e-ISSN: 2637-0611

PENERBIT PRESS
UNIVERSITI TEKNOLOGI MARA

Available online at
https://journal.uitm.edu.my/ojs/index.php/COS

Compendium of Oral Science 13(2) 2026, 16–30

Compendium of Oral Science

# Utilizing Gingival Crevicular Fluid (GCF) as a Diagnostic Tool for Endodontic Disease: A Narrative Review

Athirah Abdul Aziz[1], Noorharliana Mohamed Zohdi[2*], Hayati Ishak[2], Mohd Haikal Muhamad Halil[3]

[1]*Faculty of Dentistry, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, Jalan Hospital Sungai Buloh, 47000, Sungai Buloh, Selangor, Malaysia*
[2]*Centre for Comprehensive Care Studies, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, Jalan Hospital Sungai Buloh, 47000, Sungai Buloh, Selangor, Malaysia*
[3]*Dean's Office, Kulliyah of Dentistry, International Islamic University of Malaysia (IIUM Kuantan), Jalan Sultan Ahmad Shah, 25200, Kuantan, Pahang, Malaysia*

ARTICLE INFO

*Article history:*
Received 05 June 2026
Revised 14 July 2026
Accepted 12 August 2026
Online first 01 September 2026
Published 30 September 2026

*Keywords:*
gingival crevicular fluid
endodontic biomarker
pulpitis
apical periodontitis

*DOI:*
10.24191/ cos.v13i2.10596

ABSTRACT

**Introduction:** Accurate endodontic diagnosis remains challenging due to the subjective and indirect nature of current clinical tests. Gingival crevicular fluid (GCF), a non-invasive, serum-derived exudate, offers a promising alternative, providing a molecular window into pulpal and periapical health (Mona et al., 2021). **Methods:** This narrative review aims to summarise the current biomarkers found in gingival crevicular fluid (GCF) and synthesise evidence from peer-reviewed databases such as PubMed and Scopus, regarding gingival crevicular fluid (GCF) biomarkers associated with irreversible pulpitis and apical periodontitis from 2000 to 2026, excluding case reports. A total of 22 articles were included. **Results:** Recent studies have identified a diverse array of pro-inflammatory cytokines (IL-8, IL-23) and the anti-inflammatory/regulatory cytokine IL-10, together with inflammasomes (NLRP3) and bone remodelling markers (RANKL, OPG) that reflect inflammatory processes better (Abdel-Latif et al., 2024; Heidari et al., 2024). These biomarkers show some potential for distinguishing between pulpitis and apical diseases, as well as monitoring post-treatment healing, with cutoff values now emerging for clinical use. **Conclusion:** Integrating gingival crevicular fluid (GCF) analysis into clinical practice through diagnostics could transform endodontic decision-making, moving the field toward personalized, biologically informed care.

[2*] Corresponding author. *E-mail address*: noorharliana@uitm.edu.my

## 1. INTRODUCTION

Accurate diagnosis of pulpal and periapical disease is a cornerstone of endodontic practice, yet it remains one of the most significant clinical challenges. Conventional diagnostic protocols aid clinicians in forming a diagnosis but are inherently limited because sensibility tests primarily measure neural responsiveness rather than actual pulpal blood flow or tissue vitality (Nair, 2004). The reliability of these tests is frequently compromised by factors such as dental trauma, calcified canals, and extensive restorations.

Gingival crevicular fluid (GCF) has emerged as a promising biological medium for improving diagnostic accuracy. As a serum-derived exudate located within the gingival sulcus, gingival crevicular fluid (GCF) contains a rich spectrum of inflammatory mediators, including cytokines, enzymes, and neuropeptides (Cimasoni, 1974). The relevance of gingival crevicular fluid (GCF) to endodontics derives from its anatomical proximity to the root apex and its ability to incorporate inflammatory mediators diffusing from inflamed pulp or periapical tissues through the apical foramen or dentinal tubules (Waerhaug, 1955). By utilizing molecular analysis of gingival crevicular fluid (GCF), clinicians can move toward a more objective, biologically precise approach to identifying disease stages and monitoring healing. Gingival crevicular fluid (GCF) is highly sensitive to pulpal disease because it functions as a serum-derived exudate that captures molecular changes within the tooth's internal environment (Cimasoni, 1974; Mona et al., 2021). Due to the anatomical continuity between the dental pulp, the periapical tissues, and the gingival sulcus, inflammatory mediators can diffuse through the apical foramen, lateral canals, and dentinal tubules into the gingival crevicular fluid (GCF) (Waerhaug, 1955; Weinstein et al., 1967).

Evidence demonstrates that gingival crevicular fluid (GCF) levels of pro-inflammatory cytokines such as IL-1β and TNF-α, as well as neuropeptides such as Substance P, increase significantly during pulpitis, reflecting neurovascular and immunological shifts within the pulp chamber (Awawdeh et al., 2002; Karapanou et al., 2008). This sensitivity is further supported by the identification of protein complexes, such as the NLRP3 inflammasome, with a clinical cutoff of 3.21 ng/mL in gingival crevicular fluid (GCF) for the diagnosis of symptomatic irreversible pulpitis (Abraham et al., 2023).

While the specific timeframe for biomarker appearance in gingival crevicular fluid (GCF) following pulpal injury is not explicitly quantified in minutes or hours in the review, gingival crevicular fluid (GCF) reflects early inflammatory changes and subclinical inflammation that traditional methods like radiographs may miss.

The rapid appearance of these markers is supported by the following biological mechanisms detailed in this review, including:

(i) **Neurogenic Signalling:** Neuropeptides like Substance P and neurokinin A are associated with early inflammatory signalling and pain perception.

(ii) **Rapid Diffusion:** Inflammatory mediators (interleukins, neuropeptides, and MMPs) migrate from the inflamed pulp through the apical foramen, lateral canals, or dentinal tubules into the periodontal ligament and gingival crevicular fluid (GCF).

