Narrative Review

Investigating Diagnostic and Prognostic Biomarkers in Gingival Crevicular Fluid across Systemic Conditions

Mohammad Amin Amiri [1]; Mohammad Norouzi [2]; Shahram Hamedani [1]; Nima Farshidfar [3];

[1] Oral and Dental Disease Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2] Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
[3] Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

KEY WORDS
Gingival Crevicular Fluid;
Gingival Exudate;
Biomarkers;
Diagnosis;
Periodontal Diseases;
Systemic Diseases;

Received: 16 April 2025;
Revised: 30 June 2025;
Accepted: 21 Feburary 2026;

ABSTRACT

Gingival crevicular fluid (GCF), as a biofluid originating from gingival crevice transudate holds promising potential as a diagnostic tool for systemic diseases. Clinical investigations have revealed the substantial impact of different systemic conditions on the dynamic levels of various biomarkers present in GCF, encompassing a diverse array of molecules and agents. Based on existing evidence, this narrative review critically examines the effects of various systemic diseases on GCF composition, shedding light on the evolving landscape of GCF as a source of specific biomarkers. A systematic search was conducted in two databases (PubMed, and Scopus) using Mesh terms and proper Boolean operators until May 26, 2025. The original research articles, written in English, and focusing on human studies were screened for further assessment. A selected number of studies were included to assess the current research trends and findings in the field. Drawing from observational studies, we unveiled the various types of GCF biomarkers associated with the most commonly studied systemic diseases, including metabolic syndrome, liver diseases, rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, and acquired immunodeficiency syndrome. The connection of each disease with GCF was separately discussed. Besides, the potential applicability of the assessed biomarkers in each disease is also elaborated upon for future implications. GCF has shown a potential opportunity in terms of diagnostic and prognostic evaluation of systemic diseases. By elucidating the intricate relationship between systemic health and periodontal status, this review has emphasized the untapped potential of GCF as a diagnostic means for systemic diseases, prognostication of their progression, and evaluation of therapy responses, a crucial step toward advancing personalized medicine.

Corresponding Author: Amiri MA, Oral and Dental Disease Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-71-36263193-4 Email: mamiri1378@yahoo.com

Cite this article as:

Introduction

Gingival crevicular fluid (GCF), a liquid compartment, is a form of transudate excreted from the vessels of the gingival plexus beneath the junctional epithelium of the gingival sulcus [1-2]. The dynamic excretion rate is dependent upon multiple factors, including the circadian rhythm [3-4], the severity and grading of gingival inflammation [4], orthodontic tooth movement [4], the impact of mechanical forces [1], pocket depth [4], sex hormones [4-5], enzymes [4, 6], smoking [4, 7], and oral contraceptives [4]. Multiple studies have indicated that the volume of GCF can vary from 0.43µl to 1.56µl [8], whereas, in slightly inflamed gingiva, 0.1mg GCF was obtained [9]. The volume of GCF is relatively small, depending on the size of the gingival sulcus and the permeability of the gingival capillaries at the base of

Table 1: Description of different techniques for the collection of GCF samples [4, 142-143]

Technique	Utilization devices	Method of application
Absorbing paper strips	Filter paper strips	Paper strips are inserted into the crevice to absorb fluid. The fluid volume is quantified by protein disclosing dye, measurement of electronic capacitance, and weighing the paper before and after application.
Pre-weighed twisted threads	Sampling threads	Threads are placed into the crevice and the collected fluid is measured by weighing the sample threads.
Micro-pipettes	Capillary tubes	Capillary tubes are inserted inside the crevice and the content is later centrifuged and assessed.
Crevicular washing	Crevicular washing appliance	The appliance consists of a hard acrylic resin covering the maxilla with soft borders and grooves entering the gingival crevice. The gingival crevice is washed for a certain period from the palatal to the buccal side with a 4-6mL solution by a peristaltic pump.

the gingival sulcus, which can be affected by the level of tissue inflammation and the osmotic gradient of the vessels [3]. The molecular composition of GCF has garnered significant attention as a promising bio reservoir for various oral and systemic health conditions as it contains a complex array of biomolecules, including proteins, cytokines, enzymes, and nucleic acids [3]. These constituents reflect the dynamic state of the periodontal tissues and can serve as indicators of both local and systemic inflammatory processes. GCF proteomic studies have revealed numerous proteins associated with periodontal health and disease, offering insights into the pathogenesis and progression of conditions such as periodontitis [10-12]. Additionally, GCF has been implicated as a potential diagnostic tool for various systemic diseases, including diabetes, cardiovascular disease, and autoimmune disorders, due to the systemic dissemination of its biomarkers. Recent advances in molecular biology and analytical techniques have provided a deeper understanding of the molecular aspects of GCF, making it an attractive candidate for non-invasive and cost-effective diagnostics in the fields of dentistry and medicine. Various methods were proposed [13] to obtain a sample of GCF for research purposes, which are elaborately explained in Table 1.

Gingival crevicular fluid primarily comprises local breakdown products, including serum transudate, systemic and local inflammatory mediators, subgingival microbial species, extracellular proteins, and cells [3]. An overview of the physiological composition of GCF is presented in Table 2 [1, 3-4, 14-18]. The diverse components within GCF render it a valuable source of a broad spectrum of biomarkers specifically associated with systemic pathologies, encompassing metabolic syndrome (MetS), hepatic diseases (HD), rheumatoid arthritis (RA), diabetes mellitus (DM), cardiovascular diseases (CVDs), and acquired immune deficiency syndrome (AIDS) (Figure 1) [19]. Consequently, a thorough exploration of GCF under systemic conditions offers a pivotal opportunity to unravel the pathophysiology of periodontal diseases and their connection with systemic manifestations. This includes exploring the

Table 2: Physiological composition of GCF

Compositions		Main Components
Cellular elements	Epithelial cells	-
	Leukocytes	95-97% neutrophils, 1-2% lymphocytes, and 2-3% mononuclear cells.
	Bacteria	Campylobacter, Selenomonas, Porphyromonas, Catonella, Tannerella, Dialister, Peptostreptococcus, Streptococcus, and Eubacterium
Electrolytes	Na: K	-
	Fluoride, Calcium, Iodine, and Phosphorous	-
Organic compounds	Carbohydrates	Glucose hexosamine and hexuronic acid
	Immunoglobulins	IgG, IgA, IgM
	Complement proteins	Residual fragments of C3, and factor B
	Cytokines	Interleukins, TNF-α, CRP
	Metabolic elements	Lactic acid
	Hormones	Sprostoglandin
	Enzymes	Acid phosphates, alkaline phosphatase, Cathepsin G, B, and D, Collagenase, Elastase, Beta-glucuronidase, and Gelatinase

Abbreviations: CRP: C-reactive protein, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, TNF-α: Tumor Necrosis Factor-α

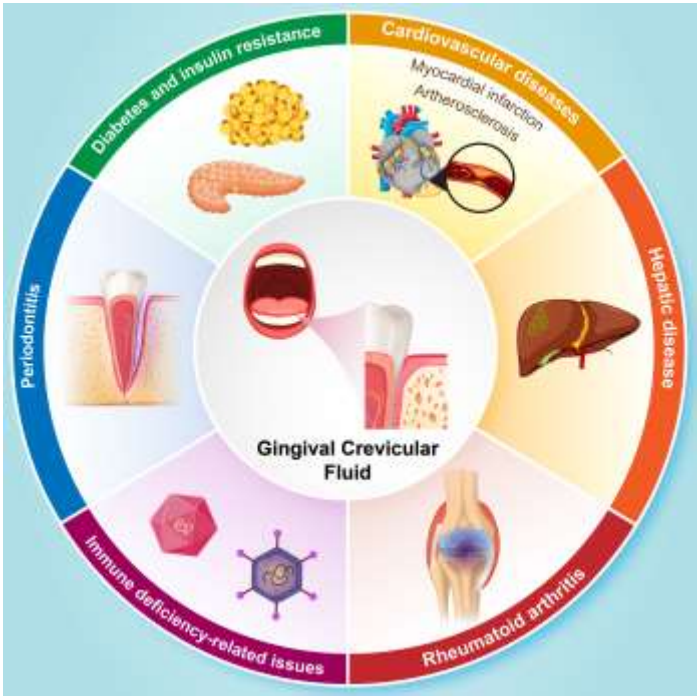

Figure 1: The evidence-based link between systemic diseases and periodontal inflammation, reflected by GCF-related biomarkers. Qualitative and quantitative alterations of GCF molecular composition attributable to general health and periodontal status

potential of GCF as a validated source of biomarkers, presenting a promising avenue for clinical point-of-care diagnostics.

Clinical investigations have revealed the substantial impact of different systemic conditions on the dynamic levels of various biomarkers present in GCF, encompassing a diverse array of molecules and agents. Notably, a majority of the GCF biomarkers significantly affected by systemic diseases are compounds and molecules associated with inflammation or immunity [20-21]. The observed disparities in biomarker levels may serve as indicators of an elevated risk for advanced periodontal inflammation, particularly periodontitis [20-21]. However, it is crucial to acknowledge that while inflammation- or immunity-related biomarkers exhibit considerable diversity, systemic diseases are demonstrated to selectively influence specific types of biomarkers in GCF [22-23]. This suggests that the spectrum of detectable biomarkers in GCF may vary among individuals, reflecting the distinct pathophysiological mechanisms of each systemic disease. This variability poses an essential consideration for future developments in personalized medicine and advanced diagnostics within the field of dentistry.

Due to the diversity of different biomarkers in GCF which can be influenced by different types of systemic diseases, as well as the vastness of the field, no literature review has comprehensively discussed the current advances and limitations in this field. Therefore, the objective of this study was to conduct a comprehensive narrative review of available evidence, exploring the potential of GCF as a diagnostic tool for detecting both systemic diseases and oral conditions. This thorough review delves into the evolving landscape of GCF biomarkers, highlighting their significance in health and disease, and discussing potential clinical applications arising from the molecular insights provided by this biofluid. We present an overview of current data on GCF-related biomarkers linked to systemic diseases. Appreciating the diagnostic role of GCF as an adjunct tool may facilitate early detection and prevention of common systemic conditions, thereby contributing to broader implications for public health policies.

Search Strategy and Screening Method

This narrative review was conducted based on the search results in two databases (PubMed, and Scopus) using the keywords: “Gingival Crevicular Fluid”, “Metabolic Syndrome”, “Liver Diseases”,” Rheumatoid Arthritis”,” Diabetes Mellitus”,” Cardiovascular Diseases”,” Acquired Immunodeficiency Syndrome”. The investigated diseases in our study represent the most

commonly studied diseases in this field. All the searched keywords were selected based on the PubMed Mesh terms. The search was conducted until May 26, 2025.

The original research articles, written in English, and focusing on human studies were considered for further assessment. However, the articles in formats other than the original article, not written in English, and conducted on subjects other than human were excluded from the study. A selected number of studies underwent further assessment to represent the current findings, advancements, and perspectives in the field.

Effect of systemic diseases on gingival crevicular fluid biomarkers

Metabolic Syndrome (MetS)

MetS is characterized by a cluster of clinical symptoms, including insulin resistance, hypertension, dyslipidemia, and obesity, ultimately leading to cardiovascular diseases and diabetes [24-25]. Given its association with various signs and symptoms affecting multiple organs, MetS has become the subject of investigation for potential oral manifestations. D'Aiuto *et al.* [26] proposed that individuals with periodontitis exhibit a 2.3 times higher likelihood of developing MetS compared to their healthy counterparts. Additionally, the results of a meta-analysis conducted by Nibali *et al.* [27] revealed that the risk of developing periodontitis in individuals with MetS is twice as high as in the general population. This suggests a plausible relationship between periodontitis and MetS, potentially reflected in changes in the levels of GCF compounds. A summary of selected studies is provided in Table 3.

Association between MetS and GCF components

GCF, being an easily accessible sample enriched with

Table 3: The summary of selected studies on the GCF in patients with metabolic syndrome

Author (year)	Study Group(s)	Measured Biomarkers	GCF Sampling Method	Evaluation Method	Outcomes
Senkal *et al.* (2024) [32]	I: MetS+ periodontitis II: MetS+ healthy periodontium III: Systemically healthy with periodontitis	Systemically/ periodontally healthy individuals	IL-20, RNAKL, OPG, RANKL/OPG	Paper strips	Among all of the assessed biomarkers, the patients with MetS and healthy periodontium did not exhibit any difference with the control group. However, the MetS group showed significant difference with the groups affected by periodontitis with and without MetS.
Nibali *et al.* (2022) [24]	I: MetS with periodontitis patients II: MetS patients without periodontitis	Aggrecan, IL-6, IL-8	Paper strips	Human XL Cytokine protein arrays ELISA	- Aggrecan, IL-6, and IL-8 were increased in patients without periodontitis compared to the patients with moderate and severe periodontitis. - Decreased levels of aggrecan in GCF of MetS patients could be an indicator of periodontitis ($p< 0.05$)
Gürkan *et al.* (2016) [22]	I: MetS+ gingivitis II: MetS+ healthy periodontium III: Systemically healthy with gingivitis IV: Systemically/ periodontally healthy individuals	RANTES, MIF, MCP-1	Paper strips	ELISA	- MCP-1 and MIF levels did not exhibit any significant differences between groups. - RANTES and MCP-1 levels of group 1 were higher than group 2. - RANTES was significantly higher in group 1 than in group 3 ($p< 0.01$). - RANTES levels in GCF are significantly elevated as a result of MetS in patients with gingivitis.
Han *et al.* (2012) [33]	I: MetS II: Periodontitis III: MetS + Periodontitis IV: Healthy individuals	MMP-8, MMP-9, and MMP-13	Absorbent paper points	ELISA	- MMP-8,9 and 13 were independently correlated with both periodontitis and MetS. - MMP-13 exhibited a significant increase in MetS subjects compared to subjects without MetS. - MMP-9 and 13 indicate significantly higher contents in women with MetS. - The elevated levels of MMP-9 and 13 can be indicators of both MetS and periodontitis among women.

