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Research Article

# Evaluation of Chemokine Profiles and Antioxidant Response in Patients with Periodontitis

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## Abstract

Periodontitis is a chronic, multifactorial, immune-mediated inflammatory disease associated with biofilm dysbiosis and progressive destruction of tooth-supporting tissues. In addition to bacteria, oral protozoa such as *Entamoeba gingivalis* and *Trichomonas tenax* have been investigated for their potential role in modulating the periodontal microenvironment. This study aimed to evaluate chemokine concentrations and copper-zinc superoxide dismutase activity in periodontal-site samples from patients with periodontitis according to the presence of protozoa. Periodontal samples were examined by direct wet-mount microscopy and Rapid Panoptic-stained smears for qualitative detection of protozoa. Participants were classified according to the presence or absence of protozoa in periodontal-site samples. Concentrations of IL-8, MCP-1, RANTES, IP-10, and MIG were determined by flow cytometry using the Cytometric Bead Array assay, whereas copper-zinc superoxide dismutase activity was assessed using the nitroblue tetrazolium reduction method. Protozoa-positive samples showed significantly higher concentrations of IL-8 and RANTES, while MCP-1, MIG, and IP-10 did not differ significantly between groups. Likewise, local copper-zinc superoxide dismutase activity did not differ significantly. These findings suggest that the presence of protozoa in periodontal sites is associated with selective modulation of inflammatory mediators involved in immune cell recruitment, particularly IL-8 and RANTES. The absence of changes in copper-zinc superoxide dismutase activity indicates that, under the conditions evaluated, this inflammatory association was not accompanied by detectable alterations in this component of local antioxidant defense.

**Keywords:** Periodontitis; Chemokines; Superoxide Dismutase; Oral Protozoa; Immunoinflammatory Response.

## Abbreviations

CBA: Cytometric Bead Array; CCL2: C-C Motif Chemokine Ligand 2; CCL5: C-C Motif Chemokine Ligand 5; CCR1: C-C Chemokine Receptor Type 1; CCR5: C-C Chemokine Receptor Type 5; CXCL8: C-X-C Motif Chemokine Ligand 8; CXCL9: C-X-C Motif Chemokine Ligand 9; CXCL10: C-X-C Motif Chemokine Ligand 10; CXCR3: C-X-C Chemokine Receptor Type 3; EDTA: Ethylenediaminetetraacetic Acid; IFN-γ: Interferon Gamma; IL: Interleukin; IL-1β: Interleukin 1 Beta; IL-6: Interleukin 6; IL-8: Interleukin 8; IP-10: Interferon Gamma-Induced Protein 10; MCP-1: Monocyte Chemoattractant Protein 1; MIG: Monokine Induced by Interferon Gamma; NBT: Nitroblue Tetrazolium; NK: Natural Killer; PCR: Polymerase Chain Reaction; RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand; RANTES: Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; Cu-Zn-SOD: Copper-Zinc Superoxide Dismutase; TNF-α: Tumor Necrosis Factor Alpha.

## Introduction

Periodontitis is a chronic, multifactorial, immune-mediated inflammatory disease associated with oral biofilm dysbiosis and characterized by the progressive destruction of tooth-supporting tissues, including the periodontal ligament and alveolar bone. Periodontitis is categorized into stages I–IV, defined primarily by disease severity and complexity of clinical management, and grades A–C, which reflect the rate of disease progression and the presence of risk-modifying factors [1].

The contemporary understanding of periodontitis pathogenesis extends beyond the concept of an infection resulting exclusively from specific pathogens. The disease is currently considered to arise from a complex interaction between dysbiotic microbial communities and a dysregulated host immunoinflammatory response. In this context, certain microorganisms, such as *Porphyromonas gingivalis*, may act as keystone pathogens that alter host–microbiota interactions, disrupt local defense mechanisms, and promote the establishment and persistence of periodontal dysbiosis [2,3].