(iii) **Proximity to Inflammation:** Because gingival crevicular fluid (GCF) is an inflammatory exudate originating from the gingival plexus near the tooth, it reflects real-time inflammatory events occurring in nearby pulpal and periapical tissues.

(iv) **Real-Time Monitoring:** Gingival crevicular fluid (GCF)- based diagnostics are considered suited for real-time assessment of pulpal and periapical status through chairside diagnostic methods.

These molecular changes occur before radiographically detectable structural bone resorption, making gingival crevicular fluid (GCF) a sensitive medium for detecting the immediate biological shift following pulpal insult.

Concentration gradient mechanics are fundamental to the production and diagnostic utility of gingival crevicular fluid (GCF) in endodontics. This process is driven by the movement of molecules from the highly inflamed internal tooth environment to the external gingival sulcus through several established biological mechanisms.

## 2. THE MECHANICS OF GINGIVAL CREVICULAR FLUID (GCF) PRODUCTION AND DIFFUSION

Gingival crevicular fluid (GCF) originates as a serum-derived exudate from the gingival plexus of blood vessels located within the gingival corium (Cimasoni, 1974). While gingival crevicular fluid (GCF) remains a transudate under physiological conditions, inflammation increases capillary permeability and osmotic pressure, thereby transitioning the fluid into an inflammatory exudate. This mechanical shift allows for a significantly higher volume of fluid and a greater diversity of molecular components, including serum-derived proteins and tissue breakdown products, to enter the gingival sulcus (Waerhaug, 1955).

The inclusion of specific pulpal biomarkers within this fluid is driven by steep concentration gradients and distinct anatomical pathways. Biomarkers such as interleukins, neuropeptides, and matrix metalloproteinases (MMPs) originate from the dental pulp and migrate toward the gingival crevicular fluid (GCF) by traveling from areas of high concentration, such as the inflamed pulp or periapical lesions, through conduits like the apical foramen, lateral canals, and dentinal tubules (Weinstein et al., 1967; Mona et al., 2021). Because gingival crevicular fluid (GCF) is situated in the immediate vicinity of the root apex and periodontium, it provides a localized "molecular window" into the real-time inflammatory status of the tooth’s internal environment (Mona et al., 2021).

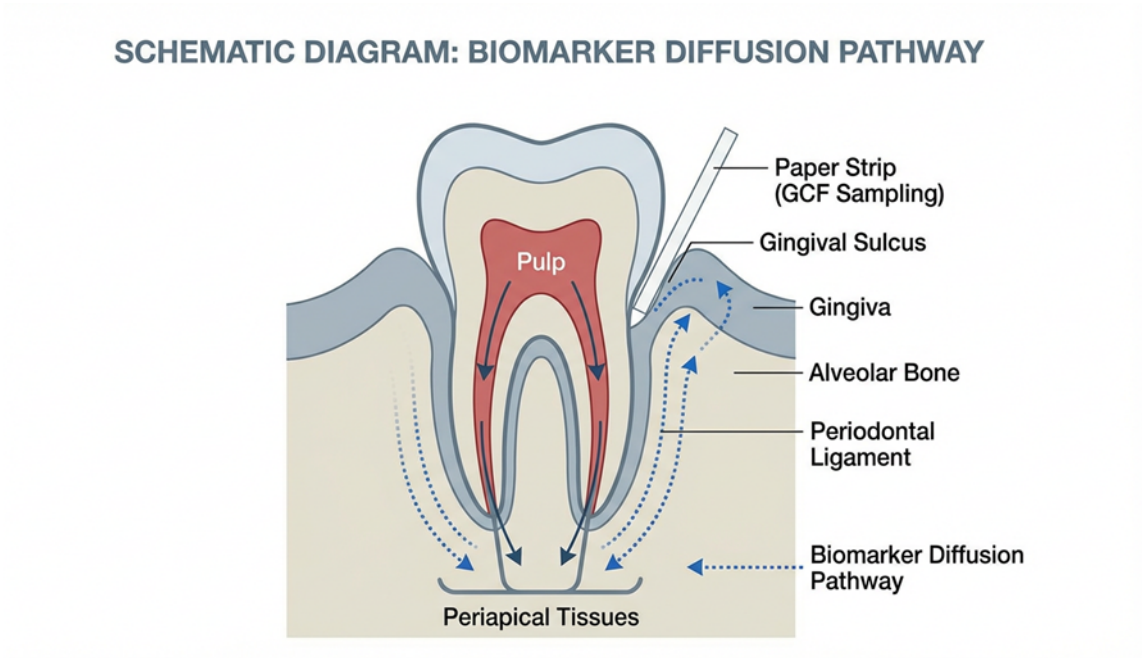



Fig. 1. Schematic diagram illustrating the diffusion pathway of biomarkers from the dental pulp and periapical tissues through the periodontal ligament and alveolar bone into the gingival crevicular fluid (GCF). The site of biomarker sampling using a paper strip at the gingival sulcus is shown. This pathway enables non-invasive assessment of pulpal and periapical status via gingival crevicular fluid (GCF) analysis.

## 3. DIAGNOSTIC SIGNIFICANCE OF THE GRADIENT

The mechanics of this gradient make gingival crevicular fluid (GCF) a highly sensitive medium for detecting subclinical inflammation and monitoring disease progression. The concentrations of specific markers, such as cytokines (IL-1β, IL-8) and neuropeptides (Substance P), vary significantly between health and disease, providing the discriminatory power needed to identify different stages of pulpitis and apical periodontitis (Awawdeh et al., 2002; Karapanou et al., 2008).

By measuring these concentrated molecular signals, clinicians gain objective insights into disease activity. This is evident in recent findings on the NLRP3 inflammasome, which quantifies a diagnostic concentration in gingival crevicular fluid (GCF) during irreversible pulpitis, offering an advantage over conventional diagnostic methods (Neha et al., 2025).

