Serum and saliva cytokine levels in a patient with brain abscess due to periodontitis

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1. INTRODUCTION
Brain abscesses most frequently occur because of bacterial dissemination from a primary lesion at a distant site or direct contiguous invasion from an adjacent site of infection. For example, neurosurgical procedures or head trauma often lead to infection by skin-colonizing bacteria, such as *S. aureus* and *S. epidermis*, or by Gram-negative bacteria. In a hematogenous spread, bacteria are associated with underlying cardiac disease (i.e., endocarditis or congenital heart defect), pulmonary disease (i.e., arteriovenous fistula), or distant foci of infection (i.e., primarily skin, paranasal sinuses, and teeth). *Staphylococcus* and *Streptococcus* species are often identified in brain abscesses after a hematogenous spread.

Brain abscesses of odontogenic origin are rare, but exist, accounting for only a small fraction of all reported brain abscesses. Three criteria for establishing the diagnosis of an odontogenic brain abscess were defined as follows: i) no alternative source of bacteremia is found; (ii) microbiological examination of sputum showed *Fusobacterium nucleatum* and *Prevotella*. No ear, nose, throat, and gastrointestinal infections were observed. Severe periodontitis was noted. Bacteriological examination of the right maxilla showed *Porphyromonas gingivalis*, and we detected a high level of serum antibody to *P. gingivalis*. Broad-spectrum antimicrobial therapy, dental calculus removal, and intraoral remediation improved the patient’s general condition. Brain MRI at the time of discharge showed a decrease in the size of the nodules, and after three months, the level of serum antibody to *P. gingivalis* decreased. Elevated pro-inflammatory cytokines such as interleukin (IL)-1α and IL-1β and chemokines such as IL-8, macrophage chemoattractant protein-1 (MCP-1), granulocyte colony-stimulating factor (G-CSF), and vascular endothelial growth factor (VEGF) levels in the saliva significantly decreased after oral treatment. Similarly, elevated serum IL-1α, IL-1β, IL-8, MCP-1, eotaxin, regulated on activation, normal T-cell expressed and secreted (RANTES), VEGF, and tumor necrosis factor-alpha (TNF-α) levels decreased after oral treatment. These findings supported our hypothesis that periodontopathic bacteria produce inflammation cytokines, pro-inflammatory cytokines migrate from systemic circulation to the brain, and their expression increases, and transition of that response into an adaptive form might have been the etiology of brain abscess in our patient.

**Key Words**: periodontitis, brain abscess, cytokines
studies reveal organisms typically found in oral microflora; and (iii) clinical or radiographic signs of active dental or periodontal disease are present. Periodontitis or caries with periapical involvement, particularly of the molar teeth, is believed to be the biggest risk factor of a central nervous system (CNS) infection. Odontogenic infection can also spread to the cranial vault via the hematogenous pathway rather than directly. Odontogenic brain abscesses are polymicrobial because of the diverse and abundant microflora the oral cavity harbors. For example, common Gram-positive bacteria reported in brain abscesses\textsuperscript{2} include *Streptococcus viridans* and *Actinomyces*\textsuperscript{7}; common anaerobes include *Peptostreptococcus*, *Prevotella*,\textsuperscript{8} and *Fusobacterium*; and common Gram-negative bacteria include *Aggregatibacter actinomycetemcomitans*\textsuperscript{9} and *Eikenella corrodens*. However, intracranial culture results poorly correlate with peripheral blood and/or oral culture results,\textsuperscript{4} which makes it uncertain how circulating bacteria cross the blood–brain barrier (BBB) and how bacterial entry into the CNS cause inflammation and complications, such as pleocytosis, BBB disruption, and neural injury.

