Case Report

Cytomegalovirus-Associated Periodontitis and Guillain-Barré Syndrome

Giorgio Tabanella* and Hessam Nowzari*

Background: *Guillain-Barré syndrome (GBS), an autoimmune disorder of the peripheral nervous system, is characterized by rapidly ascending neural paralysis, hyporeflexia, and areflexia. The polyneuropathy of the GBS affects one to four humans per 100,000 of the population annually throughout the world (adults and children). The pathogenesis of GBS remains unclear. However, there are increasing indications that the disease is triggered by a preceding well-established febrile infection by cytomegalovirus (CMV). The present report describes active CMV within the periodontium of a 37-year-old patient affected by GBS.*

Methods: *Real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) was performed to detect CMV, Epstein-Barr virus-1 (EBV-1), herpes simplex 1 (HSV-1) and 2 (HSV-2) virus, and enteroviruses (polio-, coxsackie-, echo-, and enteroviruses 68 and 71) from periodontal sites demonstrating advanced attachment loss. Healthy sites and sites with inflamed gingival tissue were not included in the study. Anaerobic bacterial culture determined the occurrence of potential major periodontal pathogens.*

Results: *Real-time RT-PCR and microbiologic analysis revealed the presence of a dual infection of CMV and specific bacterial plaque. CMV,* Porphyromonas gingivalis, Tannerella forsythensis, *and* Campylobacter *species were associated with periodontitis active sites, loss of attachment, and gingival bleeding. Furthermore, periodontal sites infected by active CMV had no visible radiographic crestal lamina dura.*

Conclusions: *The periodontium may serve as a reservoir for CMV and a source of viral replication. However, further research is needed to test whether viral replication in the periodontium precedes the GBS symptoms.* J Periodontol 2005;76:2306-2311.

KEY WORDS

Cytomegalovirus; gingival crevicular fluid; Guillain-Barré syndrome; periodontitis; reverse transcriptase-polymerase chain reaction.

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Guillain-Barré syndrome (GBS), an autoimmune disorder of the peripheral nervous system, is characterized by rapidly ascending neural paralysis, hyporeflexia, and areflexia. Cytokines released by lymphocytes and macrophages appear to damage Schwann cells, myelin, and axons, although the exact role of the different cytokines is uncertain.[1]

Of unknown origin, the acute polyneuropathy of the GBS affects one to four humans per 100,000 of the population annually throughout the world[2] (adults and children).[3,4] In 25% of those affected, respiratory failure requires ventilation.[5]

GBS is a heterogeneous disorder[6] including acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor sensory axonal neuropathy (AMSAN), and Fisher variant.[7] Of these, AIDP is the most common in Western countries. Although a clear correlation to the summer months has been reported in northern China,[8] in Argentina, two peaks at the summer and winter months were observed.[9]

Although the pathogenesis of GBS remains unclear, there are increasing indications that the autoimmune disease is triggered by a preceding well-established febrile infection.[2] The antecedent events of the GBS include *Campylobacter jejuni* (26% to 36% of patients),[10-13] cytomegalovirus (CMV), Epstein-Barr virus (EBV), and *Mycoplasma pneumoniae* infections.[14,15]

Severe axonal degeneration is more common following *C. jejuni* infection and severe sensory impairment following cytomegalovirus infection.

Active CMV replication was detected and quantified in severe periodontitis sites of a 37-year-old patient affected by GBS.