Gingival crevicular fluid (GCF) is superior to serum sampling for endodontic diagnostics primarily due to its high site-specificity and the localized concentration of inflammatory mediators. While serum reflects a systemic average of the body's inflammatory state, gingival crevicular fluid (GCF) acts as a localized exudate that captures biomarkers directly from the tooth's immediate environment. Because biomarkers like IL-1β, TNF-α, and neuropeptides diffuse from the inflamed pulp through the apical foramen and dentinal tubules, they reach the gingival sulcus in significantly higher concentrations than they would after entering the systemic circulation (Mona et al., 2021; Weinstein et al., 1967).

In the systemic bloodstream, these signals are often diluted below detectable limits or masked by systemic conditions, such as cardiovascular disease or diabetes. In contrast, gingival crevicular fluid (GCF)

provides a concentrated molecular profile unique to a single tooth, allowing precise differentiation of pulpal disease stages, including the 3.21 ng/mL cutoff for NLRP3 in symptomatic irreversible pulpitis (Abraham et al., 2023). Furthermore, gingival crevicular fluid (GCF) collection is entirely non-invasive, making it more practical for point-of-care chairside testing in a dental setting (Sagar et al., 2024).

## 4. SEARCH STRATEGY AND SELECTION CRITERIA

A systematic search of electronic databases was performed, including Science Direct, PubMed and Scopus for associated literature covering the period from 2000 to 2026. The review included primary research, such as longitudinal and cross-sectional clinical studies, while case reports were excluded. The keywords used included "gingival crevicular fluid", "endodontic biomarkers", "pulpitis" and "apical periodontitis". Search strategy using keywords and Boolean operators included:

(i) ((((gingival crevicular fluid) AND (endodontic)) AND (biomarkers)) AND (pulpitis))
(ii) ((((gingival crevicular fluid) AND (endodontic)) AND (biomarkers)) AND (apical periodontitis))

Zotero reference manager software was used to remove any duplicates. This is followed by a manual review of the abstracts. Articles were excluded when they did not meet the inclusion criteria. Then, the remaining articles were read individually, and articles that addressed topics outside this literature review were excluded. Figure 2 illustrates the method used to select the articles in this review.

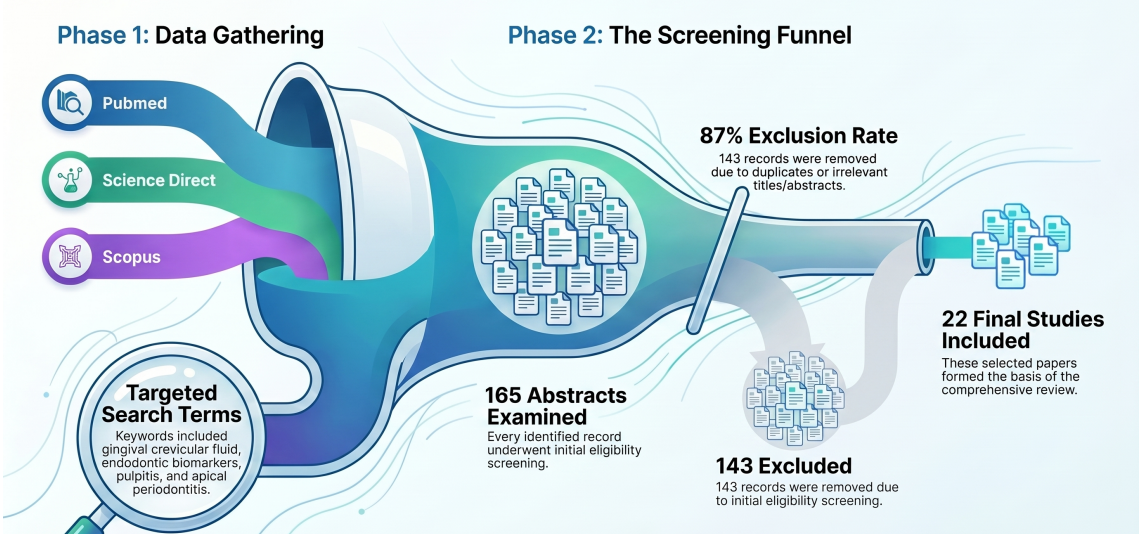



Fig. 2. Illustration of the article selection method.

## 5. LIMITATIONS OF CURRENT DIAGNOSTIC TOOLS

The limitations of conventional diagnostic approaches necessitate the development of more objective, biologically based methods. A primary challenge is the inherent subjectivity of thermal and electric pulp tests, which rely heavily on patient perception and can be influenced by age, anxiety, and previous dental experiences. These tests provide only an indirect measure of pulpal health because they assess neural responses rather than true pulpal blood flow, which can lead to false results in teeth with immature roots or significant calcification (Nair, 2004).

Radiographic methods also present diagnostic hurdles, most notably "radiographic lag," in which structural changes in bone are only detectable after substantial resorption has occurred (Gliga et al., 2023). While advanced imaging modalities such as CBCT improve anatomical resolution, they still cannot reliably distinguish active from inactive lesions or quantify the severity of inflammation. Ultimately, these clinical signs and symptoms often show poor correlation with the true histological status of the tissue, creating diagnostic uncertainty that may lead to either overtreatment or missed disease progression.