Abbreviations: CP: Chronic periodontitis, ELISA: Enzyme-linked immunoassay, IL: Interleukin, RANTES: regulated on activation, normal T-cell expressed and secreted protein, MCP-1: Monocyte chemotactic protein-1, MetS: Metabolic syndrome, MIF: Macrophage migration inhibitory factor, MMP: Matrix metalloproteinase

various biological markers, holds promise for diagnosing and assessing the severity/prognosis of MetS. In a study by Nibali *et al.* [24], GCF markers in MetS patients were analyzed concerning the presence of periodontitis. GCF samples from 95 patients were collected using periopaper strips, with 10 subjects categorized as no/mild, 34 moderate, and 51 severe periodontitis cases. Aggrecan, and Interleukins (ILs) including IL-6, and IL-8 were found to be increased in patients without periodontitis compared to those with moderate and severe periodontitis. Notably, Aggrecan exhibited a significant association with the periodontal status of the patients ($p< 0.05$). Decreased levels of Aggrecan in GCF of MetS patients could serve as an indicator of periodontitis. This decrease aligns with findings in patients with RA, suggesting common pathological pathways between periodontitis and RA [24, 28-31]. Moreover, IL-20 is also demonstrated as a biomarker increasing in patients affected by both MetS and periodontitis [32].

Examining GCF markers and MetS correlation, Gürkan *et al.* [22] assessed regulated on activation, normal T-cell expressed and secreted protein (RANTES), macrophage migration inhibitory factor (MIF), and monocyte chemotactic protein-1 (MCP-1) levels. In the MetS + gingivitis group, RANTES and MCP-1 GCF levels were higher than in MetS and healthy periodontium groups, showing significant elevations compared to healthy individuals ($p< 0.0001$, 0.005, respectively). Notably, RANTES levels in the MetS + gingivitis group were significantly higher than in systemically healthy patients with gingivitis ($p< 0.01$). However, MIF and MCP-1 levels did not exhibit significant differences between these two groups ($p> 0.05$). Elevated RANTES levels in GCF were attributed to increased local gingival inflammation caused by MetS, establishing RANTES as significantly elevated due to MetS in patients with gingivitis.

Exploring the link between MetS and periodontitis, matrix metalloproteinase (MMP) levels in GCF were considered [33]. Han *et al.* [33] evaluated MMP-8, MMP-9, and MMP-13 concentrations in 314 individuals, categorizing them into healthy cases, MetS cases, periodontitis cases, and MetS + periodontitis cases. GCF levels of MMP-8, MMP-9, and MMP-13 in patients with periodontitis were higher than in MetS patients or healthy individuals ($p< 0.001$). In the comparison between patients with and without MetS, only MMP-13 exhibited a significant increase in MetS subjects compared to those without MetS ($p< 0.002$). MMP-9 and MMP-13 levels were significantly higher in women with MetS. The study concluded that MMP-8, MMP-9, and MMP-13 were independently correlated with both periodontitis and MetS, making elevated MMP-9 and MMP-13 indicative of both MetS and periodontitis among women [33].

In summary, studies assessing GCF among patients with MetS suggest that RANTES and MMP-13 can serve as indicative biomarkers, showing marked elevation in these patients [33]. However, other molecules, including MMP-9, were increased specifically among female patients. Additionally, MetS appears to be a co-existing morbidity in patients with periodontal diseases, with Aggrecan concentration in GCF substantially decreased in patients with MetS and periodontitis [24].

Liver Diseases

GCF has been utilized in investigating chronic hepatitis C (CHC) [34]. In CHC, the progressive replacement of natural liver tissue with fibrotic tissue leads to impaired liver function and eventual development of liver cirrhosis [35-36]. Globally, around two hundred million people grapple with CHC, making it a significant global health concern [34]. CHC is known to manifest oral symptoms, impacting the overall quality of life [37-38]. The inflammatory nature of CHC suggests multifaceted interactions with periodontal tissues, contributing to periodontitis through the presence of pro-inflammatory mediators in the blood of CHC patients [36, 39]. Table 4 provides a summary of the selected studies in this context.

Association between liver diseases and GCF components

The effects of CHC on the GCF, particularly IL-1α, and IL-1β level were investigated by Surlin *et al.* [36] among 13 chronic periodontitis (CP) patients without systemic disease (group 1), 11 CP patients with asymptomatic CHC (group 2), and 11 systematically and periodontally healthy controls (group 3). The highest level of both cytokines was observed among the patients with CP and asymptomatic CHC, while the lowest level was in systemically and periodontally healthy participants. The maximum probing depth and the number of remnant teeth, were positively and negatively correlated with the GCF levels of IL-1α and IL-1β in CP patients

Table 4: The summary of selected studies on the GCF in patients with liver diseases

Author (year)	Study Group(s)	Measured Biomarkers	GCF Sampling Method	Evaluation Method	Outcomes
Surlin *et al.* (2021) [38]	I: CHC+CP patients II: CHC patients III: CP patients III: Healthy individuals	- NLRP3, - IL-18 - Caspase-1	Paper strips	ELISA	The highest levels of all three biomarkers were observed in group I ($p< 0.05$). - Group III showed significantly higher levels of all three biomarkers than the periodontally healthy groups (groups II and IV).
Surlin *et al.* (2020) [36]	I: CP patients without systemic disease II: CP patients with asymptomatic CHC III: Healthy individuals	- IL-1α - IL-1β	Paper strips	ELISA	- The highest level of both cytokines was observed in group 2 and the lowest level was in the control group. Also, the difference between all the groups was statistically significant ($p< 0.05$). -CHC can exacerbate the inflammatory status of CP via increasing shared pro-inflammatory cytokines levels (IL-1α and IL-1β).
Suzuki *et al.* (2005) [47]	HCV-positive patients	- HCV RNA	Paper strips	QIAamp viral RNA kit RT-PCR	- GCF had higher sensitivity in the detection of HCV RNA levels than saliva, although saliva provided higher amounts of sample volume.
Gammie *et al.* (2002) [49]	Random subjects	- HAV antibodies	NR	Automated IMx MEIA analytical system	- GCF can be an easy source for screening and allows for long-term follow-ups in assessing the level of immunity against HAV.
Maticic *et al.* (2001) [46]	HCV-positive patients	- HCV RNA	paper points	PCR assay	The detection rate of HCV RNA in GCF and saliva was 59% and 35%, respectively.
Pollock *et al.* (1984) [48]	Random subjects	- HBsAg - HBeAg	Paper strips	Radioimmunoassay test kits	- HBeAg can be detected in GCF.

Abbreviations: CHC: Chronic Hepatitis C, CP: Chronic Periodontitis, ELISA: Enzyme-Linked Immunosorbent Assay, GCF: Gingival Crevicular Fluid, HAV: Hepatitis A Virus, HBsAg: Hepatitis B Surface Antigen, HBeAg: Hepatitis B e Antigen, HCV: Hepatitis C Virus, IL-1α: Interleukin-1 alpha, IL-1β: Interleukin-1 beta, IL-18: Interleukin-18, NLRP3: NOD-, LRR-, and pyrin domain-containing protein 3, NR: Not Reported, PCR: Polymerase Chain Reaction, RT-PCR: Reverse Transcription Polymerase Chain Reaction

with asymptomatic CHC, respectively. It appears that CHC can take a role in the exacerbation of the inflammatory status of CP via increasing shared pro-inflammatory cytokines levels (IL-1α and IL-1β). As indicated before, the inflammatory nature of both CP and CHC could result in interactions between the two diseases which can be investigated through the fluctuation of biomarkers in GCF [38, 40].

One of the noticeable biomarkers concerning liver diseases in GCF is inflammasome. This biomarker is a multi-protein, oligomer compound which plays a role in the initial stages of inflammation [38, 41, 42]. Nod-like receptor 3 (NLRP3) complex, as an inflammasome, takes part in inflammatory and innate immunity mechanisms [38, 43-44]. NLR3 is a part of the NLPR family that has a role in the activation of Caspase 1 and regulating important pro-inflammatory cytokines, like IL-18 [38, 44]. In this regard, Surlin *et al.* [38] investigated GCF levels of NLRP3, IL-18, and Caspase-1 in patients with CHC and periodontitis. The patients were divided into four study groups: (I) 18 CHC + CP, (II) 14 only CHC, (III) 15 only CP, and (IV) 15 healthy controls. The patients affected by both CHC and CP achieved the significantly highest levels of NLRP3, IL-18, and Caspase-1.

The results of this study can be further validated by the fact that NLRP3 inflammasome and IL-18 are secreted by liver macrophages during the initiation of hepatitis C infection [45].

Another GCF-related biomarker includes hepatitis C virus ribonucleic acid (HCV RNA) [46-47] to detect HCV. Maticic *et al.* [46] investigated the presence of HCV RNA in GCF and saliva of 50 HCV-positive patients, concluding that the rate of HCV RNA for GCF and saliva was 59% and 35%, respectively. Arguably, in addition to blood, GCF could be regarded as an adjunct mean for detecting HCV. However, further investigations are required to optimize and validate the current data. Suzuki *et al.* [47] quantified HCV RNA in the saliva and GCF of 26 HCV-positive patients of whom

78% were GCF-positive while exhibiting negative results in saliva. In 77% of the cases, the GCF level of HCV was higher than the saliva level. In spite of the fact that HCV levels in neither GCF nor saliva showed a significant correlation with the viral loads in the blood samples, the reduced levels of serum HCV RNA were less likely to be detected in saliva than in GCF [47]. This indicates the higher sensitivity of GCF in the detection of HCV RNA levels than saliva.

Another liver disease, which has been evaluated via GCF, is hepatitis B (HB). Quantification of hepatitis B surface antigen (HBsAg) in GCF was conducted by Pollock *et al.* [48] and the presence of hepatitis B e antigen (HBeAg) was also reported in GCF. In another study by Gammie *et al.* [49], the level of immunity against the hepatitis A virus (HAV) was assessed by quantification of antibodies in the GCF samples. They reported that GCF can be an easy source for screening and allows for long-term follow-ups.

Based on the studies related to the implications of liver diseases on GCF biomarker levels [38, 46-49], it can be concluded that HCV infection can increase the GCF levels of inflammatory mediators, NLRP3, IL-1α, IL-1β, IL-18, and caspase in patients suffering from periodontitis [36, 38]. Moreover, quantification of antibodies against HCV [46-47], HBV [48], and hepatitis A virus (HAV) [49] in GCF seems to be a reliable approach to evaluate the sensitivity and specificity of GCF as a potential diagnostic medium.

Rheumatoid arthritis (RA)

RA stands out as an extensively studied autoimmune disease in the context of GCF-based diagnostics. It is a prevalent systemic autoimmune disorder affecting approximately 224.25 per 100’000 of the global population [50]. RA primarily targets synovial tissues, leading to synovitis and joint structural damage, resulting in chronic pain, disability, and potential severe extra-articular complications such as rheumatoid vasculitis or interstitial pneumonia [21]. Recent investigations have explored the intricate relationship between CP and systemic disorders with inflammatory mechanisms [51-52]. Notably, the levels of rheumatoid factor (RF) have been found to be significantly elevated in the saliva, serum, and subgingival plaque of CP patients [52-53]. The inflammatory nature shared by RA and CP suggests potential interactions between these conditions, mediated by inflammatory mediators and cytokines such as C-reactive protein (CRP), IL-1, IL-6, and tumor necrosis factor-α (TNF-α) [52, 54]. Given the unique characteristics of GCF as a reservoir for various biomarkers this fluid presents itself as a valuable complement for assessing the progression of RA [55]. Analyzing GCF holds promise for supporting early RA diagnosis. A summary of selected studies exploring the link between RA and GCF is presented in Table 5.

Association between RA and GCF components

To investigate the correlation between RA and biomarkers in GCF, Silosi *et al.* [20] conducted a comprehensive study focusing on matrix metalloproteinase-9 (MMP-9) levels in GCF and serum as potential reliable biomarkers for RA. The study included four distinct groups: 21 healthy individuals, 14 patients with CP, 16 patients with RA, and 12 patients with RA + CP [20]. The results revealed a significant elevation in GCF MMP-9 levels in RA patients compared to the control group [20]. Similarly, the RA+ CP group exhibited higher GCF MMP-9 levels compared to both CP and RA groups. This suggests that the inflammatory nature of RA and CP may synergistically enhance MMP-9 levels in patients with RA+CP.

Consequently, the presence of MMP-9 in GCF could be considered a valuable marker for patients with RA or RA+CP [20]. Additionally, Manoil *et al.* [14] explored the bacterial load in GCF, proposing a potential link between CP and RA through unattached GCF microorganisms. The presence of specific bacteria, such as *P. gingivalis*, was indicated as a potential indicator of RA severity, showing statistically significant correlations with gingival bleeding on probing and plaque scores [14]. This highlights the possibility of investigating GCF bacteria as a mean to assess RA activity.