Although the microbial biofilm is an essential component in initiating the periodontal response, the extent and progression of tissue destruction are strongly influenced by the magnitude and persistence of the host immunoinflammatory response. Microbial products and signals arising from tissue damage activate innate immune receptors and multiple intracellular signaling pathways, leading to the production of cytokines, chemokines, reactive oxygen species (ROS), matrix metalloproteinases, and osteoclastogenic mediators. When this response is not adequately regulated, persistent inflammatory cell infiltration, extracellular matrix degradation, disruption of the RANKL/osteoprotegerin balance, increased osteoclast activity, and, consequently, periodontal attachment loss and alveolar bone resorption may occur [4-6].

Among the cells involved in this process, neutrophils play a central role in gingival sulcus defense and are continuously recruited to periodontal tissues in response to chemotactic stimuli. Upon activation, these cells perform phagocytosis and degranulation, generate ROS, and form neutrophil extracellular traps. Although these mechanisms are essential for microbial control, excessive or persistent neutrophil activation may contribute to periodontal tissue damage through the release of proteases, matrix metalloproteinases, and oxidants [4,7]. Monocytes and macrophages also participate in the periodontal microenvironment by producing mediators such as IL-1β, IL-6, TNF-α, and various chemokines, while also influencing mechanisms associated with osteoclastogenesis. Thus, an imbalance between pro-inflammatory mechanisms and resolution processes may promote persistent inflammation and the pathological bone remodeling characteristic of the disease [5,6].

The chemokines may play an important role by regulating the recruitment, migration, and positioning of different leukocyte populations within periodontal tissues. Among these molecules, CXCL8/IL-8 is particularly involved in neutrophil recruitment and activation, whereas CCL2/MCP-1 primarily mediates monocyte migration. CCL5/RANTES is involved in the recruitment of lymphocytes and monocytes, while CXCL9/MIG and CXCL10/IP-10, IFN-γ-inducible chemokines that bind to CXCR3, are primarily associated with the recruitment of activated T lymphocytes and natural killer cells [7]. Persistent production of these mediators may contribute to sustained leukocyte infiltration and, consequently, amplification of the local inflammatory response, with further release of cytokines, proteases, ROS, and mediators associated with periodontal tissue destruction. Therefore, assessing CXCL8, CCL2, CCL5, CXCL9, and CXCL10 together may provide a more comprehensive characterization of the inflammatory profile of the

periodontal microenvironment than evaluating these mediators individually [6,7].

In addition to the inflammatory response, oxidative stress represents another relevant mechanism in the progression of periodontitis. This process results from an imbalance between the generation of reactive species and the ability of antioxidant systems to neutralize them. During the periodontal response, activated neutrophils and macrophages generate ROS as part of their antimicrobial mechanisms; however, excessive ROS production may induce lipid peroxidation, oxidation of proteins and nucleic acids, cellular dysfunction, and further activation of inflammatory pathways, contributing to persistent tissue damage [4,8].

To counteract the effects of reactive species, the body relies on both enzymatic and non-enzymatic antioxidant mechanisms. Major enzymatic antioxidant systems include superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD is one of the first lines of antioxidant defense, catalyzing the dismutation of superoxide anions into hydrogen peroxide, which other antioxidant systems can then metabolize. However, SOD activity may vary by biological compartment, inflammatory intensity, and disease stage. Thus, changes in its activity may reflect either a compensatory response to increased ROS generation or impairment or depletion of local antioxidant capacity [8]. Clinical studies have demonstrated alterations in oxidative stress-related biomarkers and antioxidant capacity in individuals with periodontitis, further supporting the involvement of redox imbalance in periodontal disease [9,10].

In addition to the bacterial components traditionally associated with periodontal dysbiosis, protozoa inhabiting the oral cavity, particularly *Entamoeba gingivalis* and *Trichomonas tenax*, have received increasing attention as potential components of the periodontal microenvironment. Recent evidence has demonstrated a higher frequency of these microorganisms in individuals with periodontal diseases than in periodontally healthy individuals, particularly for *E. gingivalis*. However, the increased detection of these protozoa in affected periodontal sites does not, by itself, establish a causal relationship with the disease. Their presence may instead be associated with the ecological conditions created by periodontal dysbiosis and inflammation, or these organisms may contribute to modulating this microenvironment [11].

