Original Article

Prevalence of herpes virus in chronic periodontitis patients with and without type 2 diabetes mellitus: A clinico-microbiological study

Jai Abooj, Siddhartha A Varma

Department of Periodontology, School of Dental Sciences, Krishna Institute of Medical Sciences "Deemed to be University," Karad, Maharashtra, India

Abstract

Background: Unfavorable modifications of tooth and its surrounding structures result in periodontal complications. Viruses, in specific herpes virus, are known to increase disease severity in periodontal patients. Periodontitis is known to be more established in type 2 diabetes mellitus (DM2) patients. Hence, the detection of the viral load, its effect on the prevalence of periodontitis and the glycemic control status of patients are to be evidenced. The study aimed to reveal the association of herpes virus with periodontal parameters and its prevalence in DM2 patients.

Materials and Methods: The cross-sectional study involved a total of 120 patients falling into three groups; Group I (healthy), Group II (periodontitis without DM2) and Group III (periodontitis with DM2) were subjected for sampling. Subgingival samples of periodontitis patients were tested for clinical parameters, and DNA extraction was performed. The presence of herpes virus (Epstein–Barr virus [EBV-1] and human *Cytomegalovirus* [HCMV]) was detected using multiplex polymerase chain reaction primers. Glycemic status of patients was recorded as glycosylated hemoglobin and scored accordingly. Chi-square test was performed to analyze the association between the categorical variables, and *t*-test/Mann–Whitney U-test/analysis of variance/Kruskal–Wallis test was used for continuous data.

Results: Significant levels of EBV-1 were detected in Group III ($n = 21$, 52.5%), followed by Group II ($n = 16$, 40%) and Group I ($n = 2$, 5%) ($P < 0.0001$). HCMV was not detected. A significant association of EBV-1 to periodontal site-specific parameters was observed in Group II patients ($P < 0.05$). EBV-1 was predominant with poor glycemic status patients.

Conclusion: This study revealed that the incidence of herpes virus infection in periodontal patients was higher in diabetic patients and the examined patients were prone to EBV-1 infections.

Keywords: Diabetes mellitus, Epstein–Barr virus, multiplex polymerase chain reaction, periodontitis

Address for correspondence: Dr. Siddhartha A Varma, Department of Periodontology, School of Dental Sciences, Krishna Institute of Medical Sciences "Deemed to be University," Karad - 415 110, Maharashtra, India.
E-mail: siddhartha_varma@yahoo.co.in

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INTRODUCTION

The most common complex disease that affects the oral cavity is periodontitis. It affects about 30%–50% of adults, with 7%–15% suffering from its severity.[1] Periodontitis is a deregulated inflammatory response that results in damage of tooth-supporting tissues. Collagen fiber

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breakdown in periodontal ligament results in periodontal pocket formation between gingiva and tooth. As this destruction progresses, it leads to deepening of pockets resulting in loss of attachment. Parallel to this progressing attachment loss, there is alveolar resorption leading to tooth mobility and tooth loss. The microbial species, host immune responses and ecosystem-based aspects are accountable for its development making it a multifactorial disease.[2] The risk factors also have an important role in patients with periodontal infection, which can be modifiable behavioral or nonmodifiable intrinsic factors.[3] According to the European Federation of Periodontology and the International Diabetes Federation report (2017), periodontitis and diabetes are chronic nontransmissible diseases that are independently allied with the mortality and are known to have a bidirectional relationship.[4] Type 2 diabetes mellitus (DM2) is a well-established risk factor for periodontitis.[5] The possible links between these two diseases are elevation in levels of oxidative stress, interleukin (IL)-1-β, tumor necrosis factor-α, IL-6, receptor activator of nuclear factor-kappa B ligand/osteoprotegerin ratio and expression of Toll-like receptor 2/4.[4]

Diabetic patients with uncontrolled diabetes and high levels of glycosylated hemoglobin (HbA1c) are subjected to high degree of attachment loss, alveolar destruction and elevated local inflammatory cytokines, compared to controlled diabetic patients.[6] This influences the subgingival atmosphere, thereby controlling the microbial profile of the individual. The evidences suggest that the occurrence of specific periodontal pathogens and the levels of HbA1c in DM2 patients with periodontitis are significantly associated.[7] Hence, in such cases, detection of the specific microorganisms subgingivally plays an important role in its local and systemic propositions. Although bacterial plaque is the most common causative factor, it is unable to explain various facts such as site specificity of the disease, variations in progression of gingivitis into periodontitis and the destruction severity among the same and different individuals with similar bacterial load. Hence, it cannot be a solitary modulator of this complex disease. These facts have also spurred the efforts to uncover a supplementary etiologic factor for periodontitis.