The comprehensive exploration of the link between GCF content and RA was conducted through the analysis of cytokines. In a systematic review, Javed *et al.* [21] investigated the cytokine profile of GCF in individuals with RA and CP. The study revealed elevated GCF levels of MMP-8, MMP-13, IL-1b, IL-4, IL-10, and TNF-α in RA patients compared to healthy individuals [21]. Additionally, Prieto *et al.* [56] examined 40 subjects, including 20 healthy individuals and 20 RA patients, evaluating the soluble neuropilin-1 (sNRP-1) level in their GCF and periodontal status. RA patients exhibited

Table 5: The summary of selected studies on the GCF in patients with rheumatoid arthritis

Author (year)	Study Group(s)	Measured Biomarkers	GCF sampling method	Method of evaluation	Outcomes
El-Wakeel et al. (2023) [59]	I: CP II: RA III: RA+CP IV: Healthy individuals	Prolactin	Paper strips	ELISA	- Before SRP treatment, groups with RA + CP showed the highest GCF levels of prolactin, followed by the group affected by CP, RA, and the control group. - After the SRP treatment, the group with RA + CP and CP showed no significant difference. This trend was also replicated for the group with RA and the control group
Manoil et al. (2021) [14]	I: RA II: Behçet's disease III: Healthy individuals	The bacterial composition of GCF	Paper strips	FISH ELISA	- The unknown link between CP and RA could be the unattached GCF bacteria. - P. gingivalis showed statistically significant correlations ($p< 0.05$) with the severity of RA as well as BOP and plaque scores of patients.
Prieto et al. (2021) [56]	I: RA II: Healthy individuals	sNRP-1	Paper strips	ELISA	- Patients with RA showed significantly higher levels of sNRP-1 in GCF ($p< 0.05$).
Xiao et al. (2021) [144]	I: RA II: Healthy individuals	TNF-α and IL-1β	Filter papers	ELISA	- RA presence was positively correlated with GCF levels of TNF-α and IL-1β levels. - Incidence of CP among the patients with RA was significantly higher than in the control group. The longer the duration of RA, the higher the incidence of CP.
Silosi et al. (2015) [20]	I: CP II: RA III: RA+CP IV: Healthy individuals	MMP-9	Paper strips	ELISA	- GCF level of MMP-9 in patients with RA was significantly higher than in the control group. - Elevated level was also observed in patients with RA + CP compared to CP and RA.
Cetinkaya et al. (2013) [145]	I: RA II: CP III: Healthy individuals	IL-1β, IL-4, IL-10 and TNF-α	Paper strips	ELISA	- GCF levels of IL-1β, IL-4, IL-10, and TNF-α did not exhibit any significant differences between the study groups. - Clinical periodontal parameters were significantly different between the groups. PI was significantly higher among the patients with RA compared to other groups; however, CAL, PD, and GI were significantly higher among patients with CP compared to other groups ($p< 0.05$).
Esen et al. (2012) [58]	I: RA + CP II: RA III: CP IV: Healthy individuals	Oxidant/antioxidant status and OSI of GCF	Paper strips	REL assay diagnostics	-GCF levels of total oxidant status among patients with RA + CP and CP were significantly higher than that in the patients with RA ($p< 0.05$). -The OSI value of GCF for the group with RA + CP was significantly higher than that in the patients with RA ($p< 0.05$).
Bıyıkog˘lu et al. (2009) [62]	I: RA + periodontitis II: RA + gingivitis III: Periodontitis IV: gingivitis V: Healthy individuals	MMP-8, MMP-13 and TIMP-1	Filter paper strips	ELISA	- MMP-8 total level was significantly lower in the healthy individuals than the groups I, II, and III ($p< 0.05$). - MMP-13 levels did not show any significant difference between the groups. - Groups I and II exhibited similar levels of TIMP-1 to groups 3 and 4. - TIMP-1 level in GCF can be markedly enhanced by the presence of RA regardless of the periodontal status of the patient. - MMP-8 and MMP-13 levels seem to be elevated by both RA and CP and the concurrent incidence of these two diseases does not significantly enhance their GCF levels.
Biyikog˘lu et al. (2006) [63]	I: RA II: CP III: Healthy individuals	PAI-2, PGE2 and IL-1β	Filter paper strips	ELISA	- The GCF levels of PAI-2, PGE2, and IL-1β were not statistically different between the study groups. - The GCF levels of t-PA were significantly higher in patients with RA compared to CP ($p< 0.05$).

Abbreviations: CP: Chronic periodontitis, ELISA: Enzyme-linked immunoassay, FISH: Fluorescent in situ hybridization, MMP: Matrix metalloproteinase, PAI-2: plasminogen activator inhibitor-2, PGE2: Prostaglandin E2, RA: Rheumatoid arthritis, Soluble neuropilin-1 (sNRP-1), TIMP-1: Tissue Inhibitor of MMP-1, TNF-α: Tumor Necrosis Factor- α, t-PA: Tissue-type Plasminogen Activator

significantly higher concentrations of sNRP-1 in GCF, suggesting its potential as a viable option for further investigations. In another study, Cetinkaya *et al.* [57] assessed anti-inflammatory and pro-inflammatory cytokine levels in GCF among RA and CP patients. Although GCF levels of IL-1β, IL-4, IL-10, and TNF-α did not show significant differences between the study groups, the use of non-steroidal anti-inflammatory drugs

(NSAIDs) by RA patients could be a crucial confounding factor in the study.

Moreover, considering the key role of oxygen metabolism in the pathogenesis of both CP and RA [58], Esen *et al.* [58] investigated the effects of CP and RA on the oxidant/antioxidant status and oxidative stress index (OSI) of GCF. GCF levels of total oxidant status in the RA + CP and CP groups were significantly higher than in patients with RA ($p< 0.05$) [58]. The OSI value of GCF for the RA + CP group was significantly higher than in patients with RA ($p< 0.05$) [58]. Therefore, while CP appears to influence the GCF's OSI concentration, RA may not exhibit the same effect on this parameter in the presence of CP [58]. The combined impact of CP and RA on other biomarkers is also implicated in a study by El-Wakeel *et al.* [59], assessing GCF levels of prolactin (PRL). They indicated that the patients with CP and RA showed the highest statistically significant GCF levels of PRL, followed by the patients with CP, patients with RA, and the control group. The last two groups showed no statistically significant difference. After the scaling and root planning (SRP) treatment, the group with RA and CP showed no difference with CP. On the other hand, the groups with RA showed no significant difference with the control group. Building upon the findings outlined above [57-58, 60-61], the co-occurrence of CP and RA appears to yield distinctive alterations linked to inflammatory biomarkers. In a study by Biyikog˘lu *et al.* [62], examining 74 patients and investigating MMP-8, MMP-13, and tissue inhibitor of MMP-1 (TIMP-1), it was observed that MMP-8 total levels were significantly lower in healthy individuals compared to those diagnosed with RA and CP ($p< 0.05$). However, MMP-13 did not exhibit any significant difference between the groups [62]. Although TIMP-1 concentrations did not show a significant fluctuation between the groups, the total amount of TIMP-1 was notably higher in groups of patients with RA + periodontitis, patients with only periodontitis, and patients with only gingivitis compared to systematically and periodontally healthy individuals [62]. Interestingly, the concurrent incidence of RA and CP did not significantly enhance the GCF levels of MMP-8 and 13 [62].

These findings align with the study by Biyikog˘lu *et al.* [63], where the coexistence of RA and CP did not significantly impact some systemic markers of RA or clinical periodontal outcomes. The total amounts of plasminogen activator inhibitor-2 (PAI-2), prostaglandin E2 (PGE2), and IL-1β did not differ significantly between RA and CP group [63].

In summary, scientific evidence points to several GCF biomarkers associated with RA, primarily inflammatory cytokines such as tumor necrosis factor- α (TNF-α) [52, 57, 64], interleukins [52, 57], and MMPs [20, 62]. Notably, the presence of a specific periodontal pathogen, P. gingivalis, was also correlated with the severity of RA [14]. However, not all the biomarkers have shown statistically significant outcomes. Another study has indicated that IL-1β, IL-4, IL-6, IL-10, IL-17A, TNF-α, IFN-γ, and chemokines RANTES/ CCL5, eotaxin, and MCP-1 exhibit no significant difference between the patients and the control group [56]. These discrepancies among the studies can be attributed to severity of the studies and sampling conditions and methods.

Diabetes mellitus

Diabetes mellitus encompasses a group of metabolic disorders characterized by elevated blood glucose levels in affected individuals [66-67]. It serves as a significant risk factor for the onset of CP, heightening susceptibility approximately threefold compared to healthy individuals [67-68]. Additionally, diabetes exacerbates the condition of periodontal disease, particularly in individuals with inadequately controlled blood glucose levels [67, 69-70]. Type 2 diabetes mellitus (T2DM), the most prevalent form of diabetes [66-67], induces altered inflammatory responses [71-72], disrupts tissue repair mechanisms [71-72], and accelerates the progression of periodontal tissue destruction [73-74]. The interplay between the two conditions is bidirectional, with CP exacerbating diabetes and diabetes-related complications significantly intensifying the severity of periodontitis [74-75].

Exploring the biomarkers present in GCF during T2DM offers insights into the biological effects of T2DM on periodontal tissues. Fluctuations in specific biomarkers can aid clinicians in assessing the severity of diabetes and its prognosis [76]. Given T2DM's demonstrated impact on impeding the healing rate of periodontal tissues, it is conceivable that diabetes negatively influences the levels of specific biological factors in GCF crucial for periodontal tissue remodeling

Table 6: The summary of selected studies on GCF in patients with diabetes mellitus

Author (year)	Study Group (s)	Measured Biomarkers	GCF sampling method	Method of evaluation	Outcomes
Păvălan *et al.* (2025) [95]	I: T2DM+HF+CP II: T2DM +CP III: CP IV: Healthy individuals	DPP-4, Gal-3	Paper strip	ELISA	- Higher levels of DPP-4 were reported in the study groups compared to the control group. - Gal-3 was elevated in patients with CP + HF regardless of T2DM.
Elazazy *et al.* (2021) [71]	I: CP patients with diabetes II: CP patients without diabetes III: Healthy individuals	microRNAs	Paper strips	-miRNeasy mini kit -miScript II RT Kit and distinct miScript primers	- MicroRNA-223 and microRNA-200b are significantly higher among patients with T2DM and periodontitis ($p< 0.05$).
Gao *et al.* (2021) [74]	I: T2DM + CP II: CP III: Healthy individuals	Calprotectin	Paper strip	ELISA	- Patients with T2DM and CP exhibit significantly higher GCF and serum levels of calprotectin compared to patients with CP or the control group.
Sande *et al.* (2020) [78]	I: Diabetic individuals II: Healthy individuals	Glucose	Test strip	Glucometer	- GCB was significantly better than FSB and was comparable to VB in estimating glucose levels
Marinho *et al.* (2019) [87]	I: T2DM+CP II: T2DM III: CP IV: Healthy individuals	GCF proteins	Paper strip	Protein Assay Kit	- Increased levels of some proteins (S100A8, S100A9, immunoglobulins, myeloperoxidase, neutrophil, azurocidin, and elastase) and decreased levels of some others (cathelicidin, annexin A1, actins) was found among T2DM + CP subjects compared to healthy individuals.
Peniche-Palma *et al.* (2019) [86]	I: T2DM (controlled) II: T2DM (poorly controlled) III: T2DM (controlled)+ CP IV: T2DM (poorly controlled) + CP V: Healthy individuals	MPO MMP-9	Paper strip	ELISA	- No relationship was found between the elevation of these biomarkers in controlled/uncontrolled T2DM compared to healthy controls
Yilmaz *et al.* (2018) [89]	I: T2DM+CP II: T2DM III: CP IV: Healthy individuals	HβD-1	Filter-paper strip	ELISA	- T2DM and periodontitis could result in altered levels of HβD-1 in GCF, leading to potential susceptibility of T2DM patients to periodontitis.
Bhavsar *et al.* (2016) [79]	I: Diabetic individuals II: Healthy individuals	Glucose	Test strip	Automated glucose analyzer	- No significant difference between GCB and VB glucose levels was reported ($P > 0.05$). The correlation of these two methods was significant.
Doğan *et al.* (2016) [146]	I: T2DM+CP II: T2DM III: CP IV: Healthy individuals	Chemerin	Paper strips	ELISA	- GCF levels of chemerin were significantly higher in patients with T2DM + CP and T2DM compared to healthy individuals. - A positive correlation was found between chemerin and HbA1c. - Chemerin could be considered as a potential inflammatory biomarker for T2DM in GCF.
Doğan *et al.* (2016) [10]	I: T2DM+CP II: T2DM III: CP IV: Healthy individuals	Vaspin Omentin-1, TNF-α	Paper strips	ELISA	- Total concentration of vaspin was significantly higher in T2DM + CP and T2DM over healthy individuals. - Total concentration of vaspin was significantly higher in patients with T2DM+CP compared to patients with CP. - Total concentration of omentin-1 was significantly lower in the presence of T2DM or CP compared to healthy individuals. - These two adipokines could be considered as potential inflammatory markers of both T2DM and CP
Mishra *et al.* (2016) [92]	I: T2DM+CP II: CP III: Healthy individuals	Visfatin	Microcapillary tubes	ELISA	- Significantly higher visfatin level was found in patients with T2DM and CP; however, T2DM has a significant role in elevated levels of visfatin.
Parihar *et al.* (2016) [76]	I: Diabetic individuals II: Healthy individuals	Glucose	Glucometer	Glucometer	A strong positive correlation ($r = 0.986$ and $r = 0.972$ in diabetic patients and $r = 0.820$ and $r = 0.721$ in non-diabetic individuals) was found between glucose levels of GCB with FSB and VB.
Yamaguchi *et al.* (2004) [80]	Healthy adults	Glucose	A custom-designed GCF-collecting device enclosed in a glucose testing tape	A novel glucometer	- Noticeable correlation coefficients of 0.7 and 0.88 between glucose levels of GCF and blood among the participants with two nationalities.
Bulut *et al.* (2001) [94]	I: Diabetic patients II: Healthy individuals	IL-1β	Filter-paper strip	ELISA	- Increased levels of IL-1β in T2DM may worsen periodontal condition due to the intensified inflammatory response to bacteria in diabetic patients compared to non-diabetic individuals.

