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Original Research Article

Macrophage Chemotactic Protein-1 and Interleukin-12 Levels in Gingival Crevicular Fluid in Patients with Periodontal Disease: A Cross Sectional Study

Njood Alshareef[1], Hala A. Abuel-Ela[2], Ibtesam K. Afifi[3]

[1]Dental intern, Faculty of Dentistry, Umm AlQura University, Saudi Arabia.
[2]Professor of Periodontology and Implant Dentistry, Faculty of Dentistry, Ain Shams University, Egypt affiliated to Umm-AlQura University, KSA.
[3]Professor of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt affiliated to Umm-AlQura University, KSA.

Corresponding Author: Njood Alshareef

ABSTRACT

Background: Periodontal diseases including gingivitis and periodontitis are among the most frequent oral diseases affecting all age groups, which can critically impact the general health. Since periodontal disease is both preventable and curable, early intervention will minimize the subsequent destruction of periodontal tissues.
Objective: Is to assess the gingival crevicular fluid level of Macrophage Chemotactic Protein-1/CCL2 and Interleukin-12 in plaque induced gingivitis patients compared to chronic periodontitis patients.
Methods: This was a cross-sectional study with a sample of 32 healthy female patients obtained from the Dental Teaching Hospital, College of Dentistry, Umm Al-Qura University. GCF samples were collected using PerioPaper Strips.
Results: Chronic periodontitis patients showed statistically significant higher mean PD (5.50mm) than patients with plaque induced gingivitis (3.06mm). Patients with chronic periodontitis revealed greater levels of MCP-1 (0.094pg/ml) in GCF compared to patients with plaque induced gingivitis (0.079pg/ml). Moreover, chronic periodontitis patients showed higher levels of IL-12 (0.11pg/ml) than plaque induced gingivitis patients (0.101pg/ml).
Conclusion: In conclusion, within the limits of the present study, IL-12 and MCP-1 may be regarded as a reliable biochemical marker for periodontal tissue destruction in CP. Further longitudinal studies with larger sample size are recommended to further elucidate the role of these biomarkers in alveolar bone resorption in periodontal disease.

Key words: Chemokines and Gingival crevicular fluid, Chronic periodontitis, Cytokines, Plaque induced gingivitis, Interleukin-12, Macrophage Chemotactic Protein-1/CCL2.

INTRODUCTION

Cytokines play a fundamental role in inflammation and they are key inflammatory mediators in periodontal disease. [1,2] Cytokines bind to specific receptors on target cells, and they initiate intracellular signaling cascades that result in phenotypic changes in the cell via altered gene regulation. [1,3]

Cytokines play a key role at all stages of the immune response in periodontal disease. [4,5] They act as mediators for both homeostasis and immunity. [6-8] T cells which are the source

of many cytokines are the dominant cell type in periodontitis lesions. [4]

The prolonged and excessive production of cytokines and other inflammatory mediators in the periodontium leads to the tissue damage that characterizes the clinical signs of the disease. However, they also have profound biologic effects that lead to tissue damage with chronic inflammation. [5]

Interleukin 12 (IL-12) is the major cytokine which induces naive T cells into Th1 cells. The major effect of IL-12 is the production of IFN-γ by Th1 cells and the regulation of transition from an early innate immune response to an adaptive immune response. [9] Interleukin-12 is a heterodimeric ligand consisting of interleukin-12 p40 and interleukin-12 p35 subunits. It is produced by myeloid cells and affects the T helper 1 differentiation of T-lymphocytes, and thereby enhances the expression of interferon-gamma. [10] Higher levels of IL-12 were found in GCF of chronic periodontitis (CP) sites compared to gingivitis (G) and healthy sites, it has a critical role in determining whether the periodontal lesion is stable or progressive, it is considered a key for better understanding of the immune response to periodontal diseases. [11]

Chemokines are signaling proteins produced mainly by myeloid cells that have important roles in recruiting specific leukocyte subpopulations to sites of ongoing tissue damage. [12,2,3] They're found in the GCF and gingival tissues, and play an important role in the immunopathogenesis of periodontal disease. Chemokines are involved in both the physiology and the pathology of bone metabolism. They are essential signals for the trafficking of osteoblast and osteoclast precursors, and consequently they are potential modulators of bone homeostasis. [13,14]