Table 1. Summary of the diagnostic trade-offs between GCF analysis and traditional pulp sensibility testing based on current evidence.

| Feature | Gingival crevicular fluid (GCF) Biomarker Analysis | Traditional Sensibility Testing | Sensitivity/ Specificity or AUC |
|---|---|---|---|
| Objectivity | **High**: Provides quantitative molecular data. | **Low**: Relies on subjective patient response. | **NLRP3**: AUC for identifying SIP is high with a cutoff of **3.21 ng/mL**. |
| Site Specificity | Localized to the sulcular environment. | Localized to the pulp chamber/apex. | **High precision** reported in specific diagnostic markers like NLRP3. |
| Sensitivity | **High**: Detects subclinical molecular changes before structural damage. | **Lower**: Requires advanced tissue damage or nerve response to trigger a result. | Often derived from **single-centre studies** or focused clinical cohorts. |
| Confounding Factors | Periodontal disease, saliva, or blood contamination. | Patient anxiety, tooth calcification, or recent trauma. | Sensitivity may decrease in **heterogeneous cohorts** with concurrent periodontal disease. |
| Speed | 10–15 minutes for Point-of-Care (POC) to weeks for Lab analysis. | **Immediate**: Results are obtained chairside instantly. | N/A |

## 6. GINGIVAL CREVICULAR FLUID (GCF) AS A DIAGNOSTIC TOOL

### 6.1. Sampling Technique and Collection

The most widely accepted and non-invasive method for collecting gingival crevicular fluid (GCF) is the absorption technique using pre-cut, sterilized absorbent paper strips or Periopaper (Cimasoni, 1974). During pre-collection preparation, the target tooth must be isolated with cotton rolls and gently dried with a directed air stream to prevent contamination from saliva, which contains its own subset of non-site-specific biomarkers. For the insertion phase, the paper strip is placed into the gingival sulcus until slight resistance is felt (Brill's Technique; Brill, 1962) and is typically left in place for 30 to 60 seconds. Then, the paper strip is placed in containers containing PBS solution to elute its components (Correa et al., 2008). Because biomarker concentrations are volume-dependent, the amount of fluid collected should be measured immediately using a calibrated electronic moisture meter, such as a Periotron 8000, to calculate the absolute amount of biomarker per unit volume of fluid (Mona et al., 2021).

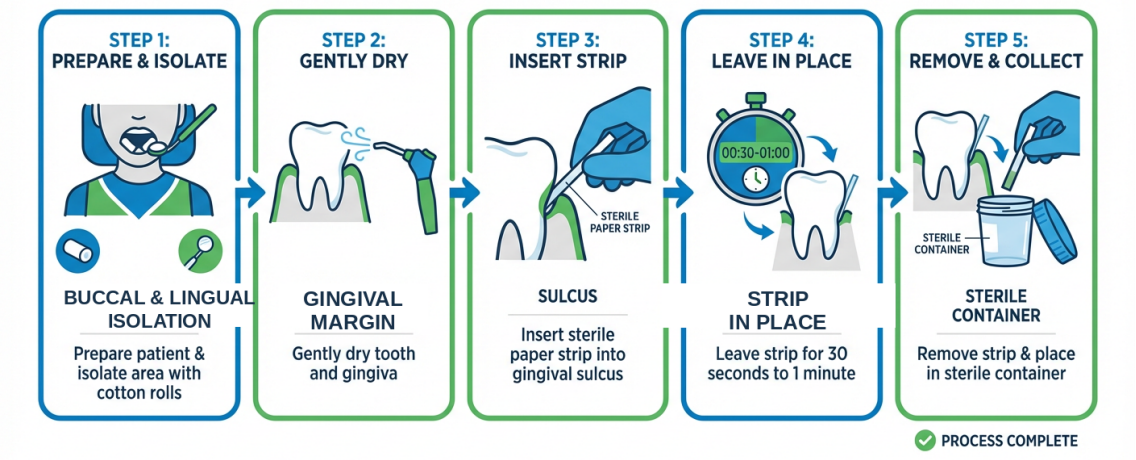



Fig. 3. Gingival crevicular fluid (GCF) collection via "Absorption Technique".(1) Preparing and isolating the sampling site with cotton rolls, (2) gently drying the tooth and gingiva, (3) inserting a sterile paper strip into the gingival sulcus, (4) leaving the strip in place for 30 seconds to 1 minute to absorb gingival crevicular fluid (GCF), and (5) removing the strip and placing it in a sterile container for subsequent analysis.

### 6.2. Optimal Collection Sites

For endodontic diagnostics, the collection site should be positioned as close as possible to the source of the inflammatory diffusion, with the mid-buccal or interproximal sulcus of the tooth in question typically utilized. The anatomic rationale for this selection is that in cases of apical periodontitis, biomarkers travel through the periodontal ligament space toward the gingival margin. If a sinus tract or periodontal pocket is associated with endodontic pathology, these specific sites may yield higher concentrations of mediators such as RANKL or IL-1β than healthy sulcular sites (Stashenko et al., 1998; Abdel-Latif et al., 2024).

### 6.3. Storage and Stable Environment

To maintain the integrity of diagnostic signals, standardized pre-analytical handling is critical. If immediate point-of-care analysis is not available, gingival crevicular fluid (GCF) samples should be eluted in a buffer and stored at -80°C (Awawdeh et al., 2002; Heidari et al., 2024). Under this ultra-low temperature, the molecular signatures of most cytokines and proteases remain reliably preserved for at least 1 month (Preianò et al., 2016). However, clinicians must be aware that even at -80°C, certain markers, such as S100A8 and S100A9, may begin to degrade after 3 months, and multiple freeze-thaw cycles must be strictly avoided to prevent loss of protein detection sensitivity.

## 7. MOLECULAR PROFILING: EVIDENCE-BASED BIOMARKERS AND SPECIFICITY

Biomarkers provide objective insight into the biological state of the pulp, complementing traditional tests. Recent research has expanded the diagnostic profile of gingival crevicular fluid (GCF) beyond basic cytokines to include complex inflammasomes and bone turnover regulators. A significant breakthrough in gingival crevicular fluid (GCF) analysis is the identification of the NLRP3 inflammasome as a marker for symptomatic irreversible pulpitis (SIP). Recent studies suggest a clinical cutoff of approximately 3.21 ng/mL in gingival crevicular fluid (GCF) for symptomatic irreversible pulpitis (SIP), with levels significantly decreasing following successful endodontic treatment (Abraham et al., 2023).

In the context of apical periodontitis (AP), the RANKL-to-OPG ratio is a critical indicator of bone resorption activity (Mona et al., 2021). Studies have shown that TIMP-1 and OPG can distinguish symptomatic from asymptomatic lesions, with area under the curve (AUC) values exceeding 0.77 (Abdul-Hadi et al., 2024). Furthermore, while pro-inflammatory markers like IL-8 indicate active disease (Karapanou et al., 2008), IL-10, an anti-inflammatory cytokine, has recently been identified as a potential diagnostic biomarker, reflecting the host's attempt to regulate the inflammatory response (Abdul-Hadi et al., 2025).