Abbreviations: CP: Chronic periodontitis, ELISA: Enzyme-linked immunoassay, HβD-1: Human β defensin 1, IL: Interleukin, T2DM: Type 2 diabetes mellitus

and healing [76-77]. A summarized overview of selected studies is presented in Table 6.

Association between diabetes mellitus and glucose level in GCF

Numerous endeavors have aimed to devise a simple, non-invasive, and painless method for accurate and validated glucose level monitoring [76, 78-82]. Among these approaches, the analysis of GCF glucose levels has exhibited promising outcomes [76, 78-82].

In a study conducted by Yamaguchi *et al.* [80], notable correlation coefficients of 0.7 and 0.88 were observed between glucose levels in GCF and blood for two patient groups of distinct nationalities (Swedish and Japanese). Parihar *et al.* [76] demonstrated a robust positive correlation between glucose levels in GCF and fingerstick blood (FSB) and venous blood (VB) (r= 0.986 and r= 0.972 in diabetic patients, and r= 0.820 and r= 0.721 in non-diabetic individuals). Furthermore, Sande *et al.* [78], through the estimation of glucose levels, asserted that utilizing GCF was significantly superior to FSB and comparable to VB. Interestingly, Bhavsar *et al.* [79] reported no significant difference between GCF and VB glucose levels, revealing a highly significant correlation between these methods (r=0.972 and r= 0.721 for diabetic and non-diabetic subjects, respectively).

Considering the advantages of GCF over FSB and VB methods, such as safety, quickness, ease of performance, non-invasiveness, and greater patient acceptance, dentists can employ it for routine examinations to diagnose diabetes [76, 78-79].

Association between diabetes mellitus and GCF components

Numerous studies conducted over the past decades have consistently indicated that diabetic patients are more susceptible to infections [83-85]. This susceptibility stems from various mechanisms through which diabetes can disrupt the normal functioning of the body, including microRNA dysregulations, impaired cytokine production, neutrophil dysfunction, and other immunological factors [83].

Consequently, investigating the impact of diabetes-induced immune dysfunction on GCF levels of immunity-related markers appears to be a promising avenue. Biomarker deregulation is evident in both CP and T2DM [71]. MicroRNAs and specific forms of gene regulators are recognized for playing a crucial role in certain pathogenic processes involved in CP development, such as genetic susceptibility, adaptive immunity, and innate immunity [53, 93]. Hence, evaluating the deregulated microRNAs in GCF of CP patients with or without diabetes could provide insights into their potential role in diabetes-related CP pathogenesis [71].

Notably, Elazazy *et al.* [71] found that GCF levels of microRNA-223 and microRNA-200b were significantly higher in patients with CP and T2DM compared to healthy individuals, suggesting these microRNAs as potential therapeutic targets and disease biomarkers. Additionally, GCF calprotectin, a major cytoplasmic protein of neutrophils, exhibited significantly higher levels in patients with T2DM compared to those with CP and healthy individuals [74].

Immune mediators, such as myeloperoxidase (MPO) and matrix metalloproteinase-9 (MMP-9), play a pivotal role in chronic disease development, leading to significant damage to collagen-rich tissues [86]. However, Marinho *et al.* [87] reported no correlation between GCF concentrations of MPO and MMP-9 in controlled/uncontrolled T2DM compared to healthy controls. On the other hand, Sereti *et al*., have indicated no significant difference regarding GCF levels of IL-8, MMP-8, and advanced glycation-end products between the type 1 diabetes and healthy groups [88]. The type of diabetes and the level of inflammation can result in such discrepancies among the studies.

Exploring the GCF protein profile in patients with both T2DM and CP, Marinho *et al.* [87] found increased abundance of certain proteins compared to healthy individuals, including S100A8, S100A9, immunoglobulins, myeloperoxidase (MPO), neutrophil, azurocidin, and elastase. Conversely, a decreasing trend was observed in proteins, like cathelicidin (an antimicrobial peptide) and annexin A1 [87]. Human β-defensin-1 (HβD-1), a key element in the appropriate interaction between epithelial surfaces and microbiota in normal conditions and involved in innate immunity [89-91], exhibited a significant decrease in T2DM patients with/ without generalized periodontitis compared to healthy individuals [89]. Consequently, altered levels of HβD-1 in GCF may contribute to the increased susceptibility of T2DM patients to periodontitis.

In addition to immunity-related biomarkers, inflammatory-related biomarkers play a pivotal role in pathogenesis, among which visfatin, a chemical media-

tor associated with both CP and T2DM [23, 92], stands out. Visfatin functions as an enhancer of inflammatory processes induced by microbial invasion [92-93]. Mishra *et al.* [92] found that patients with both CP and T2DM exhibited significantly higher GCF levels of visfatin compared to healthy individuals and patients with CP and controlled T2DM.

Another key inflammatory mediator in diabetes and periodontitis is IL-1β [83]. IL-1β, present in GCF, possesses bone resorptive features and stimulates the production of prostaglandins (PGs), collagenase, and other proteases, contributing to soft tissue degeneration [94]. Bulut *et al.* [94] reported that increased levels of IL-1β in T2DM may be linked to alterations in immune response, suggesting that T2DM intensifies the inflammatory response to bacteria, thereby worsening the periodontal condition of diabetic patients compared to non-diabetic individuals. Other pro-inflammatory biomarkers in this regard are dipeptidyl peptidases-4 (DPP-4) and Galectin-3 (Gal-3). In this regard, Păvălan *et al.* [95], assessed these biomarkers among four groups: healthy individuals, patients with periodontitis, patients with periodontitis and heart failure, patients with periodontitis, heart failure, and T2DM. The results indicated highest Gal-3 values for the patients with periodontitis and heart failure with and without T2DM. DPP-4 exhibited elevated levels in all the study groups compared to the control group.

Adipokines, a group of biomarkers critical in inflammation caused by diabetes [96-98], also play a significant role. Vaspin, an adipokine linked to the severity of diabetes [99-101], was found by Doğan *et al.* [101] to have significantly higher total concentrations in patients with T2DM + CP compared to patients with CP alone and in patients with T2DM compared to healthy individuals. In contrast, omentin-1, another adipokine with anti-diabetic, anti-atherogenic, and anti-inflammatory characteristics [101-102], exhibited significantly lower total concentrations in the presence of T2DM or CP compared to healthy controls. These adipokines, along with chemerin, recently discovered and influencing lipid and glucose metabolism and inflammation [99-101], showed significantly higher GCF levels in patients with T2DM + CP compared to patients with CP alone and in patients with T2DM compared to healthy individuals. Additionally, a positive correlation was found between chemerin and glycated hemoglobin (HbA1c) [103]. Consequently, chemerin could be considered a potential inflammatory biomarker for T2DM in GCF.

In conclusion, GCF-related biomarkers associated with diabetes mellitus encompass glucose levels, immunity-related biomarkers (e.g., calprotectin, S100A8, S100A9, immunoglobulins, MPO), and inflammatory-related biomarkers (e.g., visfatin, IL-1β, adipokines like vaspin and chemerin). Further investigation is warranted to comprehensively understand the multidirectional interactions between diabetes and periodontal status.

Cardiovascular diseases

Cardiovascular diseases stand out as prevalent conditions linked to patients' lifestyles [104]. Emerging evidence points towards a potential association between atheromatous diseases and periodontitis [105]. Recent studies [106-108] indicate that individuals with periodontal disease may be prone to aortic stiffness, vascular endothelial dysfunction, and atherosclerosis. The exploration of the interplay between these conditions [106-108] has prompted further investigations, particularly examining the potential influence of cardiovascular diseases on the GCF. A concise summary of relevant studies is provided in Table 7.

Association between atherosclerosis and GCF components

As inflammation plays a primary role in both periodontitis and atherosclerosis [109], a specific parameter detectable GCF that could indicate the risk of developing atherosclerosis is leukotrienes concentration, the pro-inflammatory mediators derived from lipids. Accordingly, Bäck *et al.* [109] investigated the possible relation between GCF leukotrienes and atherosclerotic plaques with the GCF-related molecules. During the ultrasonography, 13 patients from the study group and 5 from the control group were detected with atherosclerosis. Patients diagnosed with atherosclerosis were attributed to a significant increase in the level of cysteinyl-leukotriene in GCF. Based on the results of this study, increased levels of leukotriene in GCF could act as an indicator of the risk of developing atherosclerosis. As suggested by several studies [109-110], atherosclerosis is one of the main cardiovascular diseases associated with elevated levels of low-density lipoprotein (LDL) [110], oxidized LDL (oxLDL) [110], and the possible role of bacteria [111] and inflammatory mediators [109, 112]. The

Table 7: The summary of selected studies on the GCF in patients with cardiovascular diseases.

Author (year)	Study Group(s)	Measured Biomarkers	GCF sampling method	Method	Outcomes
Çalapkorur *et al.* (2017) [121]	I: PAD II: Healthy individuals	IL-1b, pentraxin 3, and CRP	Filter paper Strips	ELISA	- No significant difference in periodontal parameters was observed between the control and study group. - No significant difference was found between groups concerning the GCF biomarkers. - The odds ratio for developing PAD appeared to increase in the presence of periodontitis.
Ertugrul *et al.* (2017) [112]	I: CP + Atherosclerosis II: CP	CCL-28 and ADM	Paper strips	ELISA	- Reduction of antimicrobial peptides in GCF as a result of the treatment could have a positive effect on reducing local inflammation. Reduced local inflammation could positively affect the prognosis of atherosclerosis.
Hayashi *et al.* (2015) [106]	CAD	lactoferrin and α1-antitrypsin	Brush (Perio-catcher)	A diagnostic kit for periodontal disease	- There was not any significant correlation between measured biomarkers and the severity of atherosclerosis. However, the GCF levels of lactoferrin and α1-antitrypsin were significantly correlated ($p<$ 0.001).
Shah *et al.* (2014) [105]	I: CP II: Healthy individuals	oxLDL and LDL	Paper points and Paper strips	ELISA	The enhanced levels of oxLDL in patients with CP can increase the risk of developing atherosclerosis.
Ehlers *et al.* (2011) [122]	I: AMI + CP II: Healthy individuals	MMP-8	Standardized MMP-8 collection strips	Dento Analyzer	Higher MMP-8 rates could be related to periodontal inflammation ($p=$ 0.001). Measuring GCF levels of MMP-8 could shed light on the severity of the AMI.
Haba *et al.* (2011) [120]	I: CP II: CP + TIA III: Healthy individuals	CRP and IL-6	Paper points	ELISA	A significant increase in GCF levels of CRP only was observed in the group of CP + TIA. - IL-6 serum level is considered as an acceptable predictor of TIA development in patients with periodontitis. - Periodontal disease significantly affects developing TIA ($p<$ 0.001).
Chen *et al.* (2010) [116]	I: CP II: CAD III: CP + CAD IV: Healthy individuals	PAF	Filter paper strips	ELISA	- The GCF levels of PAF in groups I and III were significantly higher than group II and the control group with no significant difference between groups I and III. Group II also showed a significant difference compared to the control group.

Abbreviations: AMI: Acute myocardial infarction, CAD: coronary artery disease, CP: Chronic periodontitis, ELISA: Enzyme-linked immunoassay, (ox)LDL: (oxidized) Low-density lipoprotein, PAD: Peripheral arterial disease, PAF: Platelet-activating factor, RT-PCR: Reverse transcription polymerase chain reaction, TIA: Transient ischemic heart attack

aforementioned biomarkers can be classified as potential candidates for future investigations concerning their fluctuations in GCF.

Association between coronary artery disease (CAD) and GCF components

A potential biomarker associated with the impact of CAD on GCF is platelet-activating factor (PAF) [113-115], a pro-inflammatory mediator that stimulates smooth muscle contraction and enhances endothelial cell monolayer permeability [116-118]. Zheng *et al.* [119] emphasized PAF's crucial role in periodontitis pathogenesis. Chen *et al.* [116] investigated local and systemic PAF levels in patients with periodontitis, with/without CAD, and healthy individuals to evaluate whether PAF levels in GCF might be elevated due to periodontitis or CAD. The study included 25 patients

with periodontitis (group 1), 19 with periodontitis+ CAD (group 2), 19 with CAD (group 3), and 24 healthy individuals (group 4) [116]. GCF PAF levels were significantly ($p< 0.001$) higher in the first three groups compared to the control group. PAF levels in patients with periodontitis and patients with periodontitis + CAD were significantly higher than in patients with CAD ($p< 0.05$ and < 0.001, respectively). However, no significant difference was observed between patients with periodontitis and those with periodontitis + CAD. In conclusion, PAF is released into the serum of patients with periodontitis to an extent comparable to patients with CAD, but no additive effects were observed when both diseases were present [116].

