0.69

*A p-value of ≤ 0.05 was considered statistically significant.

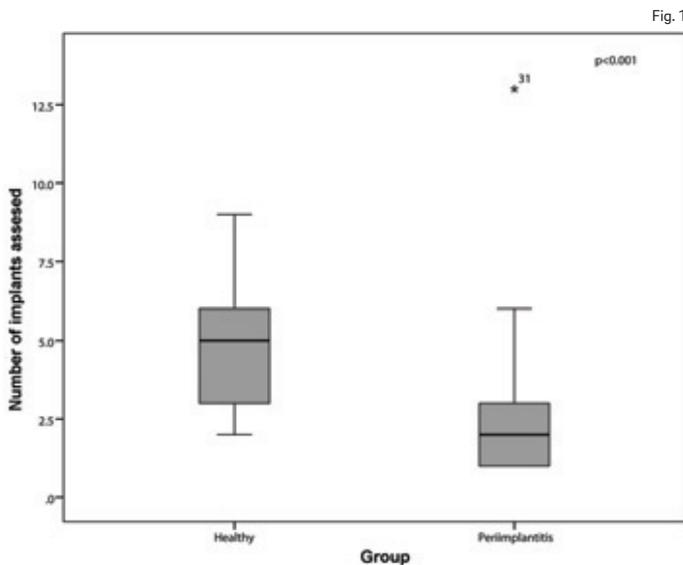


Fig. 1

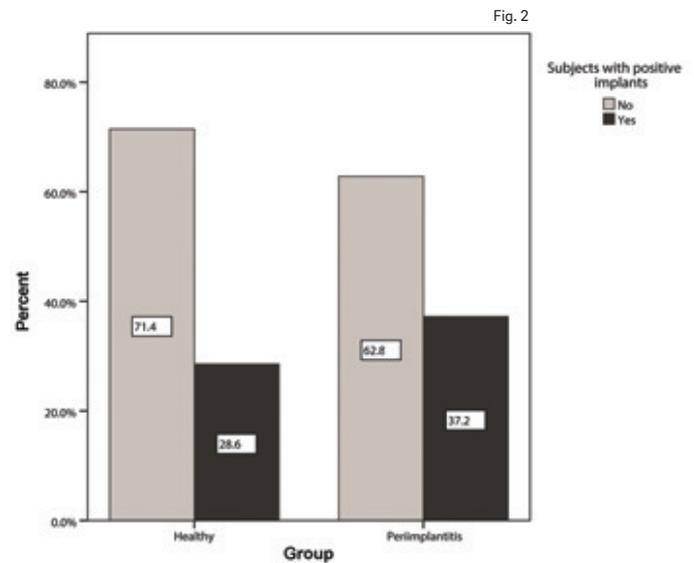


Fig. 2

Independent sample *t*-tests or nonparametric Mann-Whitney *U* tests were performed to check for any statistically significant difference in each continuous variable between the two groups of patients (healthy vs. periimplantitis).

Chi-squared tests or Fisher exact tests were used to assess whether a statistically significant change in proportions occurred in categorical variables between the two groups of patients in the same population.

All the analyses were computed using IBM SPSS Statistics software (Version 20.0; IBM Corp., Armonk, N.Y., U.S.). A *p*-value of ≤ 0.05 was considered statistically significant.

Results

At the end of the study, the total population consisted of 83 subjects (44 females, 39 males; mean age: 59.5 ± 11.3 years). Twenty-four females and 19 males were reported in the group with periimplantitis, and 20 females and 20 males were reported in the healthy patients. Seven patients belonging to the periimplantitis group and ten to the healthy group refused to undergo the microbiological analysis and for this reason were excluded from the studied sample.

The study evaluated 103 dental implants affected by periimplantitis and 197 healthy implants (mean time of loading: 5.31 ± 2.6 years). Clinical characteristics are shown in **Table 1**. In **Table 2**, all of the differences between the two groups of patients are expressed.