Viruses explain the sporadic activity of the disease in specific sites. Many bacterial infections are known to occur as superinfection to viral diseases. Herpesviruses, such as herpes simplex virus-1, Epstein–Bar virus (EBV) and *Cytomegalovirus* (CMV) alone or in alliance with other subgingival pathogens, influences not only the development but also the progression of periodontitis.[8] These viruses were found in progressive periodontitis, aggressive periodontitis, HIV-associated periodontitis, necrotizing ulcerative gingivitis, periodontal abscesses and some types of medical disorders but at varying levels.[9-12] Several studies have investigated what role, inflammatory mechanisms play in the link between periodontitis and diabetes. However, barely a handful have evaluated, related or compared the prevalence of herpes virus in chronic periodontitis patients with diabetes mellitus.

MATERIALS AND METHODS

The cross-sectional study was undertaken in a tertiary care hospital at Karad, Maharashtra, India, after the due approval of the Ethics Committee. We included a total of 120 patients satisfying the inclusion (age: 35–75 years; patients with chronic moderate periodontitis based on the criteria of American Academy of Periodontology; minimum of 15 teeth present and patients with diagnosed DM2 before 4 years of this study with good, moderate or poor glycemic control based on the HbA1c test) criteria. Individuals not meeting these criteria (patients with any other systemic condition such as cardiac diseases, nutritional deficiency and immunocompromised patients; smokers and tobacco chewers; patients with symptomatic viral infection and having received periodontal treatment or antibiotics before 6 months of study and pregnant and lactating mothers) were excluded. The study procedure was explained, and due consent was obtained before commencing. The basic demographic data, socioeconomic status, oral hygiene habits, education levels, past medical and dental history and glycemic status were recorded in a predesigned pro forma.

Forty patients periodontally healthy without any systemic diseases were categorized as Group I, Group II included forty patients with chronic moderate periodontitis without DM2 and Group III comprised forty chronic moderate periodontitis patients with DM2 [Table 1]. The periodontal status was evaluated using plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment loss as per Sillness and Loe method.[13]

Supragingival plaque was removed gently using sterile cotton pellets. It was randomly collected from Group I, and from the deepest periodontal pocket site in Group II, and Group III, with a single stroke of a sterile curette. The samples were immediately transferred into microcentrifuge

Table 1: Characteristics of participants

	Group I	Group II	Group III	Total	*P*
Mean age±SD	47.30±8.47	48.05±9.86	47.88±9.37	47.74±9.18	0.9306
Male:female	20:20	18:22	17:23	55:65	0.7906

*No significance was observed with Chi-square test and ANOVA.
SD: Standard deviation

tubes containing storage medium (TE buffer) for DNA extraction and multiplex polymerase chain reaction (PCR) studies. DNA extraction was performed using Qiagen QIAamp DNA Mini Kit (51,304, Setlab, India) as per manufacturer's specifications.

PCR was performed in a thermal cycler (Himedia, Prima-DUO™, Mumbai, India), with the constituents mixed and kept at 94°C for 30s, 60°C for 40s and 72°C for 50s. The samples were further incubated for 15 min at 78°C for primer extension. Further, the amplicons were run on a 5% agarose gel and bands visualized under UV transilluminator (Bio-Imaging Systems, NY, USA). The primers used in multiplex PCR were as follows, EP5: 5'-AACAATGGCAGCAGGTAAGC-3' and EM3: 5'-ACTTACCAAGTGTCCATAGGAGC-3' for EBV-1; CP16: 5'-GTACACGCACGCTGGTTACC-3' and CM3: 5'-GTAGAAAGCCTCGACATCGC-3' synthesized from Macrogen.

R i386.3.5.1 statistical software was used for data analysis. Continuous data are represented as mean ± standard deviation and the categorical variable are represented by the frequency table. An association between categorical variables is studied using Chi-square test. Continuous data were compared using *t*-test/Mann–Whitney U-test/analysis of variance/Kruskal–Wallis test.

RESULTS

The mean age of three different groups was not significantly different, although Group II had the highest age (48.05 ± 9.86 years), followed by Group III (47.88 ± 9.37 years) and finally Group I (47.30 ± 8.47 years). In Group I, gender was equally distributed, whereas in Group I, gender was predominantly male (45%) [Table 1]. No significant difference was observed between the average and site-specific parameters PPD and clinical attachment level (CAL) at $P \geq 0.05$ in moderate chronic periodontitis patients (Groups II and III). The significance was observed between Groups I and II ($P \leq 0.001$) and Groups I and III ($P \leq 0.001$) in terms of site-specific and average PII, GI and PPD. Fair PI was observed in Group III, followed by Groups II and I ($P \leq 0.001$); similar results were obtained for GI ($P \leq 0.001$), as shown in Table 2.