MATERIALS AND METHODS

A 37-year-old Hispanic patient was referred to the Advanced Periodontology Program, University of Southern California, for severe periodontitis characterized by discomfort, gingival redness, and open contacts (Fig. 1). The patient was affected by GBS and experienced paresthesia in the hands and feet, areflexia in all limbs for 1 week, and progression of

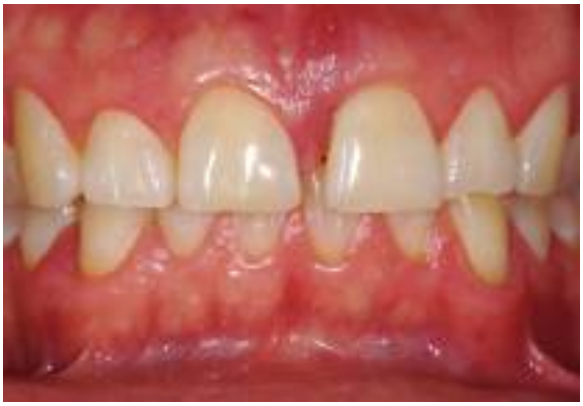

Figure 1.
Clinical view showing gingivitis and open contact.

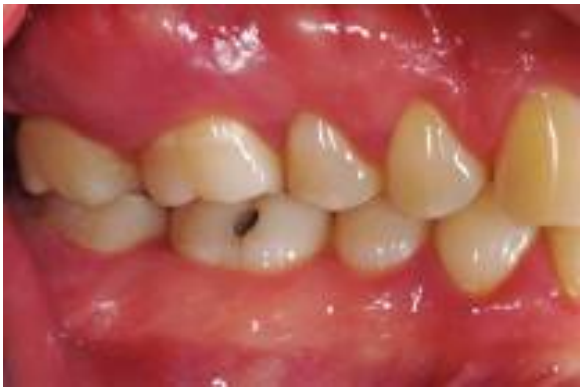

Figure 2.
Right posterior sextants.

the paresthesia over 1 month. After giving informed consent, an oral examination showed deep (>7 mm) periodontal pockets, localized severe periodontal attachment loss, and bleeding on probing (Figs. 2 and

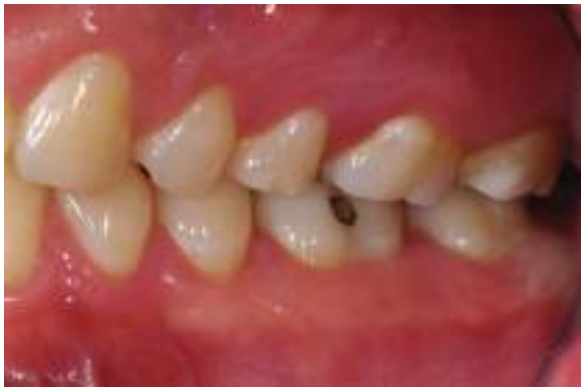

Figure 3.
Left posterior sextants.

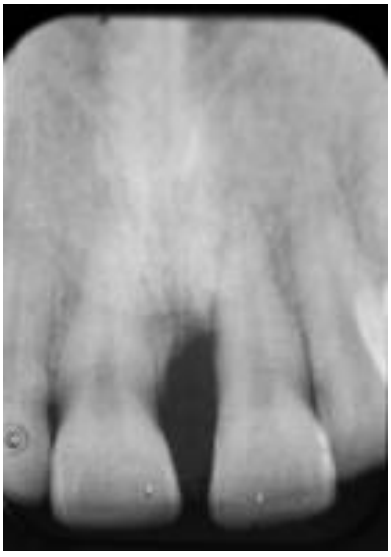

Figure 4.
Periapical radiograph showing vertical alveolar bone loss and no detectable lamina dura.