Therefore, considering that periodontitis results from interactions among microbial dysbiosis, immune cell recruitment and activation, inflammatory mediator production, and alterations in the oxidant–antioxidant balance, investigating these mechanisms in an integrated manner is relevant. The assessment of CXCL8, CCL2, CCL5, CXCL9, and CXCL10, together with SOD activity and the identification of *E. gingivalis* and *T. tenax*, may contribute to a better characterization of the periodontal microenvironment and allow the exploration of potential associations between the presence of these protozoa, the intensity of the inflammatory response, and local antioxidant defense mechanisms [7,8,11].

Thus, this study aimed to evaluate chemokine concentrations and superoxide dismutase (SOD) activity in samples obtained from periodontal sites of patients with periodontitis and to investigate their association with the presence of oral protozoa.

## Materials and Methods

### Ethical aspects

This study was submitted to and approved by the Human Research Ethics Committee under CAAE Number: 43524921.2.0000.5587. Sample collection began only after ethical approval was obtained.

All participants received detailed information regarding the objectives, procedures, potential risks, and benefits of the study and were enrolled only after providing written informed consent by signing the Informed Consent Form. Participant confidentiality was ensured by coding biological samples and collected data, preserving anonymity and data privacy throughout the study.

Participation was voluntary, and the procedures involved minimal risk. Potential adverse effects were primarily related to peripheral blood collection and periodontal sampling and included transient discomfort, mild bleeding, or hematoma formation. Trained healthcare professionals performed all procedures using sterile, disposable materials.

### Participants and experimental groups

Adult patients aged 18 years or older receiving dental care at a public dental clinic within the Family Health Unit located, Barra do Garças, Mato Grosso, Brazil, were recruited during routine dental appointments.

Patients with a clinical diagnosis of periodontitis were eligible for inclusion. The attending dentist established the periodontal diagnosis based on clinical periodontal examination

and radiographic findings. The periodontal assessment included evaluation of clinical signs of inflammation, bleeding on probing, dental biofilm accumulation, calculus, gingival alterations, and the presence of periodontal pockets [11].

We excluded individuals younger than 18 years, those who declined participation, and patients who had received antibiotics, antiparasitic agents, or antifungal drugs within the previous 90 days. Additional exclusion criteria included use of immunosuppressive drugs or systemic corticosteroids, pregnancy, the postpartum period, uncontrolled systemic diseases, or other clinical conditions that could modify the host immune response.

Participants were allocated to two experimental groups based on the presence or absence of protozoa in periodontal-site samples, as determined by microscopic examination. The Inflammation Group consisted of patients with periodontitis in whom no protozoa were detected in the periodontal samples analyzed (n = 3). The Infection Group consisted of patients with periodontitis in whom protozoa were detected in periodontal-site samples by microscopic examination (n = 4).

## Collection and processing of periodontal samples

Periodontal samples were obtained from patients with a clinical diagnosis of periodontitis following dental examination. Sampling was performed at the periodontal site presenting the greatest probing depth and clinical signs of inflammation. Biological material was collected using a sterile periodontal curette and/or a sterile swab carefully introduced into the periodontal pocket or gingival sulcus. Gentle rotational movements were performed to maximize the recovery of biological material, according to a protocol adapted from Aciöz, Ak, and Bozkaya [11].

Immediately after collection, each swab was transferred to a sterile tube containing Medium 199 to preserve the cellular components and potential protozoa present in the sample until laboratory processing. Samples were transported under refrigerated conditions. An aliquot intended for direct microscopic examination was analyzed before long-term storage, whereas the remaining material was subsequently stored at −80 °C until biochemical and immunological analyses were performed.

## Microscopic detection of protozoa

Periodontal-site samples were subjected to microscopic examinations to investigate the presence of protozoa. Two complementary approaches were used: direct wet-mount examination and microscopic analysis of Rapid Panoptic-stained preparations. Two examiners independently evaluated all slides.

For direct wet-mount examination, an aliquot of freshly collected material was placed on a concave microscope slide and immediately examined to identify structures morphologically compatible with protozoa and, when detectable, to assess their motility. Preparations were examined using optical microscopy, initially at 40× magnification.