Here, we report a case of a brain abscess patient with severe periodontitis and a high level of serum antibody to *Porphyromonas gingivalis*, one of the most etiologically predominant microorganisms in periodontal disease. We examined the serum and saliva cytokine levels in the patient and determined the brain abscess etiology on the basis of a report of different groups of bacteria inducing quantitatively distinct local immune fingerprints.\textsuperscript{10}

2. CASE REPORT

2.1 History

A 66-year-old man with a recent history of high-grade fever for a week and sudden onset of right-sided paresis was admitted to our hospital. Clinical-neurological examination showed that his consciousness level on the Japan Coma Scale was 30. In addition, his body temperature was >38°C, heart rate was 92 beats/min, respiratory rate was 29/min, and blood pressure was 203/109 mmHg.

2.2 Clinical examination

Brain magnetic resonance imaging (MRI) revealed multiple high-intensity oval nodules in the right side of the patient’s parieto-occipital lobe and corona radiate and in the left side of the thalamus (Figures. 1A,B). Blood tests showed increased leukocytes (10,532/µl; normal range: 3800–9500/µl), cerebrospinal fluid (CSF) white blood cells (235–7860 mm\textsuperscript{3}; normal: <6/mm\textsuperscript{3}), and protein content (108–50 mg/dl; normal: <40 mg/dl), indicating that the meningeal inflammation and pleocytosis were due to disruption of
BBB and bacterial entry (Table 1). In addition, the patient’s glycosylated hemoglobin A\textsubscript{1C} was 8.2\% (normal: <6.2\%) (Table 1). On the basis of these data, the patient was diagnosed with a brain abscess and administered empirical antibiotic treatment with cefotaxime sodium (2.0 g/day).

**Figure 1:** Brain MRI of the patient on admission. (A) T1-weighted brain MRI revealed multiple high-intensity oval nodules in the patient’s right parietooccipital lobe and corona radiate and in the left side of the thalamus on admission. (B) MRI sagittal section also revealed a high-intensity oval nodule in the left side of the thalamus, close to the brainstem. MRI, magnetic resonance imaging
Table 1: Laboratory findings at the time of the first dentistry medical examination

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Blood Chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC 10,532 /ml</td>
<td>T-Bil 0.7 mg/dl</td>
</tr>
<tr>
<td>RBC 428 × 10⁴ /ml</td>
<td>AST 20 IU/l</td>
</tr>
<tr>
<td>Hb 13.4 g/dl</td>
<td>ALT 14 IU/l</td>
</tr>
<tr>
<td>Ht 38.8%</td>
<td>ALP 130 IU/l</td>
</tr>
<tr>
<td>Plt. 26.2 × 10⁴ /ml</td>
<td>LDH 171 IU/l</td>
</tr>
<tr>
<td></td>
<td>γ-GTP 39 IU/l</td>
</tr>
<tr>
<td>Examination of sputum bacteria</td>
<td>TP 7.1 g/dl</td>
</tr>
<tr>
<td>Fusobacterium nucleatum (+)</td>
<td>Alb 3.2 g/dl</td>
</tr>
<tr>
<td>Prevotella Sp. (+)</td>
<td>Na 131 mEq/l</td>
</tr>
<tr>
<td>Examination of bacteria of the right</td>
<td>K 3.5 mEq/l</td>
</tr>
<tr>
<td>upper first molar</td>
<td>Cl 95 mEq/l</td>
</tr>
<tr>
<td>Porphyromonas gingivalis (+)</td>
<td>CRP 10.5 mg/dl</td>
</tr>
<tr>
<td></td>
<td>BS 234 mg/dl</td>
</tr>
<tr>
<td></td>
<td>HbA1c 8.2 %</td>
</tr>
</tbody>
</table>

Cerebrospinal fluid
WBC count 7,860 mm⁻³ (6 mm⁻³ in normal)
Protein content 250 mg/dl (<40 mg/dl in normal)

WBC, white blood cell; RBC, red blood cell; T-Bil, total bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; γ-GTP, gamma-glutamyltransferase; CRP, C-reactive protein; HbA₁C, hemoglobin A₁C

Since possible primary focus was important for treatment selection, we further clinically investigated the origin of the brain abscess. Bacteriological examination of the patient’s sputum showed *Fusobacterium nucleatum*, *Prevotella*, and indigenous bacteria from the upper respiratory tract (Table 1). Therefore, we excluded ear, nose, throat, and gastrointestinal infections, but we suspected an odontogenic source.