Considering the changes in inflammatory biomarkers of GCF in patients with periodontitis and CAD [106], Hayashi *et al.* [106] measured lactoferrin (Lf) and α1-antitrypsin (AT) levels in GCF of patients with CAD. GCF-Lf and GCF-AT concentrations were 0.29±0.36 µg/mL and 0.31±0.66µg/mL, respectively. A significant correlation was found between these two parameters ($r= 0.701$, $p< 0.001$). However, no significant correlation was observed between these parameters and atherosclerosis indicators, including carotid artery plaque score, maximum intima-media thickness, flow-mediated dilation of the brachial artery, and brachial-ankle pulse wave velocity ($p> 0.05$). Additionally, in patients with single, double, or triple-vessel CAD, the difference in GCF parameter levels was not significant ($p> 0.05$).

Association between transient ischemic heart attacks and GCF components

In a study by Haba *et al.* [120], pro-inflammatory biomarkers, including CRP and IL-6, were examined in both GCF and serum to investigate periodontal damage in patients with periodontitis and transitory ischemic attacks (TIA). A noteworthy finding was a significant ($p< 0.05$) increase in GCF levels of CRP observed exclusively in patients with both TIA and periodontal disease [120]. In an effort to identify subjects with periodontal disease at high risk for TIA development, a score function based on various parameters was evaluated. The results indicated that the score function concerning GCF levels of CRP and IL-6 was not statistically significant [120]. However, the IL-6 serum level emerged as a credible predictor for TIA development in patients with periodontitis. Additionally, periodontal disease demonstrated a statistically significant function score, underscoring the role of periodontitis in the development of TIA ($p< 0.001$) [120].

Association between peripheral arterial disease and GCF components

In a study investigating the impact of peripheral arterial diseases (PAD) on GCF biomarkers, Çalapkorur *et al.* [121] examined 40 patients with PAD and 20 healthy individuals. GCF samples, collected using filter paper, were assessed for interleukin-1 β, pentraxin 3 (an inflammatory cytokine), and C-reactive protein. Despite analyzing periodontal clinical parameters (gingival index, plaque index, bleeding point index, clinical attachment level, and pocket depth), no significant differences were observed between the groups. Moreover, there was no notable distinction among the three clinical parameters of GCF. Logistic regression analysis indicated that the odds ratio for developing PAD appeared to increase with a rate of 5.842 in the periodontitis group [121]. It was suggested that periodontitis could increase the odds ratio for developing PAD.

Association between acute myocardial infarction and GCF components

In a cohort observational study by Ehlers *et al.* [122], 20 patients with acute myocardial infarction (AMI) were compared to 20 healthy individuals with similar periodontal conditions, focusing on measuring Matrix Metalloproteinase-8 (MMP-8) levels in the GCF. Active MMP-8 (aMMP-8) concentrations were found to be significantly higher in the AMI group ($p= 0.001$) [122]. These results suggested a potential link between elevated MMP-8 in the study group and chronic periodontal inflammation [122]. Consequently, AMI appears to influence the severity of periodontal inflammation through inflammation-related biomarkers, particularly MMP-8. Therefore, assessing GCF levels of MMP-8 could serve as a valuable analysis to gain insights into the severity of the disease. Examining biomarker concentrations in GCF of patients with cardiovascular diseases reveals a predominant association with inflammatory processes, encompassing chemokines [112], interleukins [121], adipokines [112], pentraxin [121], CRP [121], and MMP-8 [122].

It is noteworthy that evaluating specific biomarkers related to a disease contributes to understanding systemic conditions and complications resulting from perio-

dontal disease. In this context, given the pivotal role of LDL and oxLDL in the pathophysiology of atherosclerosis, Schenkein *et al.* [110] have highlighted the enhancement of GCF-related levels of LDL and oxLDL in patients diagnosed with cardiovascular diseases.

AIDS

AIDS, a retroviral disease renowned for its immunosuppressive nature, is associated with secondary neoplasms, neurological manifestations, and opportunistic infections [123]. The human immunodeficiency virus (HIV), the primary cause of AIDS, is implicated as a risk factor for various oral diseases [124]. The oral cavity is considered a potential reservoir for this virus [127-130], a notion supported by recent findings revealing the detection of HIV DNA and RNA sequences in GCF and saliva of infected patients [124-127]. Therefore, evaluating the content of GCF holds promise for clinicians in the diagnosis and management of AIDS (Table 8).

Associations between AIDS and GCF components

In a study conducted by Atram *et al.* [123], the diagnostic potential of GCF as a marker for AIDS was investigated. The study involved 37 HIV-positive and 37 HIV-negative individuals, with GCF and saliva samples collected. Remarkably, HIV antibodies were detected in GCF with positive/negative predictive values, specificity, and sensitivity of 100% [123]. This suggests that GCF could be a more favorable sampling source compared to other samples like serum and saliva, as saliva contains 800-1000 times less antibody content than serum [123]. When considering the differences in sample preparation between GCF and serum, the advantages of GCF include lower cost, smaller sample requirements, reduced discomfort, non-invasiveness, safer disposal, easier collection, and improved patient compliance. GCF, as a diagnostic medium, offers essential insights into disease stage and treatment type [124].

Elizondo *et al.* [124] collected GCF samples from 78 HIV-positive and 39 HIV-negative individuals, stratified based on age-specific CD4+ T-lymphocyte count (<1 yr, 1–5 yrs, and >5 yrs) and the type of antiretroviral therapy (ART), which included non-ART (n = 6), Naive ART (n = 8), short-term ART (n= 41), and long-term ART (n= 23). GCF levels of IL-6, IL-7, IL-10, IL-12, monocyte chemotactic protein-1 (MCP-1), and granulocyte colony-stimulating factor (G-CSF) were significantly different between subgroups ($p< 0.05$). Furthermore, in the non-ART group, the GCF level of IL-8 was identified as a significant predictor of patient status ($p< 0.05$). Notably, GCF levels of G-CSF yielded similar results among short-term ART patients. These findings underscore the accuracy of certain biomarkers in determining the HIV patient's status and the type of treatment administered [124].

Another common issue among HIV-positive individuals is the loss of periodontal support, leading to tooth loss. In a study by Baqui *et al.* [128], IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) levels in GCF were assessed in 39 HIV-positive patients with varying probing depths. Notably, HIV-positive patients exhibited a statistically significant increase in all three pro-inflammatory cytokines in both shallow and deep periodontal pockets compared to systemically healthy indivi-

Table 8: The summary of selected studies on the GCF biomarkers in patients with acquired immunodeficiency syndrome

Author (Year)	Study group(s)	Measured biomarkers	GCF sampling method	Method of evaluation	Outcomes
Atram *et al.* (2015) [123]	I: HIV-positive patients II: Healthy individuals	HIV antibodies	Kimble disposable 5µl microcapillaries	ELISA	- GCF can be used as a favorable sampling source compared to serum and saliva for the diagnosis of AIDS.
Elizondo *et al.* (2017) [124]	I: HIV-positive II: Healthy individuals	IL-6, IL-7, IL-8, IL-10, IL-12, MCP-1, and G-CSF	Paper strips	Bioplex ProTM Human Cytokine 17-plex Assay	- The GCF levels of IL-8 can predict the status of patients undergoing no antiretroviral therapy. Moreover, G-CSF is found to have the same role in patients undergoing short-term antiretroviral therapy.
Baqui *et al.* (2000) [128]	I: HIV-positive patients II: Healthy individuals	IL-1b, IL-6, and TNF-a	Paper strips	ELISA	- HIV-positive patients have higher levels of pro-inflammatory cytokines in their GCF, which may be associated with the development of advanced periodontal lesions.

Abbreviations: ELISA: Enzyme-linked immunoassay, HIV: Human immune deficienc

duals. These cytokine levels were notably higher in deep pockets. This suggests a connection between these cytokines and the development of advanced periodontal disease in HIV-positive individuals, highlighting the need for enhanced follow-up and clinical monitoring of their periodontal health. Drawing from the results of the reviewed studies [123-4, 128], it is clear that AIDS serves as a risk factor for the development of periodontal diseases [129-130].

To elucidate potential correlations and mechanisms, several studies have been conducted [130-132]. One such study by Alpagot *et al.* [133] explored the association between GCF levels of transforming growth factor-beta 1 (TGF-β1) and the status of periodontitis in HIV-positive patients. Their findings indicated significantly elevated GCF levels of TGF-β1 in periodontal tissues affected by advanced periodontitis compared to non-affected sites [133]. Furthermore, TGF-β1 levels correlated with viral load and CD4+ cell count during the 6-month follow-up period ($p< 0.05$) [133]. These results can be attributed to the role of TGF-β1 as a growth factor that supports fibroblastic differentiation of periodontal ligament (PDL) progenitor cells and the maintenance of periodontal structures [134]. As a result, GCF concentrations of TGF-β1 are enhanced in periodontal disease associated with AIDS.

In summary, the studies discussed above [123-124, 128, 133] demonstrate the potential for reliable assessment of antibodies against HIV using GCF samples. Additionally, fluctuations in specific inflammation-related indicators and biomarkers [123], along with growth factors associated with periodontal tissue remodeling and regeneration [133], are evident in patients with AIDS. These findings underline the significance of GCF as a valuable diagnostic resource for HIV-related oral and systemic health.

The intimate interplay of GCF and the systemic conditions

This comprehensive review provided a summary of current evidence of the relationship between GCF biomolecules and commonly investigated systemic conditions, with a particular focus on their biological and prognostic implications. It is evident that GCF, as a reservoir of diverse biomarkers, including various other biomolecules, exhibits positive correlations with systemic diseases and the state of periodontal and other oral tissues [63-34, 123, 135].

Originating from the micro-capillaries beneath the junctional epithelium of the gingival sulcus [1-2], GCF should be recognized as a liquid bio-sample for specific molecule evaluations. We conducted an analysis of how various pathologies influence the quantitative and qualitative composition of GCF. While specific GCF biomarkers for most systemic diseases have not yet been identified for diagnostic purposes, it is evident that the commonality among these pathologies lies in their association with hyperinflammatory states, which manifests as fluctuations in biomarker levels within GCF.

It is hypothesized that these variations may be disease-specific, as illustrated in Figure 2, implying the potential for a set of biomarkers to serve as predictive models for medical conditions (Figure 2). Profiling multiple GCF biomarkers may enable the prediction of the progression or development of specific systemic diseases, though this hypothesis requires further investigation.

Building on the evidence-based theory of the bidirectional relationship between periodontitis and general health, where periodontal tissue inflammation can trigger systemic inflammatory responses in distant tissues and organs [136-137], this review underscores the importance of studying the release of inflammatory and immunologic biomarkers as a bridge connecting oral and systemic health.

Interestingly, on the other hand, the alterations impacting the inflammatory and immunologic biomarkers in GCF are not deemed to be a highly specific indicator of the development of systemic disease [138]. These biomarkers can also be released by the periodontal tissue by activation of localized inflammatory processes due to advanced periodontal disease [139-140]. This could trigger a subsequent cascade of pathological mechanisms within periodontal tissues, including periodontal attachment loss, bone resorption, and so on [139]. It has to be noted that two methods of evaluating biomarkers in GCF exist, including the quantitative assessment of the biomarker's concentration and the total amounts of the collected biomolecules [62]. These methods can deliver different diagnostic outcomes as seen in the study by Biyikog˘lu *et al.* [62] who observed that the concentration of TIMP-1 was not significant among the groups; though the total amount of collected TIMP-1 was elevated in patients with RA-CP, CP, or gingivitis compared to healthy individuals. Such differe-