For stained preparations, periodontal material was smeared onto microscope slides, then fixed and stained using the Rapid Panoptic method (Instant Prov, Newprov Ltda, Brazil), according to the manufacturer's instructions. Slides were subsequently examined by optical microscopy at 40× and 100× magnifications, with immersion oil used for the latter.

Protozoal positivity was determined based on the visualization of morphological characteristics and/or motility compatible with oral protozoa, particularly structures suggestive of *Entamoeba* spp. and *Trichomonas tenax*, given their recognized occurrence in the oral cavity and reported association with periodontal conditions. Samples were qualitatively classified as protozoa-positive or protozoa-negative.

Species-level identification was not performed at this stage; therefore, microscopic findings were interpreted as presumptive identification. Molecular methods are planned for subsequent confirmation and species-level characterization.

## Quantification of chemokines

The concentrations of IL-8/CXCL8, MCP-1/CCL2, RANTES/CCL5, IP-10/CXCL10, and MIG/CXCL9 were quantified in periodontal-site samples obtained from the Inflammation and Infection Groups using the BD Cytometric Bead Array (CBA) Human Chemokine Kit (Catalog No. 552990, BD Biosciences), according to the manufacturer's instructions.

Chemokine standards were reconstituted and serially diluted to generate standard curves covering the manufacturer-specified concentration range. The assay was performed using populations of capture beads with distinct fluorescence intensities, each coated with antibodies specific for one of the chemokines evaluated.

Samples and standards were incubated with the capture bead mixture and phycoerythrin-conjugated detection reagent according to the manufacturer's protocol. Following incubation and washing, samples were acquired using a FACSCalibur flow cytometer (BD Biosciences, USA).

Fluorescence intensity data were analyzed using FCAP Array™ software. Chemokine concentrations were calculated from the corresponding standard curves and expressed as pg/mL.

### Determination of superoxide dismutase activity

Copper-zinc superoxide dismutase (Cu-Zn-SOD) activity was determined in periodontal-site samples obtained from patients in the Inflammation and Infection Groups using the nitroblue tetrazolium (NBT) reduction method (Sigma, St. Louis, MO, USA), according to the method described by Novelli., *et al*. [12].

For the assay, 500 µL of each sample was mixed with 500 µL of a chloroform–ethanol solution (1:1, v/v), 500 µL of a reaction mixture containing NBT and EDTA, and 2 mL of hydroxylamine buffer solution. For spectrophotometer calibration, a reference solution containing 500 µL of hydroalcoholic mixture, 500 µL of chloroform–ethanol solution (1:1, v/v), 500 µL of the NBT–EDTA reaction mixture, and 2 mL of hydroxylamine buffer was used.

The reaction mixtures were incubated for 15 min at room temperature and protected from light. Absorbance was subsequently measured at 560 nm using a spectrophotometer. SOD activity was determined according to the inhibition of NBT reduction, using the following equation:

SOD inhibition (%) = [(Absorbance of the reference − Absorbance of the sample)/Absorbance of the reference] × 100

One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% under the assay conditions. Cu-Zn-SOD activity was expressed in international units (IU).

### Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, USA). Data were initially screened for potential outliers using Grubbs' test. The distribution of the variables was subsequently assessed using the Shapiro–Wilk test. For variables exhibiting an approximately normal distribution, comparisons between the two independent experimental groups were performed using the unpaired Student's *t*-test. Associations between continuous variables were assessed using Pearson's correlation coefficient. Data were considered statistically significant when $p < 0.05$.

## Results and Discussion

Analysis of chemokine concentrations in periodontal-site samples revealed significant differences in IL-8/CXCL8 and RANTES/CCL5 between patients with periodontitis based on protozoa detection (Figure 1). Patients with protozoa-positive periodontal samples showed significantly higher concentrations of IL-8/CXCL8 ($p = 0.0178$) and RANTES/CCL5 ($p = 0.0246$) than patients in whom protozoa were not detected. In contrast, no statistically significant differences were observed for MCP-1/CCL2, MIG/CXCL9, or IP-10/CXCL10 between the groups. These findings indicate that protozoal detection was associated with a selective alteration in the local chemokine profile rather than a generalized increase in all chemokines evaluated.