2.3 Treatment
After confirming that there was no indication of clinical deterioration, we began the patient’s dental examination. As shown in Figures. 2A and 2B, we observed thick dental plaque and calculus along the gum line of the patient’s mandible and maxilla, especially the bilateral first molars of the maxilla, in addition to redness, swelling, spontaneous discharge of pus, a 10 mm periodontal pocket, and level 3 tooth movement according to the Miller classification.¹¹
Figure 2: Oral observations and examination of the periodontium

(A) The white arrow indicates the right maxillary first molar. (B) The white arrow indicates the left maxillary first molar. Thick dental plaque and calculus are observed along the gum line of the mandible and maxilla, especially the bilateral maxillary first molars, where there is redness, swelling, spontaneous discharge of pus, a 10 mm periodontal pocket, and level 3 tooth movement as per the Miller classification.

Bacteriological examination showed *P. gingivalis* in the right maxilla. On dental examination, we also detected a high level of titers of serum immunoglobulin G (IgG) antibody to *P. gingivalis*. Initial treatment included dental calculus removal, intraoral remediation, and antibiotics (2.4–1.0 g/day). After confirming that the patient’s general condition improved, we performed extraction of the bilateral maxillary first molars one week after dental examination (Figure 3).
Figure 3: Clinical course of the patient after hospitalization for a month

The patient’s titers of serum IgG antibody to *P. gingivalis* decreased from 83.8 (before tooth extraction) to 58.5 (after 6 months) and then to 30.7 (after 9 months; Figure 4). Brain MRI showed an improvement in high-intensity nodules six months after tooth extraction (Figure 5), that is, clinical improvement with decreasing abscess size.

Figure 4: Serum IgG antibody titers of *P. gingivalis*

High levels of serum antibody to *P. gingivalis* were seen on admission, but the levels decreased 6 and 9 months after tooth extraction. Results are expressed as EU. IgG, immunoglobulin G; EU, enzyme-linked immunosorbent assay (ELISA) units
Figure 5: Brain MRI 6 months after tooth extraction
Brain MRI shows improvement in high-intensity nodules 6 months after tooth extraction. MRI, magnetic resonance imaging

3. METHODS
3.1 Quantitative measurement of serum and saliva cytokine levels
We measured the patient’s serum and saliva cytokine levels before and six months after tooth extraction. Briefly, peripheral blood and saliva samples were collected. Blood samples were centrifuged at 7,000 rpm and 2,000 x g to collect plasma, which was stored at −80°C. Saliva samples were collected from the sublingual space using a Salimetrics Oral Swab (SOS) (#5001.02; Funakoshi, Japan) for 30 s and centrifuged at 3,000 rpm for 15 min. The supernatants were stored at −80°C.

We measured the serum and saliva levels of 27 items, including IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, and IL-17; eotaxin; fibroblast growth factor (FGF) basic; granulocyte colony-stimulating factor (G-CSF); granulocyte macrophage colony-stimulating factor (GM-CSF); interferon-gamma (INF-γ); INF-γ-induced protein 10 (IP-10); macrophage chemoattractant protein-1 (MCP)-1; macrophage inflammatory protein-1 alpha (MIP-1α); platelet-derived growth factor (PDGF)-BB; macrophage inflammatory protein-1 beta (MIP-1β); regulated on activation, normal T-cell expressed and secreted (RANTES); tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF).