MCP-1/CCL2 is supposed to be the major chemoattractant of macrophages in periodontal disease. MCP-1/CCL2 activity in GCF increased with severity of periodontal disease. [15]

Chemokines play critical roles in acute and chronic stages of inflammation and regulating the cells chemical flow into the site of infection. [16] They are facilitating the migration and activation of specific types of leukocytes in response to the bacterial infection. [17]

They are responsible for the recruitment and subsequent activation of particular leucocytes into inflamed tissues sites, and therefore play a central role in the final outcome of the immune response by determining which subsets of leucocytes form the inflammatory infiltrate. [18]

Two groups of chemokines are functionally characterized within the context of inflammatory processes: (1) the CXC subgroup, which activates the neutrophils and (2) the CC subgroup, which is comprised of monocyte chemo attractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), and regulated on activation normal T cell expressed and secreted chemokine (RANTES), CC chemokines are chemotactic for monocytes or lymphocytes, [19,20] which determine the transition from the acute inflammation into chronic inflammatory process through class switching of macrophages (M1-M2). [21]

Macrophage Chemotactic Protein-1 (MCP-1)

One of the most investigated and disease-associated chemokines is monocyte chemoattractant protein-1 (MCP-1), which plays a crucial role in the recruitment of mononuclear phagocytes to sites of inflammation and malignancies (de la Rosa et al. 2003; Garlet et al. 2003; Yao et al. 2006; Buhling et al. 2007; Tsai et al. 2008).

MCP-1 has been associated with gingivitis, periodontitis, and bone inflammation secondary to bacterial infections in the oral cavity. [19] Patients with chronic periodontitis (CP) and aggressive periodontitis (AgP) have been reported with high levels of MCP-1 in the gingival crevicular fluid (GCF). [22,23]

MATERIALS AND METHODS

Subjects

Sixteen patients diagnosed as plaque induced gingivitis and sixteen patients with moderate to severe chronic periodontitis were selected from the screening clinic of the Dental Teaching Hospital, College of Dentistry, Umm Al-Qura University. Patients participating in this study signed an informed consent demonstrating the purpose of the study. The study proposal was reviewed by UQU-DNT Ethical Approval Committee

Selected individuals fulfilled the following criteria:

Inclusion criteria:

1. Patients free from any systemic disease as evidenced by health questionnaire using health history questionnaire.
2. Female gender.

Exclusion criteria:

1. Patients with history of periodontal surgery or antimicrobial therapy for at least 4 months prior to the initiation of the study.
2. Pregnant and breast-feeding women.
3. Smokers.

Participants were divided into two groups:

Group I: Included sixteen moderate to severe chronic periodontitis patients with clinical attachment loss ≥ 3mm.

Group II: Included sixteen plaque induced gingivitis patients.

Clinical examination

The following measurements were recorded for all patients:

1. Plaque Index (PI).
2. Bleeding on probing.
3. Probing Depth (PD).
4. Clinical attachment level (CAL).

Collection of gingival crevicular fluid (GCF):

Clinical examination, group allocation and sampling site selection were performed, and then the samples were collected on the subsequent day. This was done to prevent the contamination of the GCF samples with blood associated with the periodontal probing of the inflamed sites.

Gingival Crevicular fluid was obtained from each patient by periopaper strips (Gingival Fluid Collection Strips, ORAFLOW, Smithtown NY 11787, Catalog no. 593520) inserted in the gingival crevice pocket until mild resistance was felt and left in place for 30 seconds after isolation by cotton rolls. Then samples were stored in Eppendorf tubes until time of analysis. Only one site per-subject was selected as the sampling site.

In periodontitis patients, the site showing the highest PD and CAL with signs of inflammation along with radiographic evidence of alveolar bone loss was selected for the sampling.

Biochemical assessments of MCP-1/CCL2 and IL-12 levels:

GCF samples were collected to assess the GCF level of MCP-1/CCL2 and IL-12 in plaque induced gingivitis patients compared to chronic periodontitis patients. The quantitative determination of human MCP-1/CCL2 and IL-12 levels in GCF was done using Human MCP-1/CCL2 from Abcam, UK. MCP-1/CCL2 was measured by ELISA according to manufacturer instructions and Human IL-12 from Abcam, UK. IL-12 was measured by ELISA according to manufacturer instructions.