Table 2. Endodontic gingival crevicular fluid (GCF)

| Category | Biomarker | Mechanism / Clinical Role | Diagnostic Implication & Evidence |
|---|---|---|---|
| **Comparative Sources** | **Pulpal Blood** | Direct collection of intravascular fluid from the pulp chamber. | Provides high specificity for true vitality but is clinically invasive and limited to cases with pulp exposure (Nair, 2004). |
| | **Gingival crevicular fluid (GCF)** | Non-invasive collection of serum-derived exudate from the sulcus. | Offers a site-specific "window" into the pulp and periapical status via diffusion through the apical foramen (Mona et al., 2021). |
| **Inflammasomes** | **NLRP3** | A multi-protein complex that triggers the release of pro-inflammatory cytokines. | A specific clinical cutoff of **3.21 ng/mL** in GCF identifies symptomatic irreversible pulpitis (SIP) with high precision (Abraham et al., 2023). |
| **Cytokines** | **IL-1β, IL-6, IL-23** | Primary pro-inflammatory mediators that drive tissue destruction and apoptosis. | Significantly elevated in irreversible pulpitis and symptomatic periapical lesions compared to healthy controls (Stashenko et al., 1998; Abdul-Hadi et al., 2025). |
| **Bone Markers** | **TIMP-1 & OPG** | Regulators of the extracellular matrix and inhibitors of osteoclastogenesis. | Identified as reliable markers to differentiate between symptomatic and asymptomatic apical periodontitis (Abdul-Hadi et al., 2024). |
| | **RANKL** | A key factor in the activation and survival of bone-resorbing osteoclasts. | A high RANKL/OPG ratio indicates active bone resorption and lesion progression (Stashenko et al., 1998; Abdul-Hadi et al., 2024). |
| **Neuropeptides** | **Substance P (SP)** | A sensory neuropeptide involved in pain transmission and vasodilation. | GCF levels correlate directly with pre-operative pain levels and neurogenic inflammation in pulpitis (Awawdeh et al., 2002). |
| **Proteases** | **MMP-1, 8, & 9** | Matrix metalloproteinases that degrade collagen and the extracellular matrix. | MMP-8 reflect the severity of tissue breakdown with 0.36 ng/mL in reversible pulpitis and 1.97 ng/mL in irreversible pulpitis (Aguirre-López et al.,2020). |

## 8. SHORTCOMINGS IN GINGIVAL CREVICULAR FLUID (GCF) AS A DIAGNOSTIC TOOL IN ENDODONTICS

While gingival crevicular fluid (GCF) offers significant diagnostic potential, its clinical implementation in endodontics is hindered by several key limitations. A primary concern is its non-specificity, as gingival crevicular fluid (GCF) acts as an inflammatory exudate that reflects tissue responses from both apical and marginal periodontal tissues. Because inflammation is a non-specific response, it remains difficult to differentiate whether a biomarker elevation originates from pulp necrosis at the apex or from marginal periodontal disease (Lamster and Ahlo, 2007). More studies are needed as there is still a lack of evidence in quantifying the volume of gingival crevicular fluid (GCF) in teeth with endodontic pathology. Furthermore, gingival crevicular fluid (GCF) is unlikely to reflect the exact spatial progression of microbial spread within the pulp chamber, a critical factor for clinicians deciding between vital pulp therapy and full root canal treatment (Mora et al., 2025).

The utility of gingival crevicular fluid (GCF) is further complicated by significant confounding factors and methodological heterogeneity. Analysis can be influenced by high levels of individual variation, including gingival health, fluid volume, patient age, systemic health, and drug use (Mora et al., 2025). From a technical standpoint, gingival crevicular fluid (GCF) collection is highly susceptible to contamination by saliva and blood, which can skew the sample's molecular profile. Additionally, there is currently no standardized method for collection or analysis, making the interpretation of results difficult across different clinical studies. Finally, the low concentration of specific target proteins or biomarkers in such minute fluid volumes can limit the overall diagnostic sensitivity of the medium (Rechenberg and Zehnder, 2014).

Moreover, differentiating between periodontal and endodontic biomarkers remains challenging. At times, inflammatory biomarkers overlap, including IL-1β, IL-8, and matrix metalloproteinases (MMPs). While both conditions involve similar proinflammatory pathways, periodontal disease typically presents with elevated IL-1β levels (210 pg/mL), indicating early-stage gingivitis, and elevated MMP-8 levels (185 ng/mL), signifying bone loss and severe periodontal destruction (Sachelarie et al., 2025). On the contrary, endodontic pathologies such as apical periodontitis exhibit highly localized concentrations of these same mediators within periapical exudates or pulpal blood, though gingival crevicular fluid (GCF) remains a promising non-invasive surrogate for periradicular status despite its inherent lack of site specificity.

To address this, there is a shift towards multiplex biomarker panels that enable the detection of a broader range of inflammatory mediators. This supports the point-of-care diagnosis that focuses on real-time readings, is more affordable and user-friendly, while maintaining a degree of sensitivity and specificity. However, it falls short in diagnostic accuracy, precision, and sensitivity compared with conventional laboratory-based techniques (Walsh et al., 2025). There is also a need to determine thresholds for these point-of-care applications to aid risk categorization, thereby enabling a personalized approach to treatment planning and disease monitoring.

## 9. FUTURE DIRECTIONS OF GINGIVAL CREVICULAR FLUID (GCF) IN VITAL PULP THERAPY AND REGENERATIVE PROCEDURES

Biomarkers such as NLRP3, IL-10, TIMP-1/OPG, and MMP-1 are viable methods for monitoring healing, predicting outcomes, and differentiating true regeneration from mere symptom resolution. IL-10 is an anti-inflammatory cytokine that limits the host immune response and is associated with healing. Evidence demonstrates diagnostic potential (AUC > 0.8) for monitoring the resolution of apical periodontitis (Abdul-Hadi et al., 2025).