Serum and saliva cytokine levels were measured before and six months after tooth extraction using a Bio-Plex Pro™ Human Cytokine 27-Plex panel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a Bio-Plex® Suspension Array System (Bio-Rad Laboratories, Inc.). The limit of detection for all target cytokines was 0.2–32,000 pg/ml. Serum samples (minimum volume: 12 µl) were diluted to a ratio of 1:4 using Bio-Plex Human Serum Diluent Kits (Bio-Rad Laboratories, Inc.). Assays were performed according to the manufacturer’s instructions. Briefly, 50 µl of each sample diluted to a ratio of 1:4 at room temperature was added to a suspension of beads coated with 27 primary antibodies in each well of an assay plate, incubated for 30 min at room temperature, and then shaken at 300 rpm. After incubation, the beads were washed thrice with a washing buffer and subsequently reacted with a mixture of 27 distinct biotin-conjugated secondary antibodies for 30 min. Next, the beads were again washed, resuspended in an assay buffer containing streptavidin-phycoerythrin (Str-PE), and shaken for 10 min at room temperature. Finally, the beads were washed and resuspended in an assay buffer without Str-PE. Serum and saliva cytokine levels
were measured using a Bio-Plex® Array Reader (Bio-Rad Laboratories, Inc.). We also identified cytokine types on the basis of bead luminescence and, using phycoerythrin luminescence, determined the amount of cytokine captured by the primary antibody on the beads. Standard curves for cytokines were generated using reference cytokine levels supplied by the manufacturer. Each test sample was subjected to duplicate examination.

3.2 Standard values from healthy controls
To evaluate the standard values in this study, 25 healthy controls (12 women, 13 men; mean age, 21 years) were enrolled. Peripheral blood and saliva samples were collected from the controls. Data were expressed as mean ± standard deviation.

4. RESULTS
4.1 Serum and saliva cytokine levels
As shown in Table 2, the patient’s saliva IL-1α, IL-1β, MCP-1, and G-CSF levels, which were very high before oral treatment, significantly decreased after oral treatment. His saliva IL-8 and VEGF levels, which were also very high before oral treatment, only slightly decreased after oral treatment. Saliva IL-6 levels, which were high before oral treatment, also decreased after oral treatment, while saliva IP-10 levels, which were also high before oral treatment, increased after oral treatment. These findings indicated severe inflammation in the patient’s oral cavity.

| Table 2: Saliva cytokine levels of standard value before and after oral treatment |
|-----------------|-----------------|-----------------|-----------------|
| Saliva Cytokines | Before (pg/ml)  | After (pg/ml)   | Standard values (pg/ml) |
| IL-1α            | 1244.2          | 21.5            | 615.9 ± 313.8   |
| IL-1β            | 355.0           | 1.1             | 42.4 ± 44.7     |
| IL-6             | 14.8            | 8.5             | 12.4 ± 21.9     |
| IL-8             | 844.0           | 727.7           | 220.7 ± 94.1    |
| IL-12            | 68.0            | 77.4            | 83.8 ± 37.7     |
| IL-17            | 1.1             | 3.0             | 4.8 ± 16.8      |
| Eotaxin          | 8.3             | 9.1             | 21.9 ± 33.0     |
| FGF basic        | 0.2             | 0.2             | 6.8 ± 13.0      |
| G-CSF            | 113.9           | 2.5             | 20.8 ± 24.1     |
| IFN-γ            | 78.5            | 60.1            | 168.2 ± 322.6   |
| IP-10            | 1244.2          | 1816.4          | 1073.0 ± 1062.5 |
| MCP-1            | 61.3            | 0.2             | 51.8 ± 64.3     |
| PDGF-BB          | 3.4             | 12.4            | 28.6 ± 68.0     |
| RANTES           | 8.6             | 6.2             | 5.3 ± 9.4       |
| TNF-α            | 19.5            | 12.6            | 23.0 ± 42.9     |
| VEGF             | 2166.1          | 1598.3          | 773.2 ± 543.9   |

IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-gamma; IP-10, INF-γ-induced protein 10; MCP-1, macrophage chemotactant protein-1; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T-cell expressed and secreted; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor
Patient’s serum levels of IL-1α, IL-1β, IL-8, MCP-1, eotaxin, RANTES, VEGF, and TNF-α, which were high before oral treatment, decreased after oral treatment; serum IL-1α and IL-1β levels showed a significant decrease (Table 3). Increased pro-inflammatory cytokines like IL-1α, IL-1β, IL-6, and IL-12 and chemokines like IL-8, MCP-1, MIP-1α, and RANTES were detected in both saliva and serum; however, serum and saliva cytokine levels are not always correlated. For example, both serum and saliva IL-8, IP-10, and VEGF levels were high before oral treatment, but their levels in saliva did not decrease much after oral treatment. Serum RANTES levels also did not change with oral treatment. Interestingly, as mentioned earlier, saliva IP-10 levels were very high both before and after oral treatment, but serum IP-10 levels significantly decreased after oral treatment. In contrast, saliva G-CSF levels significantly decreased after oral treatment.

**Table 3:** Serum cytokine levels of standard value before and after oral treatment

<table>
<thead>
<tr>
<th>Serum Cytokines</th>
<th>Before (pg/ml)</th>
<th>After (pg/ml)</th>
<th>Standard values (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>452.8</td>
<td>7.2</td>
<td>264.0 ± 260.3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>597.3</td>
<td>0.2</td>
<td>99.9 ± 137.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>11.7</td>
<td>2.8</td>
<td>15.6 ± 21.9</td>
</tr>
<tr>
<td>IL-8</td>
<td>36.9</td>
<td>0.2</td>
<td>20.9 ± 18.4</td>
</tr>
<tr>
<td>IL-12</td>
<td>44.6</td>
<td>3.5</td>
<td>52.5 ± 32.8</td>
</tr>
<tr>
<td>IL-17</td>
<td>63.4</td>
<td>27.1</td>
<td>102.9 ± 57.9</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>47.5</td>
<td>0.2</td>
<td>33.4 ± 18.2</td>
</tr>
<tr>
<td>FGF basic</td>
<td>79.7</td>
<td>63.3</td>
<td>92.1 ± 43.4</td>
</tr>
<tr>
<td>G-CSF</td>
<td>54.8</td>
<td>0.2</td>
<td>47.8 ± 26.6</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>97.7</td>
<td>0.2</td>
<td>72.2 ± 65.5</td>
</tr>
<tr>
<td>IP-10</td>
<td>297.0</td>
<td>11.6</td>
<td>277.1 ± 344.7</td>
</tr>
<tr>
<td>MCP-1</td>
<td>74.4</td>
<td>25.62</td>
<td>40.1 ± 33.6</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>238.5</td>
<td>4.4</td>
<td>2175.8 ± 1463.6</td>
</tr>
<tr>
<td>RANTES</td>
<td>8015.3</td>
<td>7.9</td>
<td>6045.0 ± 3103.0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>37.9</td>
<td>6.2</td>
<td>20.7 ± 17.9</td>
</tr>
<tr>
<td>VEGF</td>
<td>161.7</td>
<td>22.8</td>
<td>128.7 ± 77.0</td>
</tr>
</tbody>
</table>

IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-gamma; IP-10, INF-γ-induced protein 10; MCP-1, macrophage chemotactant protein-1; PDGF, platelet-derived growth factor; RANTES,regulated on activation, normal T-cell expressed and secreted; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

The patient met all criteria for odontogenic brain abscesses; that is, clinically, ear, nose, throat, and gastrointestinal infections were excluded, yet severe periodontitis was obvious, and the level of serum antibody to *P. gingivalis* was higher on admission. Spread from a contiguous source of infection (e.g., sinusitis or mastoiditis) or direct inoculation into the CSF (e.g., via skull fracture or ventricular shunts) were unlikely routes for bacterial entry into the patient’s CNS; rather, bacteria presumably...
entered the patient’s brain through hematogenous dissemination. On the basis of all our findings, we determined this to be a case of brain abscess due to severe periodontitis.