Statistical Analysis:

The results were processed using SPSS program utilizing:

Independent sample t- test to compare plaque index (PI), bleeding on probing (BOP) and probing depth (PD) between 'Periodontitis' and 'Gingivitis' patients.

Independent sample t- test to compare between 'Periodontitis' and 'Gingivitis' patients regarding IL-12 and MCP-1 GCF levels.

Pearson Correlation coefficient to determine the relation between PI, BI, PD and Clinical

attachment level (CAL) in subjects with periodontitis.
Percentage of mild, moderate and severe periodontitis in periodontitis patients.

Conflicts of interest: The authors declare that they have no conflicts of interest relevant to this article.

Budget: The study was completely self-funded by the principle investigator and costs about 7100 Saudi Riyals, to cover the expenses of the ELISA kits and the perio paper strips.

RESULTS

Table (1): Independent sample t- test to compare PI between the gingivitis patients and the periodontitis patients.

Variable	Sample	N	Mean	SD	p-value
Plaque Index	Chronic Periodontitis	16	43.44	17.32	0.001
	Plaque Induced Gingivitis	16	23.03	15.15	

Table (1) showed that there were statistically significant difference in Plaque Index between the 2 groups. (p- value is <0.05).

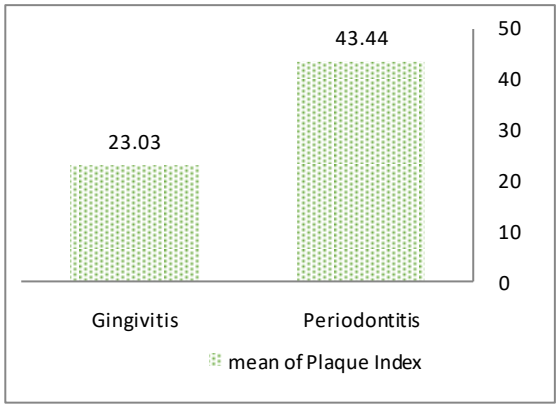

Fig (1) Mean of Plaque Index

Table (2): Independent sample t- test to Compare Bleeding Index between the gingivitis patients and the periodontitis patients.

Variable	Sample	N	Mean	SD	p-value
Bleeding on Probing	Chronic Periodontitis	16	52.30	19.07	0.000
	Plaque Induced Gingivitis	15	22.14	17.67	

Table (2) showed that there were statistically significant difference in BI between the between the 2 groups. (p- value is <0.05).

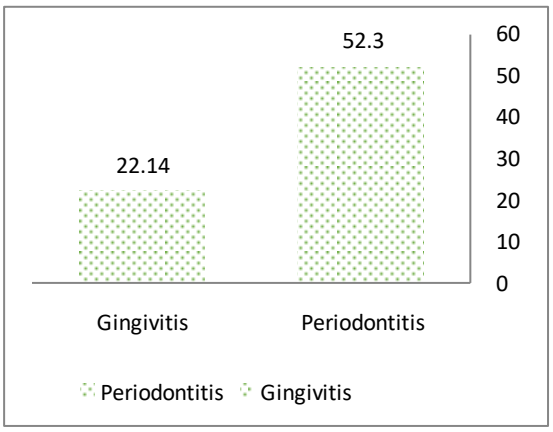

Fig (2) Mean ofBleeding Index

Table (3): Independent sample t- test to compare PD between the gingivitis patients and the periodontitis patients.

Variable	Sample	N	Mean	SD	p-value
Probing Depth	Chronic Periodontitis	16	5.50	1.41	0.000
	Plaque Induced Gingivitis	15	3.06	.59	

Table (3) showed that there were statistically significant difference in Probing Depth between the 2 groups. (p- value is <0.05).

