Review

Dicle Dent J 2024;25(1):19-24

# The use of gingival crevicular fluid as a potential biomarker for periodontal disease

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**Cite this article as**: Akdeniz C, Güler Doğru A. The use of gingival crevicular fluid as a potential biomarker for periodontal disease. *Dicle Dent J.* 2024;25(1):19-24.

**Received**: 08/02/2024 ◆ **Accepted**: 26/03/2024 ◆ **Published**: 28/03/2024

## ABSTRACT

Gingival crevicular fluid (GCF) is an extremely valuable research material in the detection of periodontal diseases. The study of GCF components contributes to clinical diagnosis and helps us understand the mechanism of periodontal diseases. GCF is important for non-invasive analysis of periodontitis as it shows markers of connective tissue and bone destruction. GCF can be used in the future as a diagnostic tool for the identification of periodontitis, but it can also help in the detection of periodontitis progression. Early detection of periodontitis progression provides clinical benefit by allowing better control of disease activity and may improve patient follow-up. The aim of this review is to investigate the various enzymes and biomediators released in the GCF in periodontal disease and to provide an update on their role in inflammation.

**Keywords**: Periodontitis, biomarker, gingival crevicular fluid, host-derived enzyme, GCF

## INTRODUCTION

The diagnosis of periodontal disease is based on traditional diagnostic parameters such as pocket depth, bleeding on probing, clinical attachment level, and assessment of alveolar bone levels. These parameters do not indicate current disease status but only reflect past periodontal destruction. Therefore, several clinical studies have been conducted to identify potential biomarkers of periodontal disease activity.[1] The diagnostic potential of GCF has been investigated by experts since the 1950s, but to date there is no consensus on its acceptance as a clinically important diagnostic tool.[2]

GCF is a biological monitoring fluid that is crucial for the identification of dental conditions, particularly gingivitis and periodontitis.[2] Deficits in GCF cause issues for proteomic and biochemical analyses.[3] Tissue inflammation and sulcular epithelium ulceration are directly correlated with GCF volume.[4] Greater GCF volume is seen in areas that are moderately or severely inflamed than in areas that are not.[5] Nevertheless, no research has demonstrated a link between a higher sulcus fluid volume and the likelihood of periodontal tissue deterioration.[4]

The progression and severity of periodontitis are linked to a combination of genetics, host response, microbial diversity, and local environmental factors.[6] The release of biological mediators from host tissue cells or the disruption of host tissues are the two main ways whereby bacterial virulence factors result in the demise of host tissue. Tissue lysis is facilitated by mediators generated as part of the host response, such as prostaglandins, cytokines, and proteinases. Furthermore, a number of enzymes generated by periodontal microbes can demolish tissue.[7] Thus far, a number of inflammatory factors, such as proteins, phosphatases, proteinases, cytokines, and products of local tissue breakdown, have been identified from GCF.[8] Prior research has examined over 65 GCF components as potential indicators of the advancement of periodontitis.[9] It has been suggested that these variables could serve as diagnostic indicators for periodontitis. GCF comprises possible indicators generated from subgingival microbial plaque as well as host tissues, serum, and other sources because it accumulates in the gingival sulcus. Variations in GCF components may serve as a possible indicator of the advancement of periodontitis.[6] Therefore, the GCF is regarded as a window for non-invasive periodontitis analysis that takes into consideration signs and indicators of bone and connective tissue degradation.[10] In addition to being a potential diagnostic tool for periodontitis, GCF can also be used to track the disease's advancement.[2] Clinically speaking, early detection of periodontitis

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advancement is advantageous since it allows for greater control over the disease's activity and may enhance patient follow-up.[11] Consequently, the targeted risk zone may be effectively managed, additionally disease progression can be stopped, and high vulnerability to disease activity in these sites can be identified with the aid of GCF collection from numerous sites.[12]

Potential biomarkers in GCF are grouped into three broad groups (**Figure**):[4]

1. Host-derived enzymes and inhibitors.
2. Inflammatory mediators and products
3. Markers of tissue lysis products

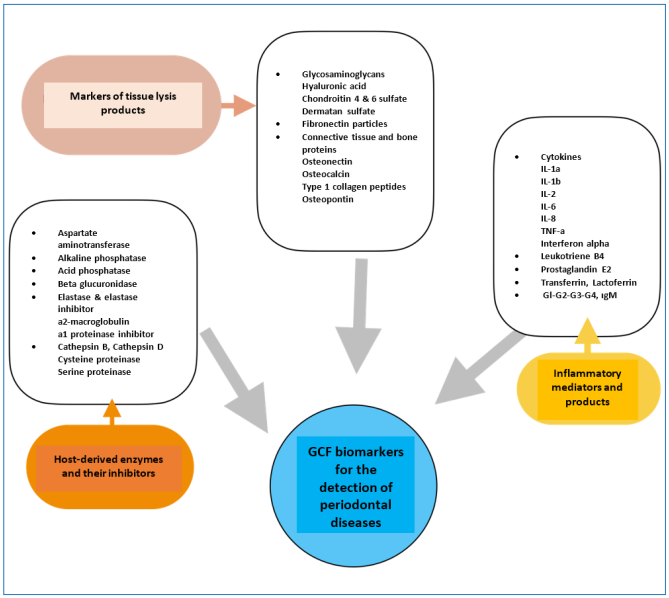


**Figure.** GCF biomarkers used for the detection of periodontal diseases.[13]

## HOST-DERIVED ENZYMES AND THEIR INHIBITORS

The regulation of periodontal tissue turnover in healthy individuals and tissue death in periodontitis is largely dependent on enzymes, particularly proteinases. The overwhelming presence of enzymes linked to tissue degradation demonstrates how crucial the pathophysiology of periodontitis is what causes the inflammatory response.[6] Some of these markers have received special attention for clinical applications, and commercial test kits have been developed.[4]

### Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST), collagenase, prostaglandin E2, beta-glucosidase, lactate dehydrogenase, arylsulfatase, and elastase are the most noticeable indicators in active periodontal lesions. In the affected areas, neutrophils, epithelial cells, dead cells, and connective tissue produce these enzymes. AST, which used to be known as serum glutamic oxaloacetate transferase (SGOT), has been used successfully to help doctors figure out what's wrong with people who have heart and liver tissue necrosis.[14]

AST levels are elevated at sites of active periodontitis. In cases of clinical attachment loss and inflammation, a significant increase in GCF AST levels has been observed.[6] In a study by Priti et al.[14] it was shown that AST levels increased as the severity of clinical inflammation increased. Chambers et al.[15] found an increase in AST levels in the GCF in their study, in which they induced experimental periodontitis using a beagle dog model.

### Alkaline Phosphatase (ALP)

Membrane-bound glycoprotein alkaline phosphatase (ALP) contributes to both the regeneration of the periodontal ligament (PDL) and the preservation of alveolar bone.[4] All types of cells, including leukocytes, osteoblasts, macrophages, and fibroblasts, produce ALP, but polymorphonuclear leukocytes (PMNL) are assumed to be the primary source. ALP is also produced by bacteria in the gingival groove or pocket, which raises the ALP levels in the GCF. It has been proposed that ALP levels in GCF could serve as a possible periodontitis diagnostic marker.[6]

ALP enzyme levels in the GCF and saliva of 23 patients with severe periodontitis and 23 healthy patients were tested by Rasaei et al.[16] ALP levels in salivary were measured as 24.78 per liter in the healthy group and 80.[17] in the periodontitis group, while ALP levels in GCF were assessed as 19.43 in the chronic periodontitis group and 12 in the healthy group. The mean of this enzyme in the gingival gingival fluid and saliva of patients with chronic periodontitis differed significantly from that of healthy subjects ($P<0.001$).

A study by Nakashima et al.[17] over a long period of time showed that high levels of ALP can be found before clinical attachment loss and that the total amount of ALP in the GCF is much higher in areas of active destruction.

### Beta Glucuronidase (BG)

Beta glucuronidase (BG) is an enzyme that helps break down non-collagen parts of the extracellular matrix. It is thought to show or predict the activity of periodontal disease.[4] Proteoglycans are broken down by BG, a lysosomal enzyme that is mostly secreted from PMNs. BG has a high sensitivity and specificity for clinical attachment loss, according to Lamster et al.[18] In a 6-month, multicenter investigation examining the correlation between BG and the advancement of periodontal disease, elevated BG levels in the GCF were linked to deeper probing depths or different thresholds of clinical attachment loss. The study's findings showed that the relative chance of the disease progressing increased six to fourteen times when the enzyme was present in high concentrations. The correlation between high PMNL cell counts in the periodontal pocket (a marker of the severity of the initial inflammatory response) and destructive periodontitis was also highlighted in this study.[4]

### Elastase

Elastase is a serine endopeptidase that breaks down a variety of collagen and non-collagen substrates. It is found in the azurophil granules of PMNs. Giannopoulou et al.[19] showed that elastase increased during experimental gingivitis and returned to baseline levels with the resumption of tooth brushing. High levels of the elastase enzyme in GCF have been reported in periodontitis, and high elastase levels are considered a risk factor for the development of periodontitis.[20] Eley and Cox[21] demonstrated in longitudinal research that a rise in GCF elastase is a sign of periodontal attachment loss. In a long-term follow-up study of adult patients with periodontitis who were getting supportive periodontal treatment, Bader et al.[22] found a link between elastase in the GCF and clinical attachment loss.

### Cathepsin B

The cysteine proteinase enzyme cathepsin B is secreted, particularly by macrophages in the GCF. Patients with periodontitis were shown to have elevated levels of GCF cathepsin B.[23] The degree of periodontitis has been directly correlated with the cathepsin B level.[21] In cases of periodontal disease, the amount of GCF cathepsin can be used as a prognostic sign as well as an indicator of attachment loss.[24] Kunimatsu et al.[25] observed that cathepsin B levels were higher in periodontitis than in gingivitis, although the amount of GCF was similar. Analysis of GCF cathepsin B levels seems to differentiate chronic gingivitis from periodontitis.[26] Also, GCF cathepsin B levels are strongly linked to clinical parameters both before and after periodontal treatment. This means that cathepsin B can be used to measure how well treatment worked.[24]

### Matrix Metalloproteinase (MMP)

PMNs are thought to be the main source of matrix metalloproteinase (MMPs), a collagenolytic enzyme that is present in many cells and is in charge of breaking down extracellular matrix constituents such collagen, proteoglycans, lamina, elastin, and fibronectin.[4,6] MMPs are essential for the remodeling of the periodontal ligament (PDL) in both pathological and physiological settings.[6] Patients with periodontal disease have tissue degradation due in part to MMP-8, MMP-9, and elastase.[27] Patients with periodontitis also have increased levels of MMP-2 and MMP-9, two MMPs involved in tissue degradation.[28] In the gingival groove, bacterial plaque causes a first infiltration of inflammatory cells, such as lymphocytes and macrophages. The inflammatory mediators produced by these activated inflammatory cells encourage fibroblasts, epithelial cells, and PMNs to manufacture MMPs.[6] Lee et al.[29] observed a substantial increase in MMP-8 activity in patients with active periodontitis in a longitudinal study comparing patients with gingivitis, stable periodontitis, and active periodontitis. MMP-3 levels were also investigated in individuals with periodontal disease in a recent study by Beklen et al.[30] In particular, it was discovered that MMP-3 generated from living gingival tissue fibroblasts activated pro-MMP-8 and pro-MMP-9 that were formed from neutrophils in GCF, underscoring the significance of MMP cascades in the etiology of periodontitis.

### Leptin

The inflammatory response is influenced by leptin, a polypeptide that regulates body fat. Adipose tissues secrete a glycosylated polypeptide called leptin, which was first shown to be a hormone linked to obesity.[31] Obesity is caused by disturbances in the leptin signaling system in both humans and animals.[6] Because leptin shares structural and functional similarities with the cytokine family, it plays a role in the host response to inflammatory and infectious stimuli. Because it promotes phagocytosis and the creation of cytokines, it also boosts the immune system.[32] Leptin is known to affect bone metabolism both through the central nervous system and directly through bone stimulatory and inhibitory activity.[30] Leptin can be detected in GCF, and it has been observed that GCF concentration is higher in individuals with healthy gingival tissues compared to individuals with periodontal disease.[33] This is because leptin concentration decreases in inflammation as a result of vascular endothelial growth factor-induced expansion of the vascular network.[34] GCF leptin levels can be utilized as a marker and have been demonstrated in studies to decline as periodontal disease progresses.[6] Moreover, leptin receptors have been found in the gingiva and GCF in both healthy and diseased conditions.[35]

## INFLAMMATORY MEDIATORS AND PRODUCTS

### Cytokines

Numerous factors influence the delicate balance between periodontal health and disease. Increased synthesis of inflammatory cytokines like IL-1, IL-6, TNF-a, PGE2, and MMP leads to periodontal damage.[36] A healthy periodontium produces large amounts of anti-inflammatory cytokines, including interleukin-1 receptor antagonist (IL-1R), IL-4, IL-10, and tissue inhibitors of matrix metalloproteinases (TIMP).[37] Alveolar bone resorption, collagen degradation, and loss of periodontal attachment are among the processes linked to periodontal deterioration that IL-1β directly affects. Patients with periodontal disease have higher levels of IL-1β in GCF, which are considerably lowered following treatment.[38] IL-1 and

TNF-a are examples of inflammatory stimuli that stimulate the release of IL-6. In addition to promoting osteoclast development, IL-6 also causes bone loss.[39] Compared to people with healthy periodontal tissues, patients with chronic periodontitis have higher levels of IL-8 protein and gene expression in their gingival tissues.[40]

There is evidence to suggest that proinflammatory cytokines, especially, IL-1β play an integral role in the etiology of periodontal disease. As the gingival index and probing depth went up, Liu et al.[41] found that IL-1β levels went up in both GCF and gingival tissue. In a longitudinal study by Engebretson et al.[42] IL-1β levels in the GCF following scaling and root planning (SRP) were evaluated in patients with periodontal disease of different severities. An important observation was that IL-1β levels were higher in shallower areas of patients with severe periodontitis compared to shallower areas in patients with mild or moderate periodontitis. This suggests that IL-1β expression in the GCF is genetically influenced and is not solely a consequence of current clinical parameters.

TNF-a promotes cell permeability by controlling the expression of adhesion molecules in vascular endothelial cells and upregulating the expression of proteolytic enzymes, collagenases, and PGE2. This results in the loss of periodontal tissue adhesion and the development of osteoclasts. PGE2 expression is elevated in inflammatory gingival tissues, and this has led to reports that it serves as a marker for the severity and progression of periodontal disease.[43]

In a study by Hwang YS.[28] IL-6, IL-8, MMP-2, MMP-9, and TNF-a levels in GCF samples obtained from periodontitis patients were found to be higher than those obtained from healthy individuals. The author stated that the use of a combination of multiple biomarkers in periodontitis may increase diagnostic accuracy.

The association between IL-1β and C-reactive protein (CRP) in GCF in Australian patients with periodontitis was assessed by Tracy et al.[44] Every biomarker was found to have an independent association in cases of clinically severe periodontitis. This suggests that those who have higher levels of biomarkers showing systemic (CRP) or local (IL-1β) inflammation may have a higher risk of developing periodontal disease.

## MARKERS OF TISSUE LYSIS PRODUCTS

### Laminin

Laminin is mainly produced by epithelial cells. Laminin is believed to be a critical component in the development of periodontal pockets, the apical migration of epithelial cells, and the course of periodontitis.[45] Some laminin isoforms are produced by PDL fibroblasts.[7] It is thought that various laminin isoforms play a role in tissue remodeling in periodontal diseases.[46]

### Osteopontin (OPN)

Osteoblasts and macrophages, while osteoclasts can also create osteopontin (OPN), a protein found in the bone matrix. Studies have shown that OPN levels in the GCF increase in periodontitis and decrease after periodontal treatment.[13,47]

### Osteocalcin (OC)

The most precise indicator of osteoblast function, it is produced by osteoblasts.[13,48] A non-collagen matrix protein called osteocalcin (OC) is present in both calcified and non-calcified tissue. It participates in bone resorption as well as mineralization structurally by binding to the two main components of bone, collagen and apatite.[6] Elevated levels of OC in GCF are linked to elevated rates of bone turnover and are observed in conjunction with heightened activity of periodontal disease.[13]

The degree of alveolar bone damage and/or healing may be correlated with the elevated GCF OC levels observed in cases of periodontitis. OC and OC fragments are anticipated to be released into the GCF from the extracellular matrix during vigorous bone resorption.[6] The average concentration of OC in GCF was found to be 10 times higher than in serum by Nakashima et al.[49] They also believed that periodontal tissues produce OC locally. Numerous studies that have looked at OC levels in GCF from patients with periodontitis demonstrate the recent interest in OC as a potential measure of bone turnover in periodontal disease. According to their research, OC levels in GCF might be an indicator of inflammation in sick locations.[50]

## CONCLUSION

GCF analysis contributes to our understanding of the role of the inflammatory response in periodontitis and provides clinicians with useful information about the location and severity of existing periodontal disease. There is a wide range of periodontal diseases that require different types of treatment. The success of periodontal treatments lies in establishing accurate diagnoses, which is best achieved with the help of GCF. The aim of this study is to develop an understanding among dentists about how to increase the use of GCF for diagnosis and treatment.

GCF has promising possibilities as a diagnostic tool as it shows biomarkers of inflammation and bone resorption in periodontal tissues, is easy to collect, can distinguish

areas of active inflammation, and allows for the diagnosis of early signs of periodontitis.

## ETHICAL DECLARATIONS

### Referee Evaluation Process

Externally peer-reviewed.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

### Financial Disclosure

The authors declared that this study has received no financial support.

### Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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