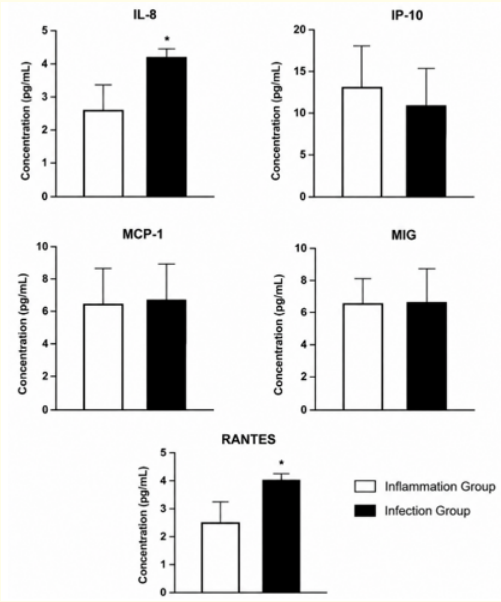


**Figure 1:** Concentrations of IL-8/CXCL8, IP-10/CXCL10, MCP-1/CCL2, MIG/CXCL9, and RANTES/CCL5 (pg/mL) in periodontal-site samples from patients with periodontitis without detectable protozoa (Inflammation Group) and patients with periodontitis and detectable protozoa (Infection Group). Data are presented as mean ± standard deviation. p < 0.05, unpaired Student's t-test.

The association between oral protozoa and periodontal disease has received increasing attention. *Entamoeba gingivalis* and *Trichomonas tenax* have been detected more frequently in individuals with periodontal alterations than in periodontally healthy individuals. Aciöz, Ak, and Bozkaya [11] reported a higher prevalence of these protozoa in patients with gingivitis and periodontitis, supporting their association with periodontal inflammatory conditions. Similarly, Yaseen., *et al*. [13], using molecular detection methods, reported an association between oral protozoal colonization and the presence and severity of periodontal disease. Nevertheless, increased detection of protozoa in diseased periodontal sites does not establish a direct causal relationship, since these microorganisms may either contribute to the inflammatory microenvironment or preferentially colonize periodontal sites where dysbiosis and inflammation are already established.

Among the chemokines evaluated, the increase in IL-8/CXCL8 is particularly relevant because CXCL8 is a major mediator of neutrophil recruitment and activation in periodontal tissues. Neutrophils are essential components of the local antimicrobial response; however, their excessive or persistent activation may contribute to periodontal tissue injury through the release of reactive oxygen species (ROS), proteases, and matrix metalloproteinases. Thus, neutrophil activity has a dual role in periodontal homeostasis: it contributes to microbial control while potentially amplifying tissue destruction when inflammatory regulation is disrupted [14].

The higher CXCL8 concentrations observed in protozoa-positive samples may therefore reflect enhanced neutrophil-directed chemotactic signaling within these periodontal sites. One possible interpretation is that oral protozoa are associated with a more pronounced innate inflammatory microenvironment. However, these findings cannot determine whether protozoa directly stimulate CXCL8 production or whether they preferentially occur in sites with greater pre-existing inflammatory activity. This distinction is important because the role of oral protozoa as active contributors to periodontal pathogenesis, rather than markers of an altered periodontal ecosystem, remains under investigation [11,15].

A similar pattern was observed for RANTES/CCL5, which was significantly increased in patients with protozoa-positive periodontal samples. CCL5 is involved in recruiting several immune cell populations, particularly T lymphocytes, monocytes, and macrophages, through receptors including CCR1 and CCR5. Its expression has been reported in inflamed gingival tissues and gingival crevicular fluid from patients with periodontitis, suggesting a role in maintaining inflammatory cell infiltration and in the interaction between innate and adaptive immune responses. CCL5 has also been associated with inflammatory mechanisms, connective tissue destruction, and periodontal bone remodeling [16].