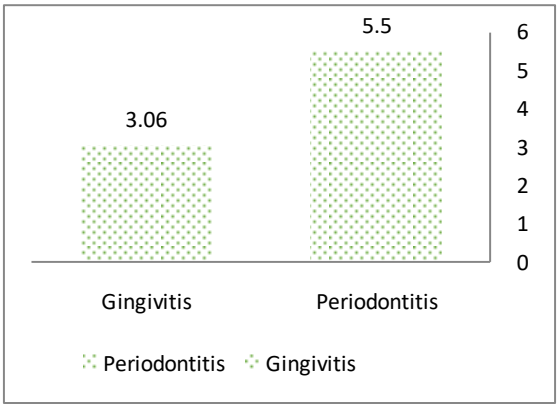

Fig (3) Mean of Probing Depth (mm)

Table (4): Independent sample t- test to compare IL-12 levels in GCF between the gingivitis patients and the periodontitis patients.

		Mean	SD	P-value
IL-12 levels in GCF	Chronic Periodontitis	.11	.024	0.856
	Plaque Induced Gingivitis	.101	.030	

Table (4) showed that there were statistically significant difference in IL-12

levels in GCF between the 2 groups (p-value is <0.05).

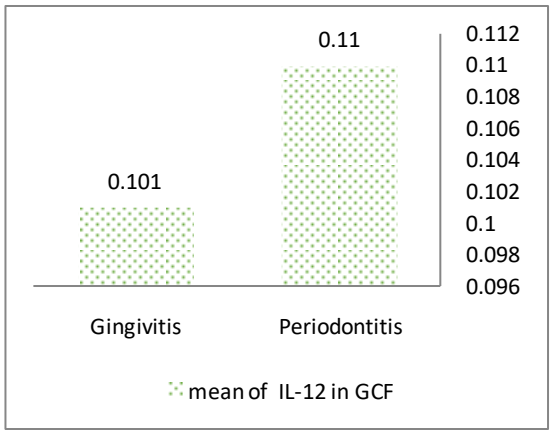

Fig (4) Mean of IL-12 in GCF (pg/ml)

Table (5): Independent sample t- test to compare MCP-1 levels in GCFbetween the gingivitis patients and the periodontitis patients.

		Mean	SD	P-value
IL-12 levels in GCF	Chronic Periodontitis	.094	Periodontitis	0.085
	Plaque Induced Gingivitis	.079	Gingivitis	

Table (5) showed that there were statistically significant difference in MCP-1 levels in GCF between the 2 groups (p-value is <0.05).

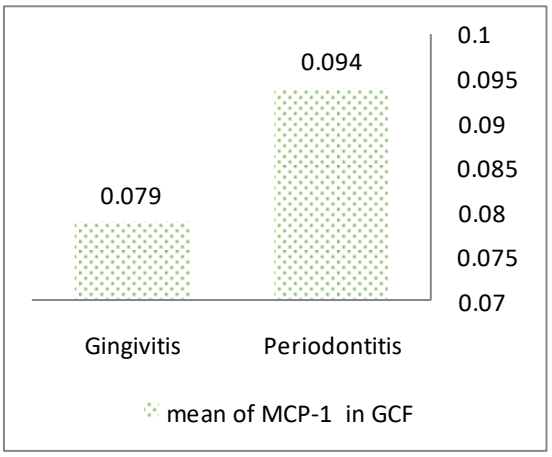

Fig (5) Mean of MCP-1 in GCF (pg/ml)

Table (6): Pearson Correlation coefficient to determine the relation between Plaque Index, Bleeding Index, Pocket Depth and CAL in periodontitis group

		Plaque Index	Bleeding Index	Pocket Depth	CAL
Plaque Index	Pearson Correlation	1	.706**	.551*	.574*
	Sig. (2-tailed)		.002	.027	.020
Bleeding Index	Pearson Correlation	.706**	1	.455	.640**
	Sig. (2-tailed)	.002		.077	.008
Pocket Depth	Pearson Correlation	.551*	.455	1	.709**
	Sig. (2-tailed)	.027	.077		.002
CAL	Pearson Correlation	.574*	.640**	.709**	1
	Sig. (2-tailed)	.020	.008	.002	

With respect to the previous table the results of the current study showed that, there was statistically significant positive correlation relation between Plaque Index, Bleeding Index, Pocket Depth and CAL in periodontitis group where the p- value was (<0.05) and Pearson Correlation was (> 0.5).