Evidence regarding the reduction of biomarkers following endodontic treatment highlights a distinct pattern that often precedes radiographic evidence of healing. Following successful nonsurgical endodontic treatment, the gingival crevicular fluid (GCF) molecular profile shifts from a pro-inflammatory state to a homeostatic one. Significant decreases in pro-inflammatory markers such as IL-8 and Substance P are observed as early as 7 days post-intervention, reflecting an immediate reduction in neurovascular stress (Neha et al., 2025). By the one-month mark, markers of active pulpitis, specifically the NLRP3 inflammasome, show a dramatic reduction. In successful cases, elevated baseline levels of these markers typically drop significantly within 30 days, reaching levels comparable to those of healthy controls (Neha et al., 2025). Throughout the 1- to 6-month monitoring period, pro-inflammatory cytokines continue to decline in successful cases, whereas failure of these markers to decrease is strongly correlated with persistent apical periodontitis.

The "biological lead time" provided by gingival crevicular fluid (GCF) allows clinicians to predict outcomes far earlier than conventional methods. A persistent elevation of markers such as IL-1β or IL-2 following root canal obturation serves as an objective indicator of treatment failure or missed canals, allowing earlier intervention before radiographic changes are visible (Jain et al., 2024). Furthermore, the reduction in molecular inflammatory activity has been shown to correlate directly with patient-reported pain reduction, providing molecular validation of clinical recovery that supports the patient's subjective experience (Viderman et al., 2026). This real-time monitoring capability makes gingival crevicular fluid (GCF)-based diagnostics suited for assessing the biological shift following a pulpal insult or treatment, before structural bone resorption is detectable on radiographs

The integration of gingival crevicular fluid (GCF) analysis into routine endodontic practice represents a promising frontier that requires further multi-disciplinary research to reach full clinical potential. Future efforts must prioritize large-scale, multi-centred studies to refine optimal biomarker panels, combining cytokines, neuropeptides, and microbial markers, that can reliably differentiate between reversible and irreversible pulpitis. Establishing a direct link between these molecular profiles and histopathological findings or long-term clinical outcomes is essential for building a robust evidence base for chairside use. Emerging microfluidic biosensors and lateral-flow immunoassays aim to analyse gingival crevicular fluid (GCF) within 10 to 15 minutes chairside. This real-time window is critical for endodontists to make immediate decisions regarding vital pulp therapy versus root canal treatment (Sagar et al., 2024).

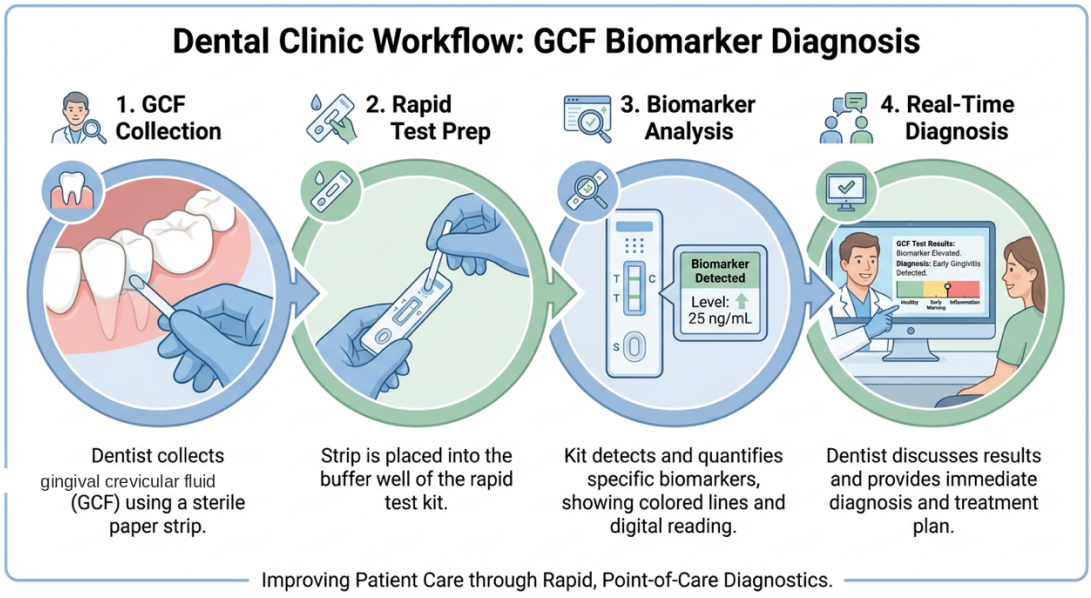



Fig. 4. Suggested workflow showing rapid pulp health diagnosis. (a) Gingival crevicular fluid (GCF) collection: Gingival crevicular fluid (GCF) is collected from the patient's gums using a sterile paper strip (b) Rapid test processing: The paper strip is placed into the buffer well rapid test kit (c) Biomarker detection: The kit detects and quantifies specific biomarkers, showing coloured lines and digital results (d) "Real-time" diagnosis: The dentist reviews the results on a screen and discusses the pulp health status as well as suitable treatment options with the patient.

To ensure these tools are reproducible across different settings, the industry must focus on standardizing sampling techniques, storage conditions, and analytical methods. Additionally, applying machine learning to develop predictive models could enable clinicians to integrate gingival crevicular fluid (GCF) data with patient demographics and radiographs to forecast the success of conservative treatments such as vital pulp therapy.

## 10. CONCLUSION

Gingival crevicular fluid (GCF) represents a biologically informative and clinically accessible medium that accurately reflects the molecular status of pulpal and periapical tissues, positioning it as a superior adjunctive diagnostic tool. By providing objective insights into disease activity and treatment response through emerging cutoff values for markers like NLRP3, gingival crevicular fluid (GCF) analysis can optimize treatment planning and improve the preservation of natural teeth.