Only two samples were positive for EBV-1 in Group I. Sixteen samples were positive for EBV-1 in Group II (chronic periodontitis patients without type 2 DM) and 21 samples positive for EBV-1 in Group III (chronic periodontitis patients with type 2 DM), as shown in Figure 1, while none of the samples from all the groups showed positive for human CMV (HCMV).

Group II patients showed a significant association ($P < 0.05$) of EBV-1 to periodontal site-specific parameters (PII, GI, PPD and CAL), and Group III displayed a significant association ($P < 0.05$) of EBV-1 with PII, PPD and CAL [Table 3]. Hence, it is evident that the presence of EBV-1 is significantly associated with increasing severity of periodontitis. The glycemic status of Group II patients indicated that out of forty samples, only ten had good glycemic control. The rest fell in streams of moderate and poor with 15 participants each [Figure 2]. The incidence of EBV-1 was highest in poor glycemic control and least in good control participants, thus indicating the prevalence of EBV-1 having statistical significance ($P < 0.0001$) with glycemic status of the participants.

DISCUSSION

Periodontitis, a critical periodontal disease, results in damage to the dentition and the cost incurred on treatment of the same to the patients.[14] High prevalence of periodontitis has been reported in 72%–85% of the general population which is alarming.[15,16] Thus, for ideal management, it is mandatory to have proper understanding of its etiology and risk factors influencing it. With an increase in acquaintance of periodontitis, the focus from bacterial plaque as the primary causative factor has shifted to an additional cause by the viruses (HCMV and EBV) having a significant impact on the periodontium.

Table 2: Distribution of clinical parameters in three different groups (I, II and III)

Factors	Group I	Group II	Group III	*P*	*Post hoc* analysis		
					I versus II	I versus III	II versus III
SS PPD	2.55±0.75	6.80±1.45	7.00±1.47	<0.0001[K]	<0.0001	<0.0001	0.6944
SS CAL	0.20±0.88	7.23±1.40	7.53±1.55	<0.0001[K]	<0.0001	<0.0001	0.6404
SS PII	1.23±1.10	1.93±0.73	1.88±0.69	0.0020[K]	0.0046	0.0051	0.8100
SS GI	0.60±0.71	2.10±0.67	2.10±0.63	<0.0001[K]	<0.0001	<0.0001	0.9892
PPD	2.55±0.75	3.68±0.46	3.61±0.42	<0.0001[A]	<0.0001	<0.0001	0.8116
CAL	0.20±0.88	3.70±0.53	4.01±0.51	<0.0001[A]	<0.0001	<0.0001	0.1093
PII	0.77±0.56	1.94±0.50	1.74±0.43	<0.0001[A]	<0.0001	<0.0001	0.1900
GI	0.64±0.54	1.78±0.69	1.68±0.49	<0.0001[A]	<0.0001	<0.0001	0.7434

[K]Kruskal- Wallis test, [A]One-way ANOVA. SS: Site specific, PPD: Probing pocket depth, CAL: Clinical attachment level, PII: Plaque index, GI: Gingival index

Table 3: Distribution of clinical parameters with respect to the prevalence of herpes virus in all the three groups (I, II and III)

Factors	EBV-1	Group I			Group II			Group III		
		n	Mean±SD	*P*	*n*	Mean±SD	*P*	*n*	Mean±SD	*P*
SS PII	+	2	1.50±0.71	-	16	2.19±0.66	0.0600[U]	21	2.10±0.62	0.0294[U]
	−	38	1.21±1.12		24	1.75±0.74		19	1.63±0.68	
SS GI	+	2	2.00±0.00	-	16	2.63±0.50	<0.0001[U]	21	2.29±0.64	0.0476[U]
	−	38	0.53±0.65		24	1.75±0.53		19	1.89±0.57	
SS PPD	+	2	4.00±0.00	-	16	7.94±1.39	<0.0001U	21	8.05±1.12	<0.0001[U]
	−	38	2.47±0.69		24	6.04±0.91		19	5.84±0.76	
SS CAL	+	2	4.00±0.00	-	16	8.44±1.15	<0.0001[U]	21	8.57±1.33	<0.0001[U]
	−	38	0.00±0.00		24	6.42±0.88		19	6.37±0.76	
PII	+	2	1.65±0.21	-	16	2.23±0.36	0.0012[t]	21	1.83±0.37	0.1692[t]
	−	38	0.72±0.54		24	1.74±0.48		19	1.64±0.47	
GI	+	2	2.45±0.07	-	16	2.34±0.50	<0.0001[t]	21	1.93±0.42	0.0002[t]
	−	38	0.54±0.35		24	1.40±0.51		19	1.40±0.41	
PPD	+	2	4.00±0.00	-	16	3.99±0.50	0.0003[t]	21	3.88±0.39	<0.0001[W]
	−	38	2.47±0.69		24	3.48±0.31		19	3.31±0.16	
CAL	+	2	4.00±0.00	-	16	4.27±0.29	<0.0001[t]	21	4.39±0.15	<0.0001[W]
	−	38	0.00±0.00		24	3.33±0.24		19	3.58±0.44	