As evident in the previous table the results showed that: there was statistically significant differences in Plaque Index, Bleeding Index, Pocket Depth between the Periodontitis group and Gingivitis where the p- value is (<0.05), while there are no statistically significant differences in IL-12 levels in GCF and MCP-1 levels in GCF between the Periodontitis group and Gingivitis where the p- value was (>0.05).

Table (7): Independent sample t- test to compare between 'Periodontitis' and 'Gingivitis' in IL-12 levels in GCF

		Mean	SD	P- value
IL-12 levels in GCF	Chronic Periodontitis	.11	.024	0.856
	Plaque Induced Gingivitis	.101	.030	
MCP-1 levels in GCF	Chronic Periodontitis	.094	.032	0.085
	Plaque Induced Gingivitis	.079	.008	
Plaque Index	Chronic Periodontitis	43.44	17.32	0.001
	Plaque Induced Gingivitis	23.03	15.15	
Bleeding Index-	Chronic Periodontitis	52.30	19.07	0.000
	Plaque Induced Gingivitis	22.14	17.67	
Pocket Depth)	Chronic Periodontitis	5.50	1.41	0.000
	Plaque Induced Gingivitis	3.06	.59	

DISCUSSION

Periodontitis is a chronic inflammatory disorder caused by specific periodontal pathogens, such as *Porphyromonas gingivalis, Tannerella forsythia and Aggregatibacter actinomycetemcomitans*. The two main forms of periodontitis namely; chronic (CP) and aggressive (AgP) are characterized by attachment loss and bone destruction. [24-26]

An orchestrated cytokine and chemokine network regulates homeostasis in the periodontium. Chemokines mediate recruitment of inflammatory cells in response to microbial and mechanical stimuli maintaining healthy levels of cell populations. [27,28] Chemokines enhance the migration and activation of leukocytes in response to bacterial infection [29] and play a crucial role in chronic inflammation via regulating the chemical flow of cells into the site of infection. [30]

Chemokines are proteins that serve as leukocyte chemoattractants in a variety of inflammatory diseases. [31,32] They affect divergent biological activities that include cell migration to sites of disease and inflammation, leukocyte activation, leukocyte secretions, and microbial activities. [33-37]

The results of our study were in accordance with Sa´nchez-Herna´ndez et al., (2011) [38] who reported that IL-12 levels in gingival tissues and serum were higher in patients with aggressive periodontitis than in patients with CP or healthy gingiva; and in CP than in healthy subjects, suggesting that IL-12 may be involved in the immuno-inflammatory response observed in the most destructive form of periodontal disease. In this regard, it has been reported that GCF IL-12 levels were higher in CP patients than those in HS. [39,40] Moreover, an elevated IL-12p35 gene expression in CP lesions compared with those in gingivitis lesions or healthy control sites was also reported. [41,42,38]

Within the limits of the present study, it can be concluded that there were statistically significant differences in plaque index, bleeding on probing and probing depth between the plaque induced gingivitis patients and the chronic periodontitis patients. Moreover, the GCF levels of IL-12 and MCP-1 were higher in the chronic periodontitis patients compared to the plaque induced gingivitis patients but with no statistically significant difference. Furthermore, there was strong and positive statistically significant correlation between plaque index, bleeding on probing, probing depth and CAL in the chronic periodontitis group where the p- value was (<0.05) and Pearson Correlation was (> 0.5).

When the mean IL-12 level in GCF was compared between both chronic periodontitis and gingivitis groups, significant difference was reported, being higher in periodontitis patients than in gingivitis patients. This might suggest a potential cellular hyperactivity that may favor periodontal destruction in CP.

In conclusion, the results of this study w serve as preliminary data suggesting that MCP-1 and IL-12 could play a profound role in periodontal inflammation. Within the limits of the present study, IL-12 may be regarded as a biochemical marker for periodontal tissue destruction in chronic periodontitis patients. Longitudinal studies with large sample size are recommended to further elucidate the role of IL-12 and MCP-1 in periodontal tissue destruction and alveolar bone resorption.

In addition, it would be interesting to investigate the Th1, Th2, and Th17 profile cytokines in both chronic and aggressive periodontitis to understand their pathogenic mechanisms and to identify potential therapeutic targets.