However, clinicians must approach gingival crevicular fluid (GCF)-based diagnostics with caution due to several inherent limitations. The medium's non-specificity makes it difficult to differentiate between apical (pulpal) and marginal (periodontal) inflammation. Furthermore, gingival crevicular fluid (GCF) cannot reflect the exact spatial progression of infection within the pulp chamber which is essential for deciding between vital pulp therapy and root canal treatment. Methodological challenges also persist, including high susceptibility to contamination by saliva or blood and the lack of standardized collection tools, which can skew the detection of low-concentration biomarkers.

To manage these shortcomings, clinicians should implement rigorous sampling protocols, such as isolating the tooth with cotton rolls and using a directed air stream to prevent salivary contamination. Introducing strict criteria that omit patients with periodontal disease will increase diagnostic precision and specificity. Standardizing collection sites ensures proximity to the source of inflammatory diffusion. Additionally, using moisture meters such as the Periotron 8000 enables precise volume quantification, helping to resolve issues of volume dependence and individual heterogeneity. When integrated with traditional radiographs and clinical tests, gingival crevicular fluid (GCF) serves as a vital "molecular window" that enhances diagnostic certainty.

## 11. ACKNOWLEDGEMENT

The authors would like to acknowledge the support of Fakulti Pergigian Universiti Teknologi MARA (UiTM), Kampus Sungai Buloh for providing the facilities and financial support for this literature review.

## 12. CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

## 13. AUTHOR'S CONTRIBUTION

Athirah Abdul Aziz conceptualised the research idea, conducted the research, wrote, and revised the article. Noorharliana Mohamed Zohdi, Hayati Ishak, and Mohd. Haikal Muhamad Halil supervised the research progress, monitored the review, revisions and approved the article for submission.

## 14. REFERENCES

Abdulhadi, Z.T., Mahdee, A.F. & Gul, S.S., 2024. Accuracy of gingival crevicular fluid biomarkers of MMP8, TIMP1, RANK, RANKL, and OPG in differentiating symptomatic and asymptomatic apical periodontitis. *Diagnostics, 14*(17), p.1872. https://doi.org/10.3390/diagnostics14171872.

Abdulhadi, Z.T., Radhi Jawad, S., Alwandawi, T.K. & Muhsin, Z.Y., 2025. Assessment of inflammatory cytokines in gingival crevicular fluid for diagnostic differentiation of apical periodontitis. *Folia Medica*, *67*(6), p.e161643. https://doi.org/10.3897/folmed.67.e161643.

Abraham, D., Singh, A. & Goyal, A., 2023. Symptomatic irreversible pulpitis induces increased levels of human NLRP3 in gingival crevicular fluid compared to saliva: a case control observational study. *Journal of Endodontics*, *49*(11), pp.1480–1486. https://doi.org/10.1016/j.joen.2023.08.013.

Aguirre-López, E.C., Patiño-Marín, N., Martínez-Castañón, G.A., Medina-Solís, C.E., Castillo-Silva, B.E., Cepeda-Argüelles, O., Aguilera-Galaviz, L.A. & Rosales-García, P., 2020. Levels of matrix metalloproteinase-8 and cold test in reversible and irreversible pulpitis. *Medicine*, *99*(52), p.e23782. https://doi.org/10.1097/MD.0000000000023782.

Awawdeh, L., Lundy, F.T., Shaw, C., Lamey, P.J., Linden, G.J. & Kennedy, J.G., 2002. Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in pulp tissue from painful and healthy human teeth. *International Endodontic Journal*, *35*(1), pp.30–36. https://doi.org/10.1046/j.1365-2591.2002.00451.x.

Brill, N., 1962. The gingival pocket fluid. Studies of its occurrence, composition and effect. *Acta Odontologica Scandinavica*, *20*(Suppl. 32), pp.1–115.

Cimasoni, G., 1974. The crevicular fluid. *Monographs in Oral Science*, 3, pp.1–122.

Correa, F.O., Gonçalves, D., Figueredo, C.M., Gustafsson, A. & Orrico, S.R., 2008. The short-term effectiveness of non-surgical treatment in reducing levels of interleukin-1beta and proteases in gingival crevicular fluid from patients with type 2 diabetes mellitus and chronic periodontitis. *Journal of Periodontology, 79*(11), pp.2143–2150. https://doi.org/10.1902/jop.2008.080132.

Essam, O., Umerji, S. & Blundell, K., 2025. Endodontic assessment, complexity, diagnosis and treatment planning. *British Dental Journal, 238*(7), pp.441–447. https://doi.org/10.1038/s41415-025-8452-6.

Gliga, A., Imre, M., Grandini, S., Marruganti, C., Gaeta, C., Bodnar, D., Dimitriu, B.A. & Foschi, F., 2023. The limitations of periapical X-ray assessment in endodontic diagnosis—A systematic review. *Journal of Clinical Medicine, 12*(14), p.4647. https://doi.org/10.3390/jcm12144647.

Jain, S., Sundar, S., Haritha, J.S. & Natanasabapathy, V., 2024. Comparison of interleukin-1β concentrations in posttreatment endodontic disease and other pulpal and periapical conditions - A clinical study. *Journal of Conservative Dentistry and Endodontics, 27*(8), pp.843–848. https://doi.org/10.4103/JCDE.JCDE_324_24.

Karapanou, V., Kempuraj, D. & Theoharides, T.C., 2008. Interleukin-8 is increased in gingival crevicular fluid from patients with acute pulpitis. *Journal of Endodontics, 34*(2), pp.148–151. https://doi.org/10.1016/j.joen.2007.10.022.

Lamster, I.B. & Ahlo, J.K., 2007. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Annals of the New York Academy of Sciences, 1098*, pp.216–229. https://doi.org/10.1196/annals.1384.027.

Milojevic, N., Krdzovic Lazic, E., Lukic, L., Puresevic, D., Mirkovic, M. & Jakovljevic, A., 2024. Inflammatory mediators' essence in apical periodontitis. *European Oral Research, 58*(3), pp.160–168. https://doi.org/10.26650/eor.20241423117.

