GINGIVAL CREAUVICAL FLUID: A REVIEW OF LITERATURE

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Abstract

Gingival crevicular fluid is a biological fluid that emerges from the gingival crevice or pocket. Its composition resembles normal serum, but its volume and composition varies in certain conditions such as gingivitis and periodontitis. It is composed of variable substances that include immunoglobulin, enzymes, local mediators, toxin cells, protein peptides, tissue breakdown products, and microorganisms. The level of this substances varies in the above-mentioned conditions and therefore it will acts as a future diagnostic tool in their non-invasive analysis. Due to limitations in its collection, which includes volume size and contamination, collecting methods need further work. This article aims at reviewing briefly about the GCF and its role as a diagnostic marker in periodontal disease.

Key words: Diagnostic tool, Gingival Crevicular Fluid, Periodontitis.

Introduction

Periodontitis is a common chronic infectious disease associated with pathogenic microorganisms affecting the supporting structures of teeth and leading to progressive destruction, finally leading tooth loss. Its diagnosis and treatment plans are commonly based on clinical parameters and radiographic findings. They represent only the past activity of periodontal disease and are not enough sensitive and specific to diagnose disease activity in individual sites or to predict future attachment loss.1

Gingival crevicular fluid (GCF) is a serum transudate as well as an inflammatory exudate emerging from the gingival plexus of blood vessels in the gingival sulcus, subjacent to the epithelium lining of the dentogingival space that has gained great interest on possible diagnostic value in periodontal disease. It contains various types of cells, enzymes, proteins and peptides derived from affected host tissues. Hence, the analysis of the GCF components can represent the disease status of individual areas and thus helping to identify potential biomarkers of periodontitis.1,2

Formation of GCF

Brill & Krasse (1958), Brill & Bjorn (1959) and Egelberg (1966) have done studies on the formation of GCF and they concluded that formation of GCF is mainly a result of an increase in the permeability of the vessels beneath the junctional and sulcular epithelium.3

Alfano in 1974 explained the mechanism of GCF formation that includes the engendering of standing osmotic gradient and the induction of inflammation. The osmotic gradient is generated by macromolecular by-products of the bacteria in the dental plaque that diffuse through the gingival crevicular epithelium to the basement membrane which prevents its further penetration. This leads to accumulation of macromolecules at the basement membrane which causes localized increase in solute concentration leading to the establishment of an osmotic gradient. Due to this osmotic gradient solvent molecule passes through the basement membrane that increases the intercellular hydrostatic pressure and cause exudation of gingival fluid.4

According to the model proposed by Pashley (1976), gingival fluid production is viewed as the result of an increased rate of capillary transudation brought about by the release of mediators of inflammation. These agents are thought to cause both increased capillary pressure and increased leakage of plasma proteins into the interstitial fluid. It is postulated that the low compliance of gingival tissue and the high hydraulic conductance of sulcular epithelium result in this interstitial fluid moving from connective tissue into the sulcus.5

Collection methods

The gingival crevicular fluid can be collected using following methods.

- Absorbent filter paper strips
- Pre-weighed twisted threads
- Micropipettes
- Crevicular washings

Absorbent filter paper strips

The strips are used in two different ways.

Intracrevicular (Brill technique): In this method specifically designed paper strips are inserted into the gingival sulcus, until the resistance is achieved. And the paper left in the sulcus for 5 to 60 seconds for the absorption of GCF. Disadvantage of this method is, the technique itself produces a degree of irritation of the sulcular epithelium that increases the production of gingival crevicular fluid.6

For avoiding this irritation, Loe and Holm: – Pedersen placed the paper strip near to the entrance of the sulcus or over the sulcus. By this way, fluid seeping out is picked up by the strip.6

Extracrevicular: Here the strips overlaid in the crevice region. Thus the physical irritation of sulcular or junctional epithelia is avoided.5

Preweighted twisted threads (Weinstein et al.)

In this method a pre-weighed thread is placed in the gingival sulcus around the tooth, and the amount of fluid...
collected is measured by weighing the thread and subtracting the previous weight.\(^5\)

**Capillary tubing or micropipettes**

The capillary tubes of known internal diameter and length are placed at the entrance of the gingival crevice. GCF from the crevice migrates into the tube by capillary action. The volume of fluid collected determined by measuring the height which the GCF has migrated into the tube. This technique provides an undiluted sample of ‘native’ GCF.\(^3\)

**Gingival washing methods**

GCF collection by gingival washing technique can be done in two different ways.