[U]Mann- Whitney [U]-test; [t]*t*-test; [W]Welch *t*-test. SS: Site specific, PPD: Probing pocket depth, CAL: Clinical attachment level, PII: Plaque index, GI: Gingival index, SD: Standard deviation

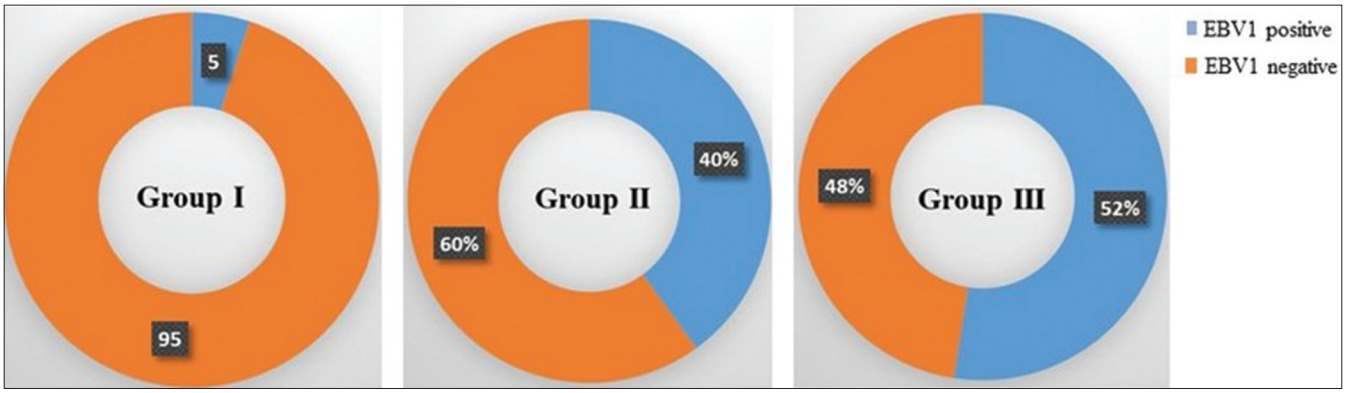

Figure 1: Prevalence of EBV1 in different groups of participants

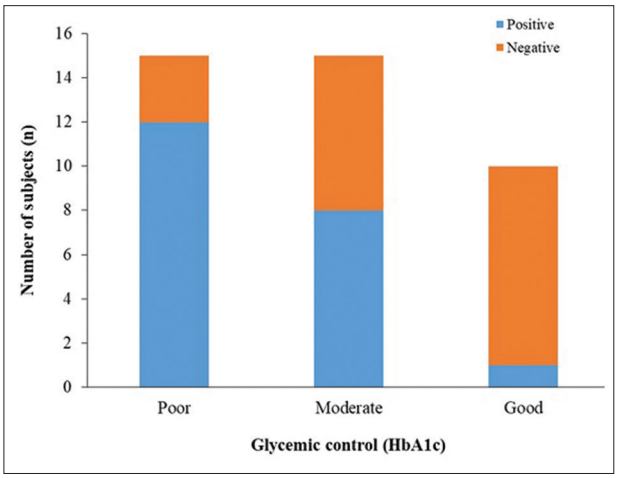

Figure 2: Glycemic status of the patients

Demographic studies from early reports suggest that the prevalence of chronic periodontitis was more in the third to seventh decade of life.[10,17] Here, the cumulative mean age was 47.74 ± 9.18 years. The study also excluded the patients with systemic disease or conditions such as cardiovascular diseases, hypertension and pregnancy, except for DM2, which are known to influence periodontitis.[18] Patients with smoking history were excluded as it imparts unfavorable effect on the occurrence and progress of periodontitis.[19]