A statistically significant difference ($p < 0.001$) between the two groups (**Fig. 1**) was observed in the total number of implants assessed per patient, with a higher number in the healthy subjects (4.7 ± 1.8) compared with patients with periimplantitis (2.4 ± 2.0). For the other evaluated variables, no statistically significant difference was detected ($p > 0.05$; **Table 2**).

EBV was present in 12 patients (**Fig. 2**) in the healthy group (28.6%) and in 16 patients in the periimplantitis group (37.2%). Of the implants affected by periimplantitis, 28.3% were positive for EBV, as were 21.9% of the healthy implants (**Table 2**). However, the differences between the two groups were not statistically significant. The highest and the median values recorded for EBV were higher among the healthy subjects in both the internal and the external implant sites. **Figures 3 and 4** show the distribution of EBV in the internal and external implant sites with lower contamination by EBV in the internal implant connections with respect to the gingival sulci.

Fig. 1

Difference in the number of implants assessed between healthy patients and patients with periimplantitis.

Fig. 2

Subjects with EBV-positive implants in the two groups.

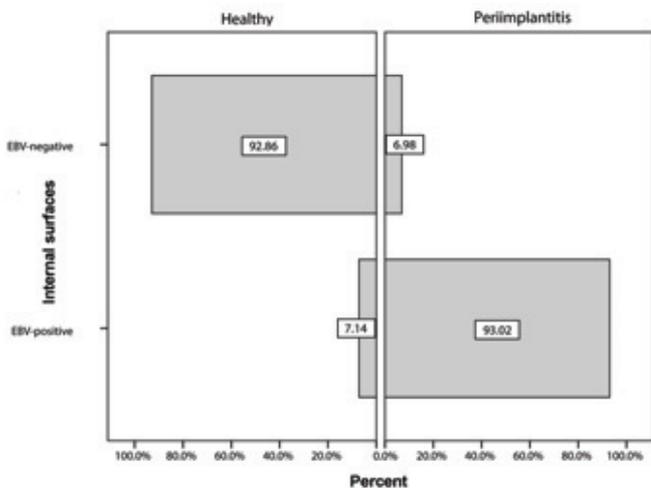


Fig. 3

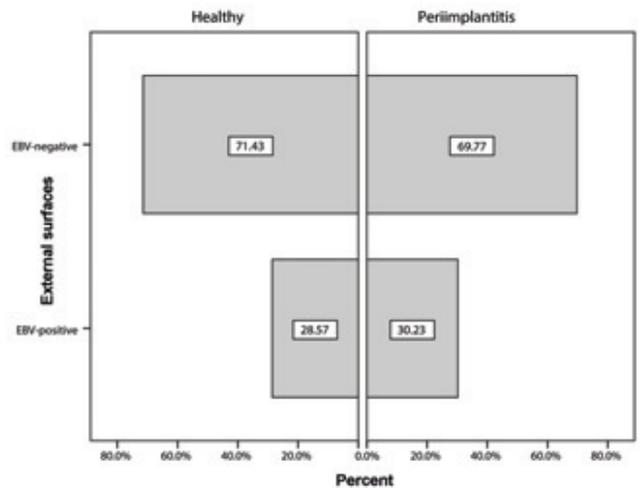


Fig. 4

Fig. 3

Distribution of EBV in internal sites in both groups.

Fig. 4

Distribution of EBV in external sites in both groups.

Discussion

Periimplantitis is one of the most controversial problems affecting the outcome of implant treatment. Different hypotheses have been proposed about its etiology and definition—even if the term “periimplantitis” appears to have been improperly used to describe any periimplant bone loss, irrespective of the complexity of the numerous factors that may contribute to loss of marginal bone around implants.¹¹

Various authors have put forward the hypothesis that periimplantitis could be associated with EBV or HCMV. These viruses have been suggested to alter the local host immune response in combination with periodontopathic bacteria, with the potential to lead to periodontal and periimplant tissue destruction.⁷

In fact, it is well known that the great majority of the human population (more than 90%) are infected with EBV. This particular virus establishes specific communication with the host, changing the expression of its own genes in different cell types, depending on its differential status. During primary infection, EBV initially infects oral epithelial cells in the lytic form and subsequently infects B cells, where the virus assumes one of three different types of latency lifelong. Occasionally, the latent infection reactivates and changes into lytic infection. The differentiation of memory B cells with the EBV genome into plasma cells after antigen stimulation activates the lytic EBV infection. The reactivation most commonly occurs in tonsillar plasma cells, as well as in tonsillar B cells. This is one of the reasons that the saliva from immunocompromised but also immunocom-

petent persons often contains infectious EBV, with or without any signs of infection. EBV can change the immune response of the host with a specific influence on the immunopathogenesis of infection. The cytokines secreted during EBV infection can influence local immunopathology.