5. DISCUSSION

Increased pro-inflammatory cytokines like IL-1α, IL-1β, IL-6, and IL-12 and chemokines like IL-8, MCP-1, MIP-1α, and RANTES were detected in both saliva and serum. In chronic periodontitis, increased pro-inflammatory cytokines, such as IL-1α, IL-1β, IL-6, and IL-12, and chemokines, such as IL-8, MCP-1, MIP-1α, and RANTES, have been reported. Saliva IL-1β levels reflect disease severity and response to therapy. Indeed, IL-1β acts on endothelial cells to promote adhesion and migration of leukocytes into inflamed periodontal tissue sites. IL-1β and TNF-α are believed to be secreted by activated parenchymal microglia and can be potent inducers of cell death. However, serum and saliva cytokine levels are not always correlated. For example, IL-8, which is a leukocyte chemotactic cytokine and plays a multifunctional role in disease pathogenesis, i.e., regulates neutrophil chemotaxis, angiogenesis, and epithelial proliferation by specific receptors, VEGF and PDGF-BB, RANTES 1 G-CSF and GM-CSF. Therefore, it is unclear whether different groups of bacteria induce quantitatively distinct local immune fingerprints, even with a specific biomarker signature associated with Gram-positive and -negative bacteria. Cytokine levels may be critical because these levels are significantly higher in saliva than in the blood.

5.2 Periodontitis as a result of chronic inflammation

Periodontal disease is initiated by a small subset of endogenous Gram-negative periodontal bacteria, such as P. gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, and Treponema denticola, which trigger innate, inflammatory, and adaptive immune responses. These bacteria induce a local inflammatory response through antigen stimulation and release of toxic products. For example, P. gingivalis becomes more invasive because of several known virulence factors, such as lipopolysaccharide (LPS), fimbrae, proteases, and outer membrane vesicles. P. gingivalis LPS causes a high innate immune response through host receptors, such as toll-like receptor 2 (TLR-2) and TLR-4, on the host cell surface, leading to secretion of IL-1, IL-6, IL-8, and TNF-α in host cells. This virulence
factor is an important pathogenic determinant in the initiation, progression, and/or severity of periodontitis. *P. gingivalis* also has major and minor fimbriae on its cell surface, and both fimbriae contribute to establishing persistent infection and the development of periodontitis with the expression of cytokines, such as IL-1, IL-6, and TNF-α.\(^{30,32}\) The defense response includes activation of both innate and acquired immunity with infiltration of gingival tissues with neutrophils and expression of antibodies to B-cells. For example, epithelial cells, periodontal ligament fibroblasts, leukocytes, osteoblasts, and dendritic cells release cytokines and chemokines, such as IL-1, IL-6, chemokine (C-X-C motif) ligand 8, and TNF-α, in addition to proteases, including matrix metalloproteinases (MMPs), prostaglandins, and other inflammatory mediators.\(^{30-32}\) These remind us an idea that periodontopathic bacteria produce inflammation cytokines, and pro-inflammatory cytokines might migrate from systemic circulation to the brain and their expression increases, causing a brain abscess.

**5.3 Brain abscess formation**

Neuroinflammation is an innate immune response in the CNS against harmful and irritable stimuli, such as pathogens and metabolic toxin waste, as well as chronic mild stress. Upon stimulation, microglia release excessive amounts of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6.\(^{33,34}\) This IL-1 family of cytokines, IL-1α, IL-1β, IL-18, and IL-33, is involved in a variety of immune reactions as well as in the initiation, regulation, and maintenance of inflammation.\(^{35-38}\) Astrocytes are immune effector cells that express cytokines (IL-1, IL-6, IL-10, INF-α and INF-β, and TNF-α and TNF-β), and chemokines and mediate inflammation and immune reactivity in the brain.\(^{39}\) Pro-inflammatory cytokines attract leukocytes and enhance their proliferation at the inflammation site. They also stimulate cytotoxicity, proteolytic enzyme release, prostaglandin synthesis, and secondary cytokine synthesis and secretion. Thus, the production of pro-inflammatory molecules might promote site-specific leakage across the BBB and establishment of a noxious peripheral adaptive immune response by targeting of neuronal antigens.