CONCLUSION

In conclusion, it can be concluded that there were statistically significant differences in Plaque Index, Bleeding Index and Probing Depth between the plaque induced gingivitis patients and the chronic periodontitis patients. Moreover, the GCF levels of IL-12 and MCP-1 were higher in

the chronic periodontitis patients compared to the plaque induced gingivitis patients but with no statistically significant difference.

Furthermore, there was strong and positive statistically significant relation between Plaque Index, Bleeding Index, Probing Depth and CAL in the chronic periodontitis group where the p- value was (<0.05) and Pearson Correlation was (>0.5).

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REFERENCES

1. Seymour GJ, Taylor JJ. Shouts and whispers: an introduction to immunoregulation in periodontal disease. *Periodontol 2000* 35:9–13, 2004.
2. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *J Clin Periodontol* 38(Suppl 11):60–84, 2011.
3. Taylor JJ, Preshaw PM, Donaldson PT: Cytokine gene polymorphism and immunoregulation in periodontal disease. *Periodontol 2000* 35:158-182, 2004.
4. Demir T, Orbak R, Tezel A, Canakç V, Kaya H. The changes in the T-lymphocyte subsets in a population of Turkish children with puberty gingivitis. *Int J Paediatr Dent*19: 206-212, 2009.
5. Bartold PM, Narayanan AS: Molecular and cell biology of healthy and diseased periodontal tissues. *Periodontol 2000* 40:29-49, 2006.
6. Birkedal-Hansen, H. Role of cytokines and inflammatory mediators in tissue destruction. Journal of Periodontal Research 28, 500–510, 1993.
7. Di Benedetto, A., Gigante, I., Colucci, S. & Grano, M. Periodontal disease: Linking the primary inflammation to bone loss. Clinical and Developmental Immunology 2013, 2013.
8. Cekici, A., Kantarci, A., Hasturk, H. & Van Dyke, T. E. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000 64, 57–80, 2014.
9. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol*3: 133-146, 2003.
10. Amcheslavsky A, Bar-Shavit Z. Interleukin-12 mediates the anti-osteoclastogenic activity of CpG-oligonucleotides. *J Cell Physiol*: 207: 244–250, 2006.
11. Tsai, I. S., Tsai, C. C., Ho, Y. P., Ho, K. Y., Wu, Y. M. & Hung, C. C. Interleukin-12 and interleukin-16 in periodontal disease. Cytokine 31, 34–40, 2005.
12. Rossi, D. & Zlotnik, A. The biology of chemokines and their receptors. *Annual Review of Immunology* 18, 217–242, 2000.
13. Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ. Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone*33: 28–37, 2003.
14. Wright LM, Maloney W, Yu X, Kindle L, Collin-Osdoby P, Osdoby P. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. *Bone* 36: 840–853, 2005.
15. Hanazawa s, kawata Y, Takeshita A, Kumada H, Okithu M, Tanaka S, Yamamoto Y, Masuda T, Umemoto T, Kitano S. Expression of monocyte chemottractant protein 1 (MCP-1) in adult periodontal disease: increased monocyte chemotactic activity in crevicular fluds and induction of MCP-1 expression in gingival tissue. *Infect immune* 61:5219-5224, 1993.
16. Baggiolini M. Chemokines and leukocyte traffic. *Nature 392*:565–568, 1998.
17. Bachmann MF, Kopf M, Marsland BJ. Chemokines: more than just road signs. *Nature Immunology 6*(2):159–164, 2006.
18. Baggiolini M, Dewald B, MoSeries B. Human chemokines: an update. Annu Rev Immunol 15:675±705, 1997.
19. Graves DT. The potential role of chemokines and inflammatory cytokines in periodontal disease progression. *Clin Infect Dis 28*(3):482–490, 1999.
20. Kabashima H, Yoneda M, Nagata K, Hirofuji T, Maeda K. The presence of chemokine (MCP-1, MIP-1alpha, MIP-1beta, IP-10, RANTES)-positive cells and chemokine receptor (CCR5, CXCR3)- positive cells in inflamed human gingival tissues. *Cytokine 20* (2):70– 77, 2002.
21. Fleetwood AJ, Dinh H, Cook AD, Hertzog PJ, Hamilton JA.GM-CSF- and M-CSF-dependent macrophage phenotypes display