The results of the present study rejected the hypothesis that periimplantitis could be associated with EBV; in fact, no statistically significant differences were found regarding the presence of EBV in healthy or periimplantitis-affected sites (28.6% of the healthy patients and 37.2% of the periimplantitis-affected patients presented at least one site with EBV).

This is in contrast to previous studies, in which a significantly higher presence of EBV was found in subgingival samples from periimplantitis lesions than from healthy periimplant sites. In fact, both Jankovic et al. and Verdugo et al. found a significantly higher presence of EBV in the periimplantitis-affected sites.^{7,8}

The absence of significant differences between the groups could lead to the rejection of the hypothesis of the pathogenic key mechanism of viruses in the incidence of periimplantitis. However, this could be because the present study had a retrospective design. This limitation could jeopardize the detection of EBV, which is thought to activate immunological response only in the early stage of disease.¹² This could be indirectly confirmed by the fact that most parts of the sites in the periimplantitis group were under the limit of quantification. At the same time, interactions between EBV and herpesviruses could be supposed to have an effect on the host response.⁷

Various authors have proposed that microbiological contamination of the internal implant connection indicates bacterial leakage along the implant–abutment interface, abutment–prosthesis interface, and restorative margins.^{13, 14} The results of the present study confirm viral leakage with the presence of EBV in the implant connection, even if with a lower detection frequency with respect to the periimplant sulci. Three periimplantitis-affected implants and three healthy implants presented with EBV with no statistically significant differences between the two groups. That slightly less of the virus was found in patients with periimplantitis-affected sites compared with healthy sites may indicate that the intensive local immune response in the case of periimplantitis reduces the amount of virus. However, in four sites, the virus was only found in the periimplant sulci. This could confirm the impressive turnover and interaction between bacteria and viruses, which could only be supposed in a retrospective study with one time point such as the present one. This particular interaction is likely mediated by cytokines produced during viral and bacterial infections. Tumor necrosis factor α , interleukin 1 and interleukin 6 have a great impact on the pathogenesis of periimplantitis. In this paper, we focused our attention on virus detection only and maybe this is the most important limitation of the study. For this reason, an observational prospective study focused also on bacteria could clarify the interactions between these microbiota and maybe the microbiological scenario of periimplantitis.

Conclusion

Within the limits of the present study, no statistically significant differences were found regarding the presence of EBV in healthy or periimplantitis-affected sites. This study failed to find a link between periimplantitis etiopathogenesis and viral infection.

Competing interests

The authors declare that they have no conflict of interests related to this study. The study was partially supported by Institute Clinident (Aix-en-Provence, France), which provided technical support, and Sweden & Martina (Padua, Italy), which provided diagnostic test kits.

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Luigi Canullo,[†]
Paolo Pesce,[‡]
Nathalie Pailler,[§]
Matteo Simonetti,[‡]
Mia Rakic,^{**}
Tanja Jovanovic^{††}

[†] Private practice in Rome, Italy, and Istituto Stomatologico Toscano, Viareggio, Italy

[‡] Department of surgical and diagnostic sciences, University of Genoa, Italy

[§] Institut Clinident, Aix-en-Provence, France

^{**} Institute for Biological Research “Siniša Stanković,” University of Belgrade, Serbia

^{††} Institute for Microbiology and Immunology, School of Medicine, University of Belgrade, Serbia

Corresponding author:

Dr. Luigi Canullo
Via Nizza 46
00198 Rome
Italy

T +39 347 620 1976
F +39 06 841 1980
luigicanullo@yahoo.com

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