The concomitant increase in CXCL8 and CCL5 may therefore indicate that protozoa-positive periodontal sites exhibit enhanced chemotactic signaling involving both neutrophilic and mononuclear inflammatory responses. Importantly, these findings demonstrate an association and should not be interpreted as evidence that the protozoa themselves induced chemokine production. Functional studies are needed to determine whether *E. gingivalis*, *T. tenax*, or their products can directly stimulate periodontal epithelial cells, neutrophils, monocytes, or macrophages to produce CXCL8 and CCL5.

In contrast, MCP-1/CCL2 concentrations did not differ significantly between the groups. CCL2 is primarily involved in monocyte chemotaxis and in recruiting and maintaining monocyte/macrophage populations within inflamed tissues. Similarly, MIG/CXCL9 and IP-10/CXCL10 did not differ significantly. These two IFN-γ-inducible chemokines share the CXCR3 receptor. They are primarily associated with recruiting activated T lymphocytes and natural killer cells and with organizing type 1 cellular immune responses.

The absence of significant changes in CCL2, CXCL9, and CXCL10, together with the increased concentrations of CXCL8 and CCL5, suggests that protozoal detection was not associated with uniform activation of all chemotactic pathways investigated. Instead, the findings suggest a potentially selective chemokine profile involving predominantly CXCL8- and CCL5-associated pathways. This observation also highlights the importance of evaluating multiple chemokines simultaneously, since the periodontal immune response comprises interconnected but partially independent signaling networks.

However, the absence of statistical significance for CCL2, CXCL9, and CXCL10 should not be interpreted as evidence that these chemokines are not involved in periodontal inflammation. The present analysis included only three patients in the Inflammation Group and four in the Infection Group. This small sample size substantially limits statistical power and the ability to detect small or moderate differences, increasing the possibility of type II error. Therefore, these findings should be considered preliminary and require confirmation in a larger population.

In addition to chemokine concentrations, Cu-Zn-SOD activity was evaluated to investigate whether the presence of protozoa was associated with alterations in local antioxidant defense. No statistically significant difference in Cu-Zn-SOD activity was observed between patients with periodontitis without detectable protozoa and those with protozoa-positive periodontal samples (Figure 2).

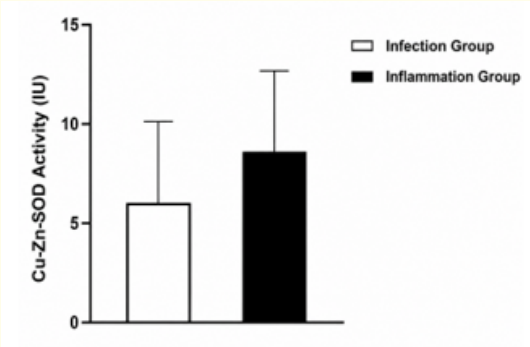


**Figure 2:** Cu-Zn-SOD activity (IU) in periodontal-site samples from patients with periodontitis without detectable protozoa (Inflammation Group; n = 3) and patients with periodontitis and detectable protozoa (Infection Group; n = 4). Data are presented as mean ± standard deviation. $p > 0.05$, unpaired Student's t-test.

SOD is a major component of the cellular antioxidant defense system and catalyzes the dismutation of superoxide anions into hydrogen peroxide. In periodontitis, antioxidant enzyme activity may vary by biological compartment, inflammatory burden, disease severity, and individual characteristics. Toczewska., *et al*. [8], for example, demonstrated that changes in enzymatic antioxidant defenses in gingival crevicular fluid and saliva are not necessarily homogeneous across biological fluids or antioxidant enzymes.

This variability has also been demonstrated in clinical studies. Veljovic., *et al*. [17] reported significantly higher salivary SOD activity in patients with periodontitis than in periodontally healthy individuals and observed changes following nonsurgical periodontal treatment. Increased SOD activity may represent a compensatory response to enhanced ROS generation, whereas reduced activity may reflect consumption or depletion of antioxidant defenses. Consequently, SOD activity alone cannot be regarded as a direct measure of the overall intensity of periodontal oxidative stress.