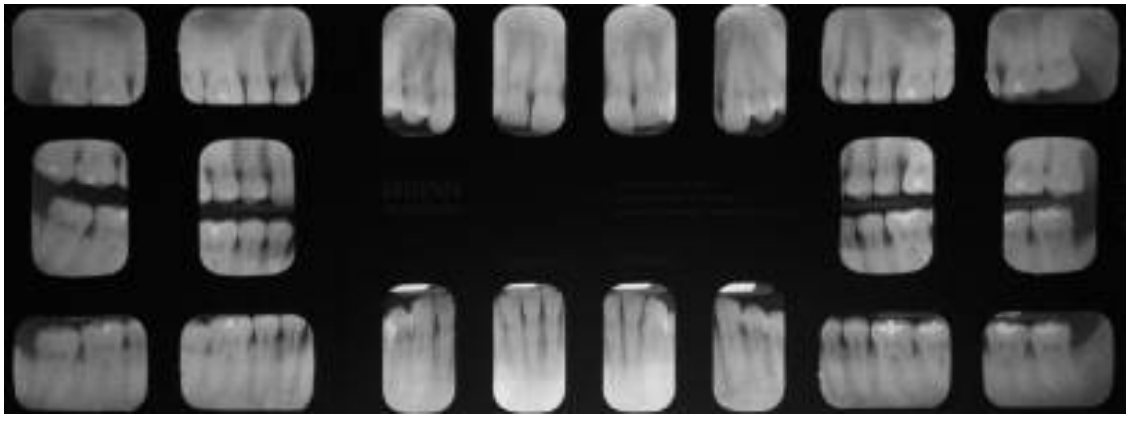

Figure 5.
Full-mouth radiographs.

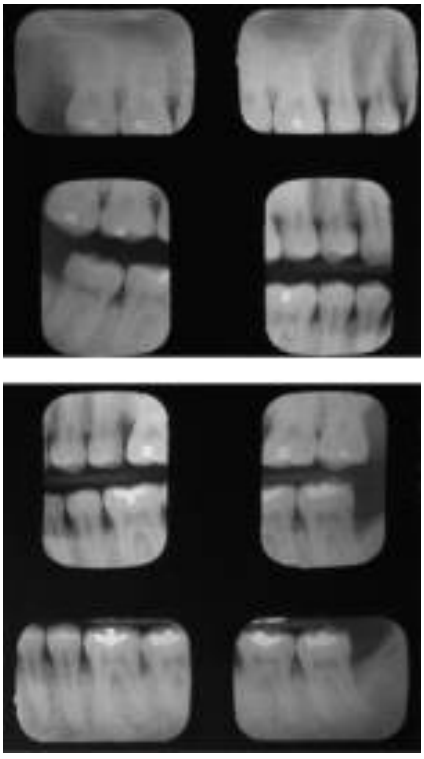

Figure 6.
Higher magnification images of periodontal sites #3 and #18 included for viral and microbiological analysis.

Table 1.

Microbiota of Periodontitis Sites

Culture	Microbiota %
A. actinomycetemcomitans	0
P. gingivalis	5.0
P. intermedia	0
T. forsythensis	8.8
Campylobacter species	10.0
Eubacterium species	8.8
Fusobacterium species	12.5
M. micros	6.3
Enteric Gram-negative rods	0
Beta hemolytic streptococci	0
Yeast	0
Eikenella corrodens	0
Staphylococcus species	0
D. pneumosintes	0

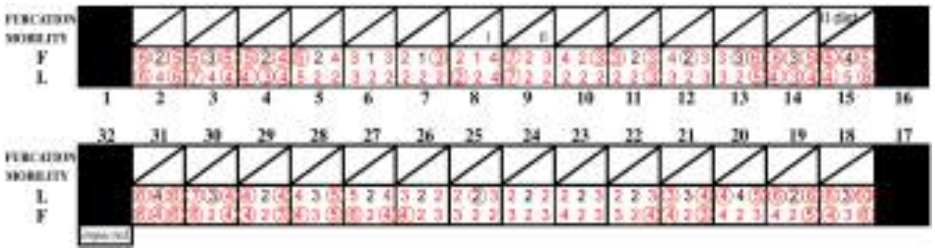

Figure 7.
Periodontal chart. The numbers refer to periodontal probing depths and the circles to bleeding on probing.