First method: - Proposed by *Tokamoli and Oppenheim*. 10μl of Hank’s balanced salt solution ejected into interdental papilla from a micro syringe and the solution is re-aspirated again. This process is repeated 12 times to allow thorough mixing of the transport solution with GCF. It can be applied either to individual interdental units or to multiple units. And the diluted GCF is collected.

Second method: - introduced by *Skapski and Lehner*. It involves the construction of a customized maxillary acrylic plate. The sulcus is then irrigated for a fixed time with a saline solution by using a peristaltic pump through palatal and buccal channels. And the diluted GCF is collected.\(^7\)

**Problems with GCF collection**

**Contamination**

GCF samples are usually contaminated by blood, saliva, or dental plaque and their presence affects the accuracy in volume determination and composition of GCF. Presence of saliva in GCF is confirmed by alpha-amylase assay.\(^8\)

**Sampling time**

Prolonged collection time of GCF will cause change in the protein concentration of the initial GCF collected.\(^3\)

**Volume determination**

Evaporation is considered to be a significant problem in accurate volume determination of GCF samples. The total volumes collected are usually 0.5-1μl. As the total sample of GCF collected is very small, the percentage of error is considered to be more significant.\(^9\)

**Components of GCF**

GCF mainly composed of\(^6\)

- Cellular components
- Organic components
- Inorganic components
- Enzymes
- Bacterial products

Components of GCF is summarized in Table 1

<table>
<thead>
<tr>
<th>Cellular Components</th>
<th>Organic Components</th>
<th>Inorganic Components</th>
<th>Enzymes</th>
<th>Bacterial Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria Epithelial cells</td>
<td>Carbohydrates Lignins</td>
<td>Proteins Lipids</td>
<td>Potassium Sodium Calcium Magnesium Fluoride</td>
<td>Acid phosphatase Alkaline phosphatase Alpha 1 antitrypsin Arylsulfatase Cholesteryl sulphate Cysteine Cytochrome C endoplasmic reticulum Acid phosphatase Alkaline phosphatase Fibroblast Growth Factors Integrins Hyaluronic Acid Various MMPs</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Metabolic products</td>
<td>Lipids</td>
<td>Cholesterol</td>
<td>Transferrin Thromboxane</td>
</tr>
</tbody>
</table>

**Table 1: Composition of GCF**

**Cellular elements**

**Bacteria**

They are derived from adjacent plaque mass. They can cause periodontal disease and initiates immune response of the host. Some of these bacterial cells are found free-floating in the GCF, while others can be seen attached to host cells, usually epithelial cells.\(^10\)

**Epithelial cells**

It is derived from oral sulcular and junctional epithelium. They represent a high turnover rate of these epithelium that makes up the gingival sulcus.\(^2\)

**Leukocytes**

They are derived from gingival plexus of blood vessels which acts as effector cells of host response. PMNs play a role in innate immunity while monocytes/macrophages and lymphocytes play roles in cell-mediated immunity.\(^10\)

**Erythrocytes**

Erythrocytes can be detected in GCF due to damage to the small blood vessels and capillaries of gingival connective tissue.\(^2\)

**Electrolytes**

The minerals like Potassium, Sodium, Calcium, Magnesium and Fluoride are detected in the GCF. Most studies have shown a positive correlation of sodium, potassium concentrations and the sodium to potassium ratio with inflammation.\(^9\)

**Organic compounds**

**Carbohydrates**

Carbohydrates which are usually seen in GCF are glucose, hexosamine and hexuronic acid. Concentration of glucose in GCF of periodontitis patients is 3-6 times higher than that of healthy individuals and their concentration decrease in case of non-inflamed gingiva.\(^11\)
Proteins and Lipids

In inflamed gingiva, due to the increased permeability, protein passes through the barrier of junctional epithelium. Brill and Bromness (1960) found that the concentration of protein in GCF was low as compared with that of serum in a ratio of 1:10. Histochemically, it was determined that crevicular exudate contains proteins similar to those found in the serum (Sueda et al., 1966).11