**5.4 Why are brain abscesses of odontogenic origin rare despite harboring so many oral pathogens?**

Mishra et al.\(^{40}\) in their study evaluated the role of the single-nucleotide polymorphism (SNP; specific alleles/genotypes) of TNF-α (−308 G>A) and IL-1β (−511 C>T) as a risk factor in the pathogenesis of brain abscesses. Activated T-lymphocytes and macrophages are known to be the principle source of cytokines, such as TNF-α and IL-1β. Thus, the increased production of these cytokines is associated with enhanced response to infection in which local induction of the cytokines facilitates microbial invasion elimination. TNF-α is also involved in early BBB breakdown, upregulation of endothelial adhesion molecules, leukocyte attraction to nerve tissue, and macrophage activation.\(^{24-27}\) Another important factor that controls BBB integrity is VEGF.\(^{41}\) Abnormal regulation of VEGF levels might be a factor in predisposing the brain to uncontrolled inflammation. Although VEGF does not cause inflammation by itself, it might modulate the immune response in the CNS by controlling BBB permeability, allowing exposure of normally sequestered CNS antigens to peripheral immune effector molecules.

In addition, the genotype distributions of intercellular adhesion molecule 1 (ICAM-1) (K469E), a member of the immunoglobulin superfamily, and MCP-1 (−2518 A>G) are also significantly different between patients
ICAM-1 and MCP-1 are known to be upregulated by cytokines, such as TNF-α and IL-1β. Under inflammatory and infectious conditions, ICAM-1 is highly inducible in many cell types, which further leads to activation of chemokines, such as MCP-1. MCP-1 belongs to the CC chemokine family and is encoded by the CCL2 gene mapped to chromosome 17q11. After infection, its production increases by macrophages, endothelial cells, and astrocytes in the CNS. Therefore, polymorphisms in these molecules seem to increase the risk for brain abscess development, and they might also play a key role in inflammation and immune surveillance, help in leukocyte migration across endothelial cells, and facilitate leukocyte recruitment into inflammatory sites.

Interestingly, on the other hand, Nikolopoulos et al. investigated the potential association of cytokine gene polymorphism with either aggressive or chronic periodontal disease. They found a statistically significant association of IL-1α (–889 C>A) and IL-1β (3953/4 C>T) with chronic periodontal disease. They also found a weak positive association between IL-1β (–511 T>C) and chronic periodontal disease. This might explain that periodontopathic bacteria produce inflammatory cytokines that are probably associated with systemic diseases such as heart disease or diabetes mellitus.

### 6. CONCLUSIONS

Cytokines, such as IL-1, IL-6, and TNF-α, play an important role in the focal immunopathology of diseases, and the genetic control of the cytokine function affects the appearance or severity of periodontitis and/or brain abscesses. Although the oral cavity harbors a diverse and abundant microflora, *P. gingivalis* is a rare cause of the origin of brain abscesses. Periodontopathic bacteria produce inflammation cytokines, pro-inflammatory cytokines migrate from systemic circulation to the brain, and their expression increases; this might have been the etiology of the brain abscess in our patient.

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### Conflict of Interest Statement:

The authors declare that the research was conducted in the absence of any commercial and financial relationships that could be construed as potential conflicts of interest.

### Author Contributions:

Conception and design of the study: OM, TC, YS, TY; Cytokine measurements: OM, DS; Figure generation: OM, DS; Draft writing of the manuscript: OM, TC, YS. All authors approved the manuscript for publication and agree on all aspects of the work.
REFERENCES

17. Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult...


34. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev