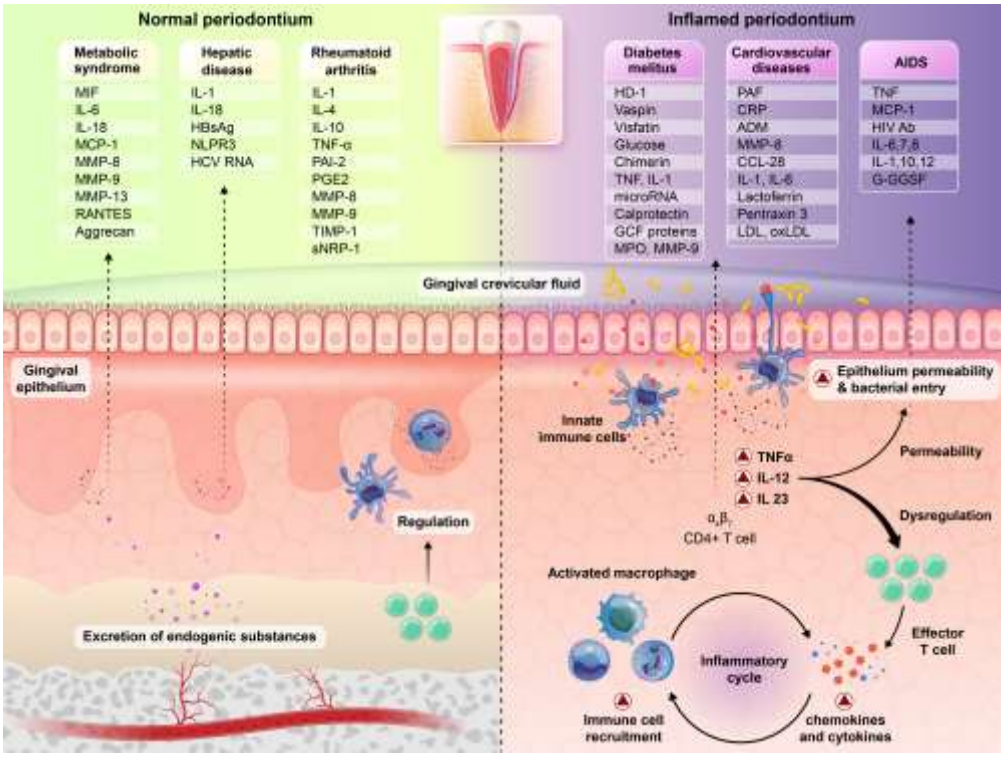

Figure 2: The impact of systemic diseases on GCF molecular biomarkers. Schematic infographic illustrating molecular mechanisms and cellular pathways within healthy and inflamed periodontium. A wide range of endogenic biomolecules associated with particular medical conditions (**Abbreviations:** α4β7: Integrin alpha-4 beta-7, ADM: Adrenomedullin, Aggrecan: Cartilage-specific proteoglycan, CCL-28: Chemokine (C-C motif) ligand 28, Chimerin: Family of GTPase-activating proteins, CRP: C-reactive protein, GCF: Gingival crevicular fluid, G-GCSF: Granulocyte colony-stimulating factor, HBsAg: Hepatitis B surface antigen, HD-1: Human defensin-1, HCV RNA: Hepatitis C virus ribonucleic acid, HIV Ab: Human immunodeficiency virus antibody, IL-1: Interleukin-1, IL-4: Interleukin-4, IL-6: Interleukin-6, IL-6,7,8: Interleukins 6, 7, and 8, IL-7: Interleukin-7, IL-8: Interleukin-8, IL-9: Interleukin-9, IL-10: Interleukin-10, IL-10,12: Interleukins 10 and 12, IL-12: Interleukin-12, IL-18: Interleukin-18, IL-23: Interleukin-23, LDL: Low-density lipoprotein, MCP-1: Monocyte chemoattractant protein-1, MIF: Macrophage migration inhibitory factor, MMP: Matrix metalloproteinase, MMP-8: Matrix metalloproteinase-8, MMP-9: Matrix metalloproteinase-9, MMP-13: Matrix metalloproteinase-13, MPO: Myeloperoxidase, NLRP3: NOD-, LRR- and pyrin domain-containing protein 3, oxLDL: Oxidized low-density lipoprotein, PAF: Platelet-activating factor, PAI-2: Plasminogen activator inhibitor-2, PGE2: Prostaglandin E2, RANTES: Regulated upon activation, normal T cell expressed and secreted (CCL5), sNRP-1: Soluble neuropilin-1, TIMP-1: Tissue inhibitor of metalloproteinases-1, TNF: Tumor necrosis factor, TNF-α: Tumor necrosis factor-alpha, Vaspin: Visceral adipose tissue-derived serine protease inhibitor, Visfatin: Visceral fat-derived adipokine).

nce in the biomarkers' level was merely dependent upon the methodological variations, which should be carefully taken into consideration when designing the study protocol. The essential steps required to validate specific GCF molecules as biomarkers of systemic diseases are presented on Figure 3.

Limitations and future prospects

All the aforementioned studies highlight GCF as a pivo-

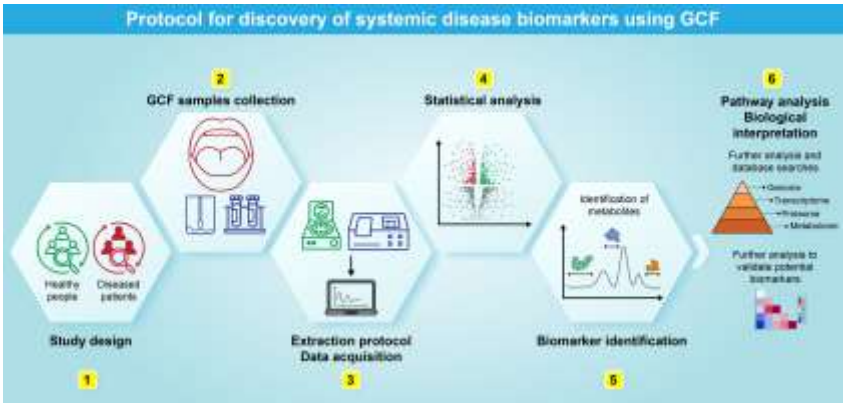

Figure 3: Schematic representation illustrating the research protocol to detect and validate specific GCF biomarkers for diagnosis and monitoring of systemic diseases, including multi-target molecular and genetic analysis

tal resource for supporting clinicians and researchers in investigating the intricate relationship between systemic diseases and periodontal structures, whether in a unidirectional or bidirectional manner. Given the accessibility and biomarker-rich nature of GCF, it can serve as a valuable tool for detecting systemic imbalances and oral tissue pathologies. Despite the siginificant outcomes in biomarkers levels, study desrepancies should also be noted [65, 141]. Therefore, conducting standard protocols for assessment and verification of biomarkers is recommended to standardize the extraction and detection procedures.

We recommend further multicenter clinical observational studies and *in vivo* investigations to assess the sensitivity and specificity of GCF in diagnosing and prognosticating systemic diseases.

To further apply GCF biomarkers for economical and effective screening, diagnosis, and monitoring of the systemic diseases, accurate implementation of omics analysis and AI-driven approaches can serve as a frontier for the future investigations. Including the current data into large-scale datasets will enable the machine and deep learning methods to provide valid and reliable models in this regard. For instance, generative adversarial networks (GANs) show promise in developing multi-omics interactions and improve phenotype prediction accuracy for various systemic diseases [138]. Application of different AI systems in both classification and generating possible disease-like phenotypes will enhance the applicability of GCF biomarkers for accurate diagnosis and monitoring. Implementing these approaches will provide the opportunities for the future role of GCF in personalized medicine.

Conclusion

Within the constraints of this comprehensive review article, we have established that various systemic diseases, including metabolic syndrome, liver diseases, cardiovascular diseases, infections, and autoimmune diseases, can induce significant qualitative and quantitative alterations in the GCF compartment. Most of the biomarkers within the GCF-systemic health axis are of inflammatory and immunologic origin. The potential role of GCF in the clinical diagnostic process of systemic diseases is of paramount importance, considering its underexplored potential in personalized point-of-care diagnostics. Further, in-depth data derived from longitudinal studies, standardization protocols, or multicenter trials can significantly enhance our understanding of the potential of GCF in screening, diagnosis, and longitudinal monitoring of systemic diseases.

Funding

This study did not receive any specific funding.

Data Availability Statement

No underlying data was collected or produced in this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Bibi T, Khurshid Z, Rehman A, Imran E, Srivastava KC, Shrivastava D. Gingival crevicular fluid (GCF): a diagnostic tool for the detection of periodontal health and diseases. Molecules. 2021; 26: 1208.

[2] Brill N, Krasse BO. The passage of tissue fluid into the clinically healthy gingival pocket. Acta Odontol Scand. 1958; 16: 233–245.

[3] Khurshid Z, Mali M, Naseem M, Najeeb S, Zafar MS. Human gingival crevicular fluids (GCF) proteomics: an overview. Dent J (Basel). 2017; 5:12.

[4] Subbarao KC, Nattuthurai GS, Sundararajan SK, Sujith I, Joseph I, Syedshah YP. Gingival crevicular fluid: an overview. J Pharm Bioallied Sci. 2019; 11: S135.

[5] Cakmak O, Tasdemir Z, Aral CA, Dundar S, Koca HB. Gingival crevicular fluid and saliva stress hormone levels in patients with chronic and aggressive periodontitis, J Clin Periodontol. 2016; 43: 1024–1031.

[6] McCulloch CAG. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol. 1994; 21: 497–506.

[7] Tymkiw KD, Thunell DH, Johnson GK, Joly S, Burnell KK, Cavanaugh JE, et al. Influence of smoking on gingival crevicular fluid cytokines in severe chronic periodontitis. J Clin Periodontol. 2011; 38: 219–228.

[8] Challacombe SJ, Russell MW, Hawkes J. Passage of intact IgG from plasma to the oral cavity via crevicular

fluid. Clin Exp Immunol. 1978; 34: 417.

[9] Cimasoni G. Crevicular fluid updated. Monogr Oral Sci. 1983; 12:

[10] Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000. 2016; 70: 53–64.

[11] Curtis MA, Gillett IR, Griffiths GS, Maiden MFJ, Sterne JAC, Wilson DT, et al. Detection of high-risk groups and individuals for periodontal diseases: laboratory markers from analysis of gingival crevicular fluid. J Clin Periodontol. 1989; 16: 1–11.

[12] de Oliveira RR, Schwartz-Filho HO, Novaes Jr AB, Garlet GP, Freitas de Souza R, Taba Jr M, et al. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: Cytokine profile in gingival crevicular fluid, preliminary results. J Periodontol. 2009; 80: 98–105.

[13] Nazar Majeed Z, Philip K, Alabsi AM, Pushparajan S, Swaminathan D. Identification of Gingival Crevicular Fluid Sampling, Analytical Methods, and Oral Biomarkers for the Diagnosis and Monitoring of Periodontal Diseases: A Systematic Review. Dis Markers. 2016; 2016: 1804727.

[14] Manoil D, Bostanci N, Mumcu G, Inanc N, Can M, Direskeneli H, et al. Novel and known periodontal pathogens residing in gingival crevicular fluid are associated with rheumatoid arthritis. J Periodontol. 2021; 92: 359-370.

[15] Asikainen S, Doğan B, Turgut Z, Paster BJ, Bodur A, Oscarsson J. Specified species in gingival crevicular fluid predict bacterial diversity. PLoS One. 2010; 5: e13589.

[16] Grbic JT, Singer RE, Jans HH, Celenti RS, Lamster IB. Immunoglobulin isotypes in gingival crevicular fluid: possible protective role of IgA. J Periodontol. 1995; 66: 55–61.

[17] Andrianus EP, Masulili SLC, Lessang R, Tadjoedin FM, Hutomo DI, Wulandari P. Immunoglobulin G Levels in the Gingival Crevicular Fluid of Menopausal Patients with Periodontitis. Pesqui Bras Odontopediatria Clin Integr. 2021; 1-6:

[18] Huerto CG, Toro JJ, LaSalma G. Levels of IgM, IgG and IgA antibodies in gingival crevicular fluid of subjects with severe chronic periodontitis. Afr J Infect Dis Res. 2016; 3: 84–89.

[19] Padisar P, Hashemi R, Naseh M, Nikfarjam BA, Mohammadi M. Assessment of tumor necrosis factor alpha (TNFα) and interleukin 6 level in gingival crevicular fluid during orthodontic tooth movement: a randomized split-mouth clinical trial. Electron Physician. 2018; 10: 7146–7154.

[20] Silosi I, Cojocaru M, Foia L, Boldeanu MV, Petrescu F, Surlin P, et al. Significance of circulating and crevicular matrix metalloproteinase-9 in rheumatoid arthritis-chronic periodontitis association. J Immunol Res. 2015; 2015: 218060.

[21] Javed F, Ahmed HB, Mikami T, Almas K, Romanos GE, Al-Hezaimi K. Cytokine profile in the gingival crevicular fluid of rheumatoid arthritis patients with chronic periodontitis. J Investig Clin Dent. 2014; 5: 1–8.

[22] Gürkan A, Eren G, Çetinkalp Ş, Akçay YD, Emingil G, Atilla G. Monocyte chemotactic protein-1, RANTES and macrophage migration inhibitory factor levels in gingival crevicular fluid of metabolic syndrome patients with gingivitis. Arch Oral Biol. 2016; 69: 82–88.

[23] Pradeep AR, Raghavendra NM, Prasad MVR, Kathariya R, Patel SP, Sharma A. Gingival crevicular fluid and serum visfatin concentration: their relationship in periodontal health and disease. J Periodontol. 2011; 82: 1314-1319.

[24] Nibali L, Stephen A, Hagi-Pavli E, Allaker R, Di Pino A, Terranova V, et al. Analysis of gingival crevicular fluid biomarkers in patients with metabolic syndrome. J Dent. 2022; 118: 104065.

[25] Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. Eisenberg, The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol. 2010; 56: 1113–1132.

[26] D'Aiuto F, Sabbah W, Netuveli G, Donos N, Hingorani AD, Deanfield J, et al. Association of the metabolic syndrome with severe periodontitis in a large U.S. population-based survey. J Clin Endocrinol Metab. 2008; 93: 3989–3994.

[27] Nibali L, Tatarakis N, Needleman I, Tu YK, D'Aiuto F, Rizzo M, et al. Clinical review: Association between metabolic syndrome and periodontitis: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;

98: 913–920.

[28] Roughley PJ, Mort JS. The role of aggrecan in normal and osteoarthritic cartilage. J Exp Orthop. 2014; 1: 8.

[29] Murphy G, Nagase H. Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat Clin Pract Rheumatol. 2008; 4: 128–135.

[30] Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. Nat Rev Rheumatol. 2017; 13: 606–620.

[31] Ruscitti P, Cipriani P, Liakouli V, Iacono D, Pantano I, Margiotta DPE, et al. Occurrence and predictive factors of high blood pressure, type 2 diabetes, and metabolic syndrome in rheumatoid arthritis: findings from a 3-year, multicentre, prospective, observational study. Clin Exp Rheumatol. 2020; 39: 995–1002.

[32] Senkal R, Yemenoglu H, Kose O, Karakas SM, Yilmaz A, Akyildiz K, et al. The role of interleukin-20 on inflammatory stress and periodontal tissue destruction in patients with metabolic syndrome and periodontitis. BMC Oral Health. 2024; 24: 1423.

[33] Han DH, Shin HS, Paek D, Kim HD. Gingival crevicular fluid levels of matrix metalloproteinases cross-sectionally related to periodontitis and metabolic syndrome in community Koreans. J Clin Periodontol. 2012; 39: 1125-1131.

[34] Feldman M, Friedman LS. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 10th ed. Elsevier Saunders: Philadelphia; 2015.

[35] Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005; 115: 209–218.

[36] Surlin P, Gheorghe DN, Popescu DM, Martu AM, Solomon S, Roman A, et al. Interleukin-1α and-1β assessment in the gingival crevicular fluid of periodontal patients with chronic hepatitis C. Exp Ther Med. 2020; 20: 2381-2386.

[37] Coates EA, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, et al. Hepatitis C infection and associated oral health problems. Aust Dent J. 2000; 45: 108–114.

[38] Surlin P, Lazar L, Sincar C, Gheorghe DN, Popescu DM, Boldeanu VM, et al. NLRP3 Inflammasome Expression in Gingival Crevicular Fluid of Patients with Periodontitis and Chronic Hepatitis C. Mediators Inflamm. 2021; 2021: 6917919.

[39] Gheorghe DN, Foia L, Toma V, Surdu A, Herascu E, Popescu DM, et al. Hepatitis C Infection and Periodontal Disease: Is there a Common Immunological Link?, J Immunol Res 2018. 2018; 8720101.

[40] Surlin P, Gheorghe DN, Foia L, Surdu A, Toma V, Solomon SM. Periodontal Implications of Hepatitis C Infection. Available at: https://www.intechopen.com/chapters/60952

[41] Isaza-Guzmán DM, Medina-Piedrahíta VM, Gutiérrez-Henao C, Tobón-Arroyave SI. Salivary Levels of NLRP3 Inflammasome-Related Proteins as Potential Biomarkers of Periodontal Clinical Status. J Periodontol. 2017; 88: 1329–1338.

[42] Marchesan JT, Girnary MS, Moss K, Monaghan ET, Egnatz GJ, Jiao Y, et al. Role of inflammasomes in the pathogenesis of periodontal disease and therapeutics. Periodontol 2000. 2020; 82: 93–114.

[43] Wang L, Hauenstein AV. The NLRP3 inflammasome: Mechanism of action, role in disease and therapies. Mol Aspects Med. 2020; 76: 2020; 100889.

[44] Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. Int J Mol Sci. 2019; 20: 3328.

[45] Negash AA, Ramos HJ, Crochet N, Lau DTY, Doehle B, Papic N, et al. Hagedorn, IL-1β production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathog. 2013; 9: e1003330.

[46] Matičičr M, Poljak M, Kramar B, Seme K, Brinovec V, Meglič-Volkar J, et al. Detection of Hepatitis C Virus RNA From Gingival Crevicular Fluid and Its Relation to Virus Presence in Saliva. J Periodontol. 2001; 72: 11–16.

[47] Suzuki T, Omata K, Satoh T, Miyasaka T, Arai C, Maeda M, et al. Quantitative detection of hepatitis C virus (HCV) RNA in saliva and gingival crevicular fluid of HCV-infected patients. J Clin Microbiol. 2005; 43: 4413–4417.

[48] Pollock JJ, Andors L, Gulumoglu A, Ells PF. Direct measurement of hepatitis B viral antibody and antigen markers in gingival crevicular fluid. Oral Surg Oral Med Oral Patho. 1984; 57: 499–503.

[49] Gammie A, Morris R, Wyn-Jones AP. Antibodies in crevicular fluid: An epidemiological tool for investigation

of waterborne disease. Epidemiol Infect. 2002; 128: 245–249.

[50] Shi G, Liao X, Lin Z, Liu W, Luo X, Zhan H, et al. Estimation of the global prevalence, incidence, years lived with disability of rheumatoid arthritis in 2019 and forecasted incidence in 2040: results from the Global Burden of Disease Study 2019. Clin Rheumatol. 2023; 42: 2297–2309.

[51] Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, et al. Association between periodontal pathogens and systemic disease. Biomed J. 2019; 42: 27-35.

[52] Xiao F, Li C, Lin Y, Peng Z, Xu X, Wen Y, et al. Increased risk of periodontitis occurrence in patients with rheumatoid arthritis and its association with the levels of il-1β and tnf-α in gingival crevicular fluid, Ann Palliat Med. 2021; 10: 9078–9087.

[53] Smolik I, Robinson D, El-Gabalawy HS. Periodontitis and rheumatoid arthritis: epidemiologic, clinical, and immunologic associations. Compend Contin Educ Dent. 2009; 30: 188–190, 192, 194 passim; quiz 198, 210.

[54] Saini R, Marawar PP, Shete S, Saini S. Periodontitis, a true infection. J Glob Infect Dis. 2009; 1: 149–150.

[55] Söderlin MK, Persson GR, Renvert S, Sanmartin Berglund J. Cytokines in gingival crevicular fluid in elderly rheumatoid arthritis patients in a population-based cross-sectional study: RANTES was associated with periodontitis. J Periodontal Res. 2021; 56: 907–916.

[56] Prieto D, González C, Weber L, Realini O, Pino-Lagos K, Bendek MJ, et al. Soluble neuropilin-1 in gingival crevicular fluid is associated with rheumatoid arthritis: An exploratory case-control study. J Oral Biol Craniofac Res. 2021; 11: 303–307.

[57] Cetinkaya B, Guzeldemir E, Ogus E, Bulut S. Proinflammatory and Anti-Inflammatory Cytokines in Gingival Crevicular Fluid and Serum of Patients With Rheumatoid Arthritis and Patients With Chronic Periodontitis. J Periodontol. 2013; 84: 84–93.

[58] Esen Ç, Alkan BA, Kırnap M, Akgül Ö, Işıkoğlu S, Erel Ö. The Effects of Chronic Periodontitis and Rheumatoid Arthritis on Serum and Gingival Crevicular Fluid Total Antioxidant/Oxidant Status and Oxidative Stress Index. J Periodontol. 2012; 83: 773–779.

[59] El-Wakeel NM, Shalaby ZF, Abdulmaguid RF, Elhamed SSa, Shaker O. Local gingival crevicular fluid, synovial fluid, and circulating levels of prolactin hormone in patients with moderately active rheumatoid arthritis and stage III and IV periodontitis before and after non-surgical periodontal treatment: a controlled trial. Clin Oral Investig. 2023; 27: 2813–2821.

[60] Salvi GE, Carollo-Bittel B, Lang NP. Effects of diabetes mellitus on periodontal and peri-implant conditions: update on associations and risks. J Clin Periodontol. 2008; 35: 398–409.

[61] Lakschevitz F, Aboodi G, Tenenbaum H, Glogauer M. Diabetes and Periodontal Diseases: Interplay and Links. Curr Diabetes Rev. 2011; 7: 433–439.

[62] Bıyıkoğlu B, Buduneli N, Kardeşler L, Aksu K, Pitkala M, Sorsa T. Gingival Crevicular Fluid MMP-8 and -13 and TIMP-1 Levels in Patients With Rheumatoid Arthritis and Inflammatory Periodontal Disease. J Periodontol. 2009; 80: 1307–1314.

[63] Biyikoğlu B, Buduneli N, Kardeşler L, Aksu K, Öder G, Kütükçüler N. Evaluation of t-PA, PAI-2, IL-1β and PGE2 in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. J Clin Periodontol. 2006; 33: 605–611.

[64] Bıyıkoğlu B, Buduneli N, Aksu K, Nalbantsoy A, Lappin DF, Evrenosoğlu E, et al. Periodontal therapy in chronic periodontitis lowers gingival crevicular fluid interleukin-1beta and DAS28 in rheumatoid arthritis patients. Rheumatol Int. 2013; 33: 2607–2616.

[65] Söderlin MK, Persson GR, Renvert S, Sanmartin Berglund J. Cytokines in gingival crevicular fluid in elderly rheumatoid arthritis patients in a population-based cross-sectional study: RANTES was associated with periodontitis. J Periodontal Res. 2021; 56: 907–916.

[66] Salvi GE, Carollo-Bittel B, Lang NP. Effects of diabetes mellitus on periodontal and peri-implant conditions: update on associations and risks. J Clin Periodontol. 2008; 35: 398–409.

[67] Abreu IS, Euzebio Alves VT, Benedete APS, Bueno da Silva HA, França BN, Saraiva L, et al. Gingival crevicular fluid levels of protease-activated receptors type 1 and type 2 in diabetic patients with periodontitis, J Periodontal Res. 2016; 51: 577–585.

[68] Tsai C, Hayes C, Taylor GW. Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. Community Dent Oral Epidemiol. 2002; 30: 182–192.

[69] Lakschevitz F, Aboodi G, Tenenbaum H, Glogauer M. Diabetes and periodontal diseases: interplay and links. Curr Diabetes Rev. 2011; 7: 433–439.

[70] Mealey BL, Oates TW. Diabetes mellitus and periodontal diseases. J Periodontol. 2006; 77: 1289-1303.

[71] Elazazy O, Amr K, Abd El Fattah A, Abouzaid M. Evaluation of serum and gingival crevicular fluid microRNA-223, microRNA-203 and microRNA-200b expression in chronic periodontitis patients with and without diabetes type 2. Arch Oral Biol. 2021; 121: 104949.

[72] Santamaria-Jr M, Bagne L, Zaniboni E, Santamaria MP, Jardini MAN, Felonato M, et al. Diabetes mellitus and periodontitis: Inflammatory response in orthodontic tooth movement. Orthod Craniofac Res. 2020; 23: 27-34.

[73] Nascimento GG, Leite FRM, Vestergaard P, Scheutz F, López R. Does diabetes increase the risk of periodontitis? A systematic review and meta-regression analysis of longitudinal prospective studies. Acta Diabetol. 2018; 55: 653–667.

[74] Gao H, Xu J, He L, Meng H, Hou J. Calprotectin levels in gingival crevicular fluid and serum of patients with chronic periodontitis and type 2 diabetes mellitus before and after initial periodontal therapy. J Periodontal Res. 2021; 56: 121–130.

[75] Taylor JJ, Preshaw PM, Lalla E. A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J Periodontol. 2013; 84: S113-S134.

[76] Parihar S, Tripathi R, Parihar AV, Samadi FM, Chandra A, Bhavsar N. Estimation of gingival crevicular blood glucose level for the screening of diabetes mellitus: A simple yet reliable method. J Oral Biol Craniofac Res. 2016; 6: 198–203.

[77] Llambés F, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. World J Diabetes. 2015; 6: 927–935.

[78] Sande AR, Guru S, Guru R, Gaduputi S, Thati DK, Siddeshappa ST. Gingival crevicular blood glucose levels. Is it a reliable tool for screening diabetes in a dental office? J Contemp Dent Prac. 2020; 21: 421–425.

[79] Bhavsar M, Brahmbhatt N, Sahayata V, Bhavsar N. Gingival crevicular blood for screening of blood glucose level in patients with & without diabetes: A chair-side test. Int J Dent Hyg. 2016; 14: 92–97.

[80] Yamaguchi M, Kawabata Y, Kambe S, Wårdell K, Nystrom FH, Naitoh K, et al. Non-invasive monitoring of gingival crevicular fluid for estimation of blood glucose level. Med Biol Eng Comput. 2004; 42: 322–327.

[81] Gupta A, Gupta N, Garg R, Jain N, Atreja G, Walia SS. Developing a chair side, safe and non-invasive procedure for assessment of blood glucose level using gingival crevicular bleeding in dental clinics. J Nat Sci Biol Med. 2014; 5: 329–332.

[82] Strauss SM, Wheeler AJ, Russell SL, Brodsky A, Davidson RM, Gluzman R, et al. The Potential Use of Gingival Crevicular Blood for Measuring Glucose to Screen for Diabetes: An Examination Based on Characteristics of the Blood Collection Site. J Periodontol. 2009; 80 907–914.

[83] Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 Diabetes and its Impact on the Immune System. Curr Diabetes Rev. 2020; 16: 442–449.

[84] Lee MS. Role of innate immunity in diabetes and metabolism: recent progress in the study of inflammasomes. Immune Netw. 2011; 11: 95–99.

[85] Nilsson J, Bengtsson E, Fredrikson GN, Björkbacka H. Inflammation and immunity in diabetic vascular complications. Curr Opin Lipidol. 2008; 19: 519–524.

[86] Peniche-Palma DC, Carrillo-Avila BA, Sauri-Esquivel EA, Acosta-Viana K, Esparza-Villalpando V, Pozos-Guillen A, et al. Levels of Myeloperoxidase and Metalloproteinase-9 in Gingival Crevicular Fluid from Diabetic Subjects with and without Stage 2, Grade B Periodontitis. Biomed Res Int. 2019; 2019; 5613514.

[87] Marinho MC, Pacheco ABF, Costa GCV, Ortiz ND, Zajdenverg L, Sansone C. Quantitative gingival crevicular fluid proteome in type 2 diabetes mellitus and chronic periodontitis. Oral Dis. 2019; 25: 588–595.

[88] Sereti M, Roy M, Zekeridou A, Gastaldi G, Giannopoulou C. Gingival crevicular fluid biomarkers in type 1 diabetes mellitus: A case–control study, Clin Exp Dent Res. 2021; 7: 170–178.

[89] Yilmaz D, Caglayan F, Buber E, Könönen E, Aksoy Y, Gursoy UK, et al. Gingival crevicular fluid levels of human beta-defensin-1 in type 2 diabetes mellitus and periodontitis. Clin Oral Investig. 2018; 22: 2135–2140.

[90] Greer A, Zenobia C, Darveau RP. Defensins and LL-37: a review of function in the gingival epithelium. Periodontol 2000. 2013; 63: 67–79.

[91] Zhu C, Tan H, Cheng T, Shen H, Shao J, Guo Y, et al. Human β-defensin 3 inhibits antibiotic-resistant Staphylococcus biofilm formation. J Surg Res. 2013; 183: 204-213.

[92] Mishra V, Shettar L, Bajaj M, Math AS, Thakur SL. Interlinking periodontitis and type 2 diabetes mellitus by assessment of crevicular visfatin levels in health and in disease before and after initial periodontal therapy. J Clin Diagnos Res. 2016; 10: ZC67–ZC71.

[93] Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. J Clin Periodontol. 2011; 38 Suppl 1: 85-105.

[94] Bulut U, Develioglu H, Taner IL, Berker E. Interleukin-1 beta levels in gingival crevicular fluid in type 2 diabetes mellitus and adult periodontitis. J Oral Sci. 2001; 43: 171–177.

[95] Păvălan A, Boldeanu MV, Nicolae FM, Gheonea TC, Rogoveanu I, Florescu C, et al. Clinical Periodontal Evaluation and Assessment of Dipeptidyl-Peptidase-4 and Galectin-3 in Gingival Crevicular Fluid of Periodontitis Patients with Heart Failure and Diabetes. J Clin Med. 2025; 14: 3345.

[96] Kim WK, Bae KH, Lee SC, Oh KJ. The Latest Insights into Adipokines in Diabetes. J Clin Med. 2019; 8: 1874.

[97] Oh KJ, Lee DS, Kim WK, Han BS, Lee SC, Bae KH. Metabolic Adaptation in Obesity and Type II Diabetes: Myokines, Adipokines and Hepatokines. Int J Mol Sci. 2016; 18: 8.

[98] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011; 11: 85–97.

[99] Gulcelik NE, KarakayaJ, Gedik A, Usman A, Gurlek A. Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. Eur J Endocrinol. 2009; 160: 65–70.

[100] Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA. 2005; 102: 10610–10615.

[101] Doğan ŞB, Dede FÖ, Ballı U, Sertoğlu E. Levels of vaspin and omentin-1 in gingival crevicular fluid as potential markers of inflammation in patients with chronic periodontitis and type 2 diabetes mellitus, J Oral Sci. 2016; 58: 379–389.

[102] El-Mesallamy HO, El-Derany MO, Hamdy NM. Serum omentin-1 and chemerin levels are interrelated in patients with Type 2 diabetes mellitus with or without ischaemic heart disease. Diabet Med. 2011; 28: 1194-1200.

[103] Doğan ŞB, Ballı U, Dede FÖ, Sertoğlu E, Tazegül K. Chemerin as a Novel Crevicular Fluid Marker of Patients With Periodontitis and Type 2 Diabetes Mellitus. J Periodontol. 2016; 87: 923–933.

[104] Choy JC, Podor TJ, Yanagawa B, Lai JCK, Granville DJ, Walker DC, et al. The regulation and consequences of immune-mediated cell death in atheromatous diseases. Cardiovasc Toxicol. 2003; 3: 269–282.

[105] Shah R, Thomas R, Mehta DS. Oxidized-low density lipoprotein in gingival crevicular fluid of patients with chronic periodontitis: A possible link to atherogenesis. Acta Odontol Scand. 2014; 72: 154–156.

[106] Hayashi S, Yamada H, Fukui M, Ito HO, Sata M. Correlation between arteriosclerosis and periodontal condition assessed by lactoferrin and α1-antitrypsin levels in gingival crevicular fluid. Int Heart J. 2015; 56: 639–643.

[107] Seinost G, Wimmer G, Skerget M, Thaller E, Brodmann M, Gasser R, et al. Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. Am Heart J. 2005; 149: 1050–1054.

[108] Kapellas K, Jamieson LM, Do LG, Bartold PM, Wang H, Maple-Brown LJ, et al. Associations between periodontal disease and cardiovascular surrogate measures among Indigenous Australians. Int J Cardiol. 2014; 173: 190–196.

[109] Bäck M, Airila-Månsson S, Jogestrand T, Söder B, Söder PÖ. Increased leukotriene concentrations in gingival crevicular fluid from subjects with periodontal disease and atherosclerosis. Atherosclerosis. 2007; 193: 389-394.

[110] Schenkein HA, Berry CR, Burmeister JA, Brooks CN, Best AM, Tew JC. Locally produced anti-phosphorylcholine and anti-oxidized low-density lipoprotein antibodies in gingival crevicular fluid from aggressive periodontitis patients. J Periodontol. 2004; 75: 146–153.

[111] Filardo S, Di Pietro M, Schiavoni G, Minniti G, Ortolani E, Romano S, et al. Chlamydia pneumoniae clinical isolate from gingival crevicular fluid: A potential atherogenic strain. Front Cell Infect Microbiol. 2015; 5: 1–9.

[112] Ertugrul AS, Bozoglan A, Taspinar M. The effect of nonsurgical periodontal treatment on serum and gingival crevicular fluid markers in patients with atherosclerosis. Niger J Clin Pract. 2017; 20: 361–368.

[113] Ninio E. Phospholipid mediators in the vessel wall: involvement in atherosclerosis. Curr Opin Clin Nutr Metab Care. 2005; 8: 123–131.

[114] Ghesquiere SAI, Hofker MH, de Winther MPJ. The role of phospholipases in lipid modification and atherosclerosis. Cardiovasc Toxicol. 2005; 5: 161–182.

[115] Subbanagounder G, Leitinger N, Shih PT, Faull KF, Berliner JA. Evidence that phospholipid oxidation products and/or platelet-activating factor play an important role in early atherogenesis : in vitro and In vivo inhibition by WEB 2086. Circ Res. 1999; 85: 311–318.

[116] Chen H, Zheng P, Zhu H, Zhu J, Zhao L, El Mokhtari NE, et al. Platelet-activating factor levels of serum and gingival crevicular fluid in nonsmoking patients with periodontitis and/or coronary heart disease. Clin Oral Investig. 2010; 14: 629–636.

[117] Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu Rev Biochem. 2000; 69: 419–445.

[118] Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit Rev Clin Lab Sci. 2003; 40: 643–672.

[119] Zheng P, Chen H, Shi S, Jepsen S, Eberhard J. Periodontal parameters and platelet-activating factor levels in serum and gingival crevicular fluid in a Chinese population. J Clin Periodontol. 2006; 33: 797–802.

[120] Haba D, Teslaru S, Ungureanu D, Hodorog D, Alecu C, Benghiac AG, et al. Evaluation of serum and gingival crevicular fluid C-reactive protein and IL-6 levels in patients with periodontitis and transient ischemic attacks. Romanian J Morphology Embryology. 2011; 52: 1243–1247.

[121] Çalapkorur MU, Alkan BA, Tasdemir Z, Akcali Y, Saatçi E. Association of peripheral arterial disease with periodontal disease: analysis of inflammatory cytokines and an acute phase protein in gingival crevicular fluid and serum. J Periodontal Res. 2017; 52: 532–539.

[122] Ehlers V, Willershausen I, Kraft J, Münzel T, Willershausen B. Gingival crevicular fluid MMP-8-concentrations in patients after acute myocardial infarction. Head Face Med. 2011; 7: 2–7.

[123] Atram P, Gotmare S, Patil P, Rathod V, Saify F. Gingival crevicular fluid: As a diagnostic marker in HIV positive patients. J Int Soc Prev Community Dent. 2015; 5: 24.

[124] Elizondo JE, del R. Rocha-Pizaña M, Treviño AC, Violant D, Álvarez MM, Rivas-Estilla AM. Potential gingival crevicular fluid and serum biomarkers by stage of HIV infection. Cytokine. 2017; 91: 96–103.

[125] Pavan P, Pereira VT, Souza RC, Souza CO, Torres SR, Colombo APV, et al. Levels of HIV-1 in subgingival biofilm of HIV-infected patients. J Clin Periodontol. 2014; 41: 1061–1068.

[126] Huang CB, Alimova YV, Ebersole JL. HIV-1 reactivation in HIV-latently infected dendritic cells by oral microorganisms and LPS. Cell Immunol. 2011; 268: 105–111.

[127] Huang CB, Alimova YV, Strange S, Ebersole JL. Polybacterial challenge enhances HIV reactivation in latently infected macrophages and dendritic cells. Immunology. 2011; 132: 401–409.

[128] Baqui AAMA, Meiller TF, Jabra-Rizk MA, Zhang M, Kelley JI, Falkler WA. Enhanced interleukin 1β, interleukin 6 and tumor necrosis factor α in gingival crevicular fluid from periodontal pockets of patients infected with human immunodeficiency virus 1. Oral Microbiol Immunol. 2000; 15: 67–73.

[129] Salvi GE, Lawrence HP, Offenbacher S, Beck JD. Influence of risk factors on the pathogenesis of periodontitis. Periodontol 2000. 1997; 14: 173–201.

[130] Aria SM, Ferreira S, Ouza Gonçalves LS, Torres SR, Nogueira SA, Meiller TF. Lactoferrin levels in gingival crevicular fluid and saliva of HIV-infected patients with

chronic periodontitis, J Investig Clin Dent. 2015; 6: 16–24.

[131] Mellanen L, Lähdevirta J, Tervahartiala T, Meurman JH, Sorsa T. Matrix metalloproteinase-7, -8, -9, -25, and -26 and CD43, -45, and -68 cell-markers in HIV-infected patients' saliva and gingival tissue. J Oral Pathol Med. 2006; 35: 530–539.

[132] Klimiuk A, Waszkiel D, Choromańska M, Jankowska A, Zelazowska-Rutkowska B. The saliva immunology mechanisms and periodontal status in HIV infected subjects. Adv Med Sci. 2006; 51 Suppl 1: 46–48.

[133] Alpagot T, Konopka K, Bhattacharyya M, Gebremedhin S, Düzgüneş N. The Association Between Gingival Crevicular Fluid TGF-β1 Levels and Periodontal Status in HIV-1 + Patients. J Periodontol. 2008; 79: 123–130.

[134] Fujii S, Maeda H, Tomokiyo A, Monnouchi S, Hori K, Wada N, et al. Effects of TGF-β1 on the proliferation and differentiation of human periodontal ligament cells and a human periodontal ligament stem/progenitor cell line. Cell Tissue Res. 2010; 342: 233–242.

[135] Gümüş P, Buduneli E, Biyikoğlu B, Aksu K, Saraç F, Buduneli N, et al. Gingival crevicular fluid and serum levels of APRIL, BAFF and TNF-alpha in rheumatoid arthritis and osteoporosis patients with periodontal disease, Arch Oral Biol. 2013; 58: 1302–1308.

[136] Wu DT, Cho YW, Spalti MD, Bishara M, Nguyen TT. The link between periodontitis and Alzheimer's disease – emerging clinical evidence, Dentistry Review. 2023; 3: 100062.

[137] Zi MYH, Longo PL, Bueno-Silva V, Mayer MPA. Mechanisms involved in the association between periodontitis and complications in pregnancy, Front Public Health. 2015; 2: 290.

[138] Vidanagamachchi SM, Waidyarathna KMGTR. Opportunities, challenges and future perspectives of using bioinformatics and artificial intelligence techniques on tropical disease identification using omics data. Front Digit Health. 2024; 6: https://doi.org/10.3389/fdgth.2024.1471200.

[139] Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012; 55: 21–31.

[140] Ngo LH, Darby IB, Veith PD, Locke AG, Reynolds EC. Mass spectrometric analysis of gingival crevicular fluid biomarkers can predict periodontal disease progression. J Periodontal Res. 2013; 48: 331–341.

[141] Mendiratta VK, Soi S, Puri A, Sharma M, Gupta A, Radhakrishnan R, et al. Systematic review and meta analysis of substance P in gingival crevicular fluid as a diagnostic biomarker for tooth pain. Discover Applied Sciences. 2025; 7: 1191.

[142] Takamori K. The growth stimulating factors for lactobacillus appeared in tissue fluid from gingival crevice. Bulletin of Tokyo Medical and Dental University. 1963; 10: 533-541.

[143] Weinstein E, Khurana H, Mandel ID. Lactate dehydrogenase isoenzymes in gingival fluid. Arch Oral Biol. 1972; 17: 375-IN29.

[144] Xiao F, Li C, Lin Y, Peng Z, Xu X, Wen Y, et al. Increased risk of periodontitis occurrence in patients with rheumatoid arthritis and its association with the levels of IL-1β and TNF-α in gingival crevicular fluid. Ann Palliat Med. 2021; 10: 9078–9087.

[145] Cetinkaya B, Guzeldemir E, Ogus E, Bulut S. Proinflammatory and anti-inflammatory cytokines in gingival crevicular fluid and serum of patients with rheumatoid arthritis and patients with chronic periodontitis. J Periodontol. 2013; 84: 84–93.

[146] Doğan ŞB, Ballı U, Dede FÖ, Sertoğlu E, Tazegül K. Chemerin as a Novel Crevicular Fluid Marker of Patients With Periodontitis and Type 2 Diabetes Mellitus. J Periodontol. 2016; 87: 923–933.