differential dependence on type I interferon signaling. *J Leukoc Biol 86* (2):411–421, 2009.
22. Emingil G, Atilla G, Huseyinov A. Gingival crevicular fluid monocyte chemoattractant protein-1 and RANTES levels in patients with generalized aggressive periodontitis. *J Clin Periodontol 31*(10): 829–834, 2004.
23. Kurtis B, Tuter G, Serdar M, Akdemir P, Uygur C, Firatli E, Bal B. Gingival crevicular fluid levels of monocyte chemoattractant protein-1 and tumor necrosis factor-alpha in patients with chronic and aggressive periodontitis. *J Periodontol 76*(11):1849–1855, 2005.
24. Lindhe J, Ranney R, Lamster I. Consensus report: chronic periodontitis. *Ann Periodontol*4: 38–38, 1999.
25. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 366:1809–1820, 2005.
26. Van Dyke TE, Sheilesh D. Risk factors for periodontitis. J Int Acad Periodontol 7: 3–7, 2005.
27. Garlet GP, Avila-Campos MJ, Milanezi CM, Ferreira BR, Silva JS. Actinobacillus actinomycetemcomitans induced periodontal disease in mice: patterns of cytokine, chemokine, and chemokine receptor expression and leukocyte migration. *Microbes Infect*7(4): 738–747, 2005.
28. Graves D. Cytokines that promote periodontal tissue destruction. *J Periodontol*79 (8 Suppl):1585–159, 2008.
29. Bachmann MF, Kopf M, Marsland BJ. Chemokines: more than just road signs. *Nature Immunology 6*(2):159–164, 2006.
30. Baggiolini M. Chemokines and leukocyte traffic. *Nature 392*:565–568, 1998.
31. Callewaere C, Banisadr G, Rostene W, Parsadaniantz SM. Chemokines and chemokine receptors in the brain: implication in neuroendocrine regulation. *J Mol Endocrinol*38:355–363, 2007.
32. Mines M, Ding Y, Fan GH. The many roles of chemokine receptors in neurodegenerative disorders: emerging new therapeutical strategies. *Curr Med Chem*14:2456–2470, 2007.
33. Callahan MK, Ransohoff RM. Analysis of leukocyte extravasation across the blood–brain barrier: conceptual and technical aspects. *Curr Allergy Asthma Rep*4:65–73, 2004.
34. Banisadr G, Rostene W, Kitabgi P, Parsadaniantz SM. Chemo- kines and brain functions. *Curr Drug Targets Inflamm Allergy*4:387–399, 2005.
35. Rebenko-Moll NM, Liu L, Cardona A, Ransohoff RM. Chemokines, mononuclear cells and the nervous system: heaven (or hell) is in the details. *Curr Opin Immunol*18:683–689, 2006.
36. Ubogu EE, Cossoy MB, Ransohoff RM. The expression and function of chemokines involved in CNS inflammation. *Trends Pharmacol Sci*27:48–55, 2006.
37. Li M, Ransohoff RM. Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology. *Prog Neurobiol*84:116–131, 2008.
38. Sánchez-Hernández PE1, Zamora-Perez AL, Fuentes-Lerma M, Robles-Gómez C, Mariaud-Schmidt RP, Guerrero-Velázquez C. IL-12 and IL-18 levels in serum and gingival tissue in aggressive and chronic periodontitis. Oral Diseases 17: 522–529, 2011.
39. Tsai IS, Tsai CC, Ho YP, Ho KY, Wu YM, Hung CC. Interleukin-12 and inter leukin-16 in periodontal disease. *Cytokine*31:34–40, 2005.
40. Yucel OO, Berker E, Gariboglu S, Otlu H. Interleukin- 11, interleukin-1beta, interleukin-12 and the pathogenesis of inflammatory periodontal diseases. *J Clin Periodontol* 35: 365–370, 2008.
41. Honda T, Aoki Y, Takahashi N. Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. *Clin Chim Acta*395: 137–141, 2008.
42. Ohyama H, Kato-Kogoe N, Kuhara A. The involvement of IL-23 and the Th17 pathway in periodontitis. *J Dent Res*88: 633–638, 2009.

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