3). Radiographs revealed vertical alveolar bone loss and no detectable lamina dura between the two maxillary central incisors (Fig. 4). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Subgingival crevicular fluid was obtained from sites demonstrating advanced attachment loss (areas #3, #8 through #9, and #18; Figs. 4 through 7). Samples were not pooled from healthy or gingivitis sites. After removing supragingival plaque using sterile cotton pellets, one endodontic fine paper point was inserted to the depth of each periodontitis site and retained for 20 seconds. Subgingival specimens were collected and tested for herpesvirus and enteroviruses using real-time quantitative reverse transcriptase-polymerase chain reaction (real-time RT-PCR) technology. A culture was used to examine potential periodontopathic microorganisms.

Herpesvirus Examination

RT-PCR was employed to detect CMV, EBV-1, herpes simplex 1 (HSV-1) and 2 (HSV-2) virus, and enteroviruses (polio-, coxsackie-, echo-, and enteroviruses 68 and 71). cDNAs were amplified using oligonucleotides to target highly conserved regions of CMV, EBV-1, HSV,[16-18] and enterovirus genomes.[19] The primers and the sensitivity and specificity of the PCR techniques used to detect genomes of CMV, EBV-1, HSV, and enteroviruses are described elsewhere.[19-23] Viral nucleic acid (CMV) was extracted in accordance with the protocol provided by the manufacturer.† Briefly, a frozen paper strip carrying a viral sample was placed in 200 μl lysis buffer containing 25 μl protease and 28 μl RNA carrier

† QIAamp MinElute virus kit, Qiagen, Valencia, CA.

Table 2.

Virological Findings

Area	Human CMV (copies/ml)	EBV-1 (copies/ml)	HSV-1 (copies/ml)	HSV-2 (copies/ml)	Enteroviruses (copies/ml)	Periodontal Probing Depth (mm)
#3	325	–	–	–	–	>7
#8 through #9	275	–	–	–	–	>7
#18	750	–	–	–	–	>10

vortexed and incubated at 56°C for 15 minutes. The eluted sample was then precipitated with 97% ethanol and transferred to a column.‡ After 5 minutes, the column was centrifuged at 60,000 × g for 1 minute and washed with 500 μl wash buffer to remove unbound non-nucleic acid compound. For elution of nucleic acid, 30 μl buffer was applied to the column, incubated at room temperature for 1 minute, and centrifuged at 20,000 × g for 1 minute to collect eluted nucleic acid. To demonstrate that the PCR mix contained only RNA and was not contaminated by DNA from the sample, the RNA was added directly to the PCR as template. Because RNA cannot support amplification by PCR, any product from this reaction must come from contaminating DNA. The gel electrophoresis demonstrated no product when the isolated RNA was added as template. First, 12.5 μl 2× master mix§ was mixed with 0.5 μl of each primer, 10.5 μl RNase-free water, and 1 μl extracted viral RNA template. In parallel, serial dilutions of positive controls were amplified to obtain sample concentration. Positive and negative controls for CMV, EBV, and HSV included infected and non-infected leukocytes from human peripheral blood. Arola et al.[19] described controls for enteroviruses. Subsequently, a reaction tray was placed in a thermocycler.‖ The thermocycling reaction included denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 2 minutes. The final cycle was completed with 7 minutes of extension at 72°C.

Microbial Examination

Microorganisms were mechanically dispersed from the paper points with a vortex mixer at the maximal setting for 45 seconds and 10-fold serially diluted in VMG I anaerobic dispersion solution (0.25% tryptose, 0.25% thione E peptose, and 0.5% Nacl). Using a sterile bent glass rod, 0.1 ml aliquots of 10^3 dilutions were plated onto non-selective 4.3% brucella agar¶ supplemented with 0.3% bactoagar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione. Aliquots diluted in VMGA III medium (5% bactogelatin and 0.05% thione E peptone) were plated onto TSBV medium (tryptic soy, serum, bacitracin, and vancomycin) for culture of *Actinobacillus actinomycetemcomitans*, enteric Gram-negative rods, and yeasts. The non-selective blood agar was incubated at 35°C in an anaerobic chamber# containing 85% N_2 10% H_2 5% CO_2 for 10 days. TSBV medium was incubated in 10% CO_2 in air at 35°C for 4 days. Presumptive identification of organisms included *A. actinomycetemcomitans*, *Prevotella intermedia/Prevotella nigrescens*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Campylobacter* species, *Fusobacterium* species, *Micromonas micros*, enteric Gram-negative rods, and *Candida* species.