Mohammad-Rahimi, H., Sohrabniya, F., Ourang, S.A., Dianat, O., Aminoshariae, A., Nagendrababu, V., Dummer, P.M.H., Duncan, H.F. & Nosrat, A., 2024. Artificial intelligence in endodontics: Data preparation, clinical applications, ethical considerations, limitations, and future directions. *International Endodontic Journal, 57*(11), pp.1566–1595. https://doi.org/10.1111/iej.14128.

Mona, M., Abbasi, Z., Kobeissy, F., Chahbandar, A. & Pileggi, R., 2021. A bioinformatics systems biology analysis of the current oral proteomic biomarkers and implications for diagnosis and treatment of external root resorption. *International Journal of Molecular Sciences, 22*(6), p.3181. https://doi.org/10.3390/ijms22063181.

Mora, M., Pacheco-Yanes, J. & Sigurdsson, A., 2025. The future of endodontic diagnosis. *JADA Foundational Science, 4*, p.100050. https://doi.org/10.1016/j.jfscie.2025.100050.

Mutlak, S.S., Hasan, N.A. & Al-Hijazi, A.Y., 2017. The profile of tooth and gingival crevicular fluid matrix metalloproteinase-1 in different dental diseases. *DOAJ (Directory of Open Access Journals), 6*(7), pp.57–66.

Nair, P.N., 2004. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology and Medicine*, *15*(6), pp.348–381. https://doi.org/10.1177/154411130401500604.

Nair, M.K. & Nair, U.P., 2007. Digital and advanced imaging in endodontics: a review. *Journal of Endodontics, 33*(1), pp.1–6. https://doi.org/10.1016/j.joen.2006.08.013.

Neha, N., Abraham, D., Goyal, A., Gupta, A., Sharma, R. & Malhotra, R.K., 2025. GCF NLRP3 as a biomarker for assessing endodontic treatment outcomes in symptomatic irreversible pulpitis: A comparative nonrandomized, observational cross-sectional study. *Journal of International Society of Preventive & Community Dentistry, 15*(5), pp.415–425. https://doi.org/10.4103/jispcd.jispcd_31_25.

Preianò, M., Maggisano, G., Lombardo, N., Montalcini, T., Paduano, S., Pelaia, G., Savino, R. & Terracciano, R., 2016. Influence of storage conditions on MALDI-TOF MS profiling of gingival crevicular fluid: Implications on the role of S100A8 and S100A9 for clinical and proteomic based diagnostic investigations. *Proteomics, 16*(6), pp.1033–1045. https://doi.org/10.1002/pmic.201500328.

Rechenberg, D.-K. & Zehnder, M., 2014. Molecular diagnostics in endodontics. *Endodontic Topics, 30*(1), pp.51–65. https://doi.org/10.1111/etp.12057.

Sachelarie, L., Stefanescu, C.L., Murineanu, R.M., Grigorian, M., Zaharia, A., Scrobota, I. & Hurjui, L.L., 2025. Role of salivary biomarkers IL-1β and MMP-8 in early detection and staging of periodontal disease. *Medicina, 61*(4), p.760. https://doi.org/10.3390/medicina61040760.

Sagar, S., et al., 2024. Point-of-care diagnostic devices for endodontic applications – current trends. *Journal of Oral Health Research*.

Viderman, D., Kalikanov, S., Mukazhan, D. & Nurmukhamed, B., 2026. Neurophysiological, radiological, and molecular biomarkers of pain-related conditions: An umbrella review. *Journal of Clinical Medicine, 15*(2), p.550. https://doi.org/10.3390/jcm15020550.

Waerhaug, J., 1955. The source of mineral salts in subgingival calculus. *Journal of Dental Research, 34*(5), pp.563–568.

Walsh, D., Quigley, R., Ekperuoh, A. & Duncan, H.F., 2025. Objectively diagnosing pulpitis: opportunities and methodological challenges in the development of point-of-care assays. *International Journal of Molecular Sciences, 27*(1), p.355. https://doi.org/10.3390/ijms27010355.

Weber, M.-T., Stratz, N., Fleiner, J., Schulze, D. & Hannig, C., 2015. Possibilities and limits of imaging endodontic structures with CBCT. *Swiss Dental Journal SSO, 125*(3), pp.293–311. https://doi.org/10.61872/sdj-2015-03-150.

© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) license (http://creativecommons.org/licenses/by/4.0/).

## 15. APPENDIX

## ABOUT THE AUTHORS

*Athirah Abdul Aziz is* a resident postgraduate in the Doctor in Restorative Dentistry program at the Faculty of Dentistry, University of Teknologi MARA (UiTM), Sungai Buloh Campus. She is currently researching endodontic biomarkers in the assessment of vital pulp therapy. She can be reached through her email at abdulazizathirah@yahoo.com.

*Noorharliana Mohamed Zohdi* is a Senior Academician and Consultant in Restorative Dentistry at the Centre of Comprehensive Care Studies, at the Faculty of Dentistry, University of Teknologi MARA (UiTM), Sungai Buloh Campus. Her main research focuses on regenerative endodontics, restorative dentistry, dental education and technologies, cariology, and ICDAS implementation. She can be reached at noorharliana@uitm.edu.my.

*Hayati Ishak is* a Senior Academician and Consultant in Restorative Dentistry at the Centre of Comprehensive Care Studies, Faculty of Dentistry, University of Teknologi MARA (UiTM), Sungai Buloh Campus. Her main research focuses on restorative dentistry, dental materials, and cariology. She can be reached at hayati66@uitm.edu.my.

*Mohd Haikal Muhamad Halil is* the Dean of the Faculty of Dentistry, International Islamic University of Malaysia (IIUM) in Kuantan, Pahang. Besides overseeing the faculty, he focuses his research on restorative dentistry, biomaterials, dental technologies and education, and endodontics. He can be reached at drhaikal@iium.edu.my.