Systematic reviews and meta-analyses have also demonstrated increased oxidative damage biomarkers and impaired antioxidant capacity in saliva and gingival crevicular fluid from individuals with periodontitis. Alterations in markers such as malondialdehyde, 8-hydroxy-2′-deoxyguanosine, total oxidant status, and total antioxidant capacity indicate that periodontal oxidative stress involves a complex redox network that cannot be adequately characterized by a single antioxidant enzyme [18].

Accordingly, the absence of a significant difference in Cu-Zn-SOD activity between the groups does not exclude the possibility of protozoa-associated alterations in periodontal redox homeostasis. Such alterations may involve other antioxidant pathways or oxidative damage markers without producing detectable changes in SOD activity. Furthermore, the limited sample size may have reduced the ability to identify differences between the groups.

An important aspect of the present findings is that significant alterations in CXCL8 and CCL5 occurred without a simultaneous detectable change in Cu-Zn-SOD activity. Within the limitations of this preliminary analysis, this pattern suggests that protozoal detection was more clearly associated with alterations in local chemotactic signaling than with measurable changes in Cu-Zn-SOD-mediated antioxidant defense. This finding should not, however, be interpreted as evidence that inflammatory and oxidative mechanisms operate independently. These systems are biologically interconnected, particularly because neutrophils recruited by chemokines such as CXCL8 constitute an important source of ROS within periodontal tissues.

Interpreting these findings also requires consideration of methodological limitations. Protozoa were identified by direct microscopic examination and morphological assessment. Although

microscopy enables the detection of structures compatible with oral protozoa, it does not provide definitive species-level identification. Molecular confirmation using PCR will therefore be important to establish the presence and identity of *E. gingivalis* and *T. tenax* with greater specificity, as employed in previous studies investigating their association with periodontal disease [11,13].

Furthermore, clinical and behavioral factors that can influence periodontal inflammation, including probing depth, bleeding on probing, plaque index, smoking, diabetes, and periodontitis severity, should be incorporated into subsequent analyses as the sample size increases. Controlling these potential confounding variables will be essential to determine whether the association between protozoal detection and increased CXCL8 and CCL5 concentrations persists independently of periodontal disease severity and other host-related factors.

Overall, the present findings indicate that protozoal detection in periodontal sites was associated with a local inflammatory profile characterized by increased CXCL8 and CCL5 concentrations. In contrast, CCL2, CXCL9, and CXCL10 concentrations and Cu-Zn-SOD activity did not differ significantly according to protozoal detection. These results suggest that oral protozoa may selectively modulate chemotactic pathways rather than cause generalized chemokine activation or detectable changes in Cu-Zn-SOD-mediated antioxidant defense. Given the limited sample size and the current use of microscopy for protozoal detection, these findings should be considered preliminary. Larger studies incorporating molecular protozoal identification and integrated clinical, inflammatory, and oxidative parameters are required to determine whether oral protozoa actively contribute to modulating periodontal inflammation or primarily represent markers of a microenvironment favorable to their colonization.

## Conclusion

The findings indicate that the presence of protozoa in periodontal sites is associated with specific alterations in the local inflammatory response of patients with periodontitis. Higher IL-8/CXCL8 and RANTES/CCL5 concentrations in protozoa-positive samples suggest selective enhancement of chemotactic pathways involved in immune cell recruitment and activation. In contrast, Cu-Zn-SOD activity did not differ significantly between groups, indicating that detectable changes in this component of local antioxidant defense did not accompany these inflammatory alterations.

Overall, these findings suggest that oral protozoa may modulate the periodontal inflammatory microenvironment, particularly through pathways involving CXCL8 and CCL5. However, the present data do not establish a causal relationship between protozoal presence and the observed inflammatory changes.

These findings should be interpreted in light of the study's preliminary nature, the small sample size, and the microscopic identification of protozoa without species-level molecular confirmation. Further studies with larger populations, molecular identification of *Trichomonas tenax* and *Entamoeba gingivalis*, and additional oxidative stress and antioxidant biomarkers are needed to clarify the relationship between oral protozoa and the host inflammatory response in periodontitis.

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## Conflict of Interest

The authors declare no conflict of interest.

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