RESULTS

RT-PCR and microbiologic analysis revealed the presence of a dual infection of CMV and specific bacterial plaque (Tables 1 and 2). CMV, *P. gingivalis*, *T. forsythensis,* and *Campylobacter* species were associated with active periodontitis sites, loss of attachment, periodontal probing depth, and gingival bleeding. Furthermore, periodontal sites infected by active CMV had no visible radiographic crestal lamina dura.

DISCUSSION

CMV infection has been associated with Guillain-Barré syndrome, meningoencephalitis, and retinitis. GBS is the most common cause of acute neuromuscular paralysis in developed countries. A number of studies have shown indicators of poor prognosis to be advanced age,[24-29] need for mechanical ventilation, shorter latency to nadir, particular antecedent infections,[10] and cytomegalovirus.[30]

To the best of our knowledge, this is the first report describing active CMV-associated periodontitis and Guillain-Barré syndrome. CMV infection seems to be a relevant well-established antecedent infection in 5% of patients affected by Guillain-Barré syndrome in a study from Japan[31] and in 11% to 22% of patients in studies from Europe.[12,32,33] Periodontal herpesviruses

‡ MinElute, Qiagen.
§ QuantiTect SYBR Green RT-PCR Master Mix, Qiagen.
‖ iCycler, Bio-Rad Laboratories, Hercules, CA.
¶ BBL Microbiology Systems, Cockeysville, MD.
# Coy Laboratory Products, Ann Arbor, MI.

have been linked to various forms of aggressive periodontitis,[34-36] Papillon-Lefèvre syndrome,[37] Fanconi's anemia,[38] Trisomy 21,[39] and renal transplant complications.[40]

In the present report, periodontal disease occurred with a markedly localized pattern of destruction. The maxillary central incisors and the maxillary and mandibular molars exhibited advanced periodontal attachment loss, whereas the mandibular anterior sextant demonstrated only gingivitis and no clinical or radiographic evidence of periodontal attachment loss. The tissue tropism of CMV infections might explain the segmental pattern of breakdown (maxillary and mandibular molars) and the mirror-like bone destruction in contralateral teeth of this patient.[41] However no conclusion can be drawn based on this study because samples from healthy and gingivitis sites were not included. Furthermore, because presumptive identification of organisms included periodontopathogen species upon culture, this method might have missed non-cultivable species like spirochetes.

However, the present report confirms the coinfection of CMV, *T. forsythensis, Campylobacter* species,[42] and *P. gingivalis*.[43] Also, since *C. jejuni* infection has been linked to GBS by different authors,[10-13] it would be interesting in the future to analyze *Campylobacter* species in the gingival crevicular fluid and their possible role in the pathogenesis of GBS.

Herpesvirus periodontal infections may cause immune suppression and induce cytokine production. The interaction between CMV and bacteria is probably bidirectional, with bacterial enzymes or other inflammation-inducing products activating herpesviruses, which subsequently might increase bacterial pathogen counts. Although it should be cautioned that evidence of the presence of CMV in various disease entities does not prove causality by itself, real-time quantitative RT-PCR differentiates active versus persistent infection. The viral load in the gingival crevicular fluid may indicate the extent of infection, virus-host interactions, and the response to antiviral therapy, all of which can play a role in the treatment regimen selected. Although we could speculate that gingival tissue may serve as a reservoir for the virus and a potential source of viral replication prior to the onset of GBS, further research should test whether viral replication in the periodontium precedes GBS symptoms.

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