The precise and quick method of viral detection by PCR was performed. EBV-1 was highly detected subgingivally of Group III patients, followed by Groups II and I. Similarly, Contreras *et al.* found EBV-1 in 79% of patients with chronic periodontitis in the American population.[20] Similarly, EBV-1 was found in 44% of cases by Konstantinidis *et al.* in chronic periodontitis patients in Greece population,[21] and Kubar *et al.* also found 56% of prevalence of EBV-1 in periodontitis patients.[22] This difference in detection is attributed to the difference in geographical distribution and ethnicity of the population as evaluated by Haffajee *et al.*[23]

Here, HbA1c values were utilized for categorizing (good, moderate and poor control) patients according to their glycemic control in Group III based on ADA 2009

guidelines.[24,25] Casarin *et al.* evaluated the influence of glycemic control on EBV-1 and HCMV infection in periodontal pocket of DM2 patients. EBV-1 was found more commonly present in diabetic patients as compared to HCMV, which is in line with our findings. The reason for this can be that the CMV infections are more predominant in the gingival region and also more common in sites undergoing active periodontitis.[10] In our study the sample was obtained from subgingival plaque region and the stage of periodontitis was pronounced. Poor glycemic control harbors higher levels of EBV-1 (81%) compared to those with good (42.9%) or moderate (61.1%) glycemic control.[26] Thus, the results of our study concur with previous findings where the prevalence of EBV-1 was highest in chronic periodontitis patients with DM 2 (Group III) and least in healthy patients (Group I). It was also observed that the prevalence of EBV-1 was highest in participants with poor glycemic control ($n = 12$) and was least in participants with good control ($n = 1$). Therefore, DM2 modifies the subgingival flora as this alters the neutrophil levels which are accorded for their role in chemotactic, phagocytic and microbicidal actions. This altered immune response ultimately results in accumulation of high concentration of viral and bacterial substrate.[27,28]

Subgingival samples were collected from the deepest pocket site, as the destruction and progression of periodontitis are active at the supra- and subgingival region.[29] Further, the site-specific and average PII, GI and PPD values of the healthy group (Group I) were highly significant to the chronic periodontitis patients (Groups II and III). It is indicative that the presence of EBV-1 is significantly associated with increasing severity of periodontitis and has a strong association with clinical parameters (probing depth and attachment loss).[30] It is in accordance with the reports by Chalabi *et al.*, where EBV-1 was more prevalent in pockets with PD >6 mm as compared to PD <3 mm.[31] Various reports suggest that the incidence of chronic periodontitis is more in the third to seventh decade of life.[17,32] Therefore, in this study, the age considered was 35–75 years, the mean age being 47.74 ± 9.18 with a total of 120 patients. The current study also confirms the higher pocket depth in EBV-1-positive sites than in virus-negative sites.[33] Shah *et al.* also found a positive association of EBV with increased GI, PPD and CAL, which is also reported in the present study.[10]

Thus, viral proteins or substrates that are expressed on cell membrane act as a bacterial receptor, further promoting the colonization of bacteria. As reported by Shah *et al.*, the bacterial plaque increases the gingival inflammation, it further smoothens the entry of herpes virus (EBV-1) into the periodontium, thus aggravating the periodontal disease.[10] There is an increase in the hard- and soft-tissue destruction due to the release of cytokines and chemokines. Moreover, there is a decrease in tissue turnover rate. Hence, the occurrence of EBV increases in individuals with poor glycemic status and sites with greater clinical attachment loss.

One of the limitations of this study was that the results were qualitative (showing presence or absences of EBV-1 and HCMV) and not quantitative (viral load). Hence, quantitative results from real-time PCR will prove to be helpful. Although the virus (like EBV) and diabetes with poor glycemic status have an important role in development and progression of periodontitis, the present treatment modalities are still, primarily focused on the removal of bacterial plaque. A substantial amount of work is required to develop an effective antiviral agent, maybe in the form of vaccines along with proper guidelines for using it in routine practice. In addition, as the current study was a cross-sectional study and established a relation, further studies required to establish causation.

CONCLUSION

A higher incidence of herpes virus infection was visible in moderate chronic periodontitis patients than healthy individuals. Between the evaluated viral strains, EBV-1 was predominant herpes virus detected in periodontitis patients and it was seen to be associated with the glycemic status of type 2 DM patients. The prevalence of other herpes viruses in other chronic diseases, specifically type 2 DM, has to be fostered, thereby reporting the role of glycemic diet in viral infections under chronic diseases can be accorded.

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Conflicts of interest

There are no conflicts of interest.

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