Proteins namely α and β globulins, transferrin, albumin, immunoglobulins such as IgG, IgM and IgA, complement components such as C1, C3, C4, C5 have been reported to be present in GCF. Other proteins like fibrinogen, ceruloplasmin and beta-lipoprotein is also detected in GCF.11

Gingival crevicular fluid also contains many classes of phospholipids as well as neutral lipids.16 8-Isoprostan, a lipid peroxidation product, its level in GCF will increase, as the disease process progresses from healthy condition to gingivitis and chronic periodontitis.11

Metabolic and bacterial products

Lactic acid, urea, hydroxy proline, endotoxins, prostaglandins and cytotoxic substances like hydrogen sulphide are detected in GCF.11

GCF as a diagnostic biomarker of periodontal disease:

The potential biomarkers in the GCF have been grouped into three general categories.12

1. Host-derived enzymes
2. Inflammatory mediators and products
3. Tissue-breakdown products.

1. Host-derived enzymes in GCF

Aspartate aminotransferase (AST)

AST is a cytoplasmic enzyme and it mainly distributed in heart, liver and skeletal muscle. The extracellular release of AST is associated with cell damage and cell death. The level of AST elevated at sites with active periodontitis.12

Alkaline phosphatase (ALP)

ALP is a membrane-bound glycoprotein produced by different type of cells like leukocytes, osteoblasts, macrophages, and fibroblasts present in the periodontium and gingival crevice. Bacteria present in the sulcus or pocket also can produce ALP and contribute to ALP levels in GCF. Its level is increased in patients with gingivitis and periodontitis.13

Beta-glucuronidase

Beta-glucuronidase is a lysosomal enzyme produced by macrophages, fibroblasts and endothelial cells of healthy or chronically inflamed gingiva.18 Its level is increased in individuals with adult periodontitis by six-fold compared with healthy individuals.12

Neutrophil elastase

Neutrophil elastase (NE) is a serine proteinase confined to the azurophilic granules of polymorph neutrophils. It acts upon elastin, proteoglycans, hemoglobin, fibrinogen and collagen.18 Its assessment in GCF provides an indication of the intracrevicular PMN activity and alveolar bone loss.12

Cathepsin B

It is an enzyme belonging to the class of cysteine proteinases mainly produced by macrophages. Its level is elevated in patients with periodontal disease and the level increases with the progression of periodontal disease. It is used in distinguishing periodontitis from gingivitis and in planning treatment and monitoring treatment outcomes.14

Trypsin-like enzymes

Porphyromonas gingivalis is a key stone pathogen associated with periodontal lesions in adults. The presence of this enzyme increases the potential of this organism to cause the destruction of periodontal tissues.12

Matrix metalloproteinases (MMPs)

MMP- 8 and MMP-9 are the main collagen-degrading enzymes in GCF and saliva, and they cause collagen degradation in inflamed tissue during gingivitis and periodontitis. Therefore, these enzymes act as indicators for periodontal inflammation.15

TIMPs

MMPs are counteracted by tissue inhibitor of matrix metalloproteinases (TIMPs) and there by restrict the extracellular matrix breakdown. The balance between MMPs and TIMPs play an important role in maintaining the integrity of periodontal tissues. In healthy periodontal tissues, TIMP levels are generally higher than in inflamed periodontal tissue, while in periodontitis MMP level levels exceed TIMP levels.15

Leptin

Leptin is a polypeptide hormone which is involved in the host response and stimulates the immune system by enhancing pro inflammatory cytokine production and phagocytosis by macrophages. It has a protective role over the periodontal tissue and the progression of periodontal disease cause a substantial decrease of its concentration in GCF.15

Hepatocyte growth factor

Hepatocyte growth factor stimulate excessive proliferation of epithelial cells and also prevent the regeneration of connective tissue attachments. Thus, it plays an important role in periodontitis. Its level in GCF of periodontitis patients is found to be 10-fold higher than that of healthy subjects.16

Myeloperoxidase

Myeloperoxidase (MPO) is a constituent of the azurophilic granules of PMNs that oxidizes chloride ions to
hypochlorous acid and it is a potent bactericidal oxidant. During periodontitis, large number of neutrophils are recruited from blood vessels and therefore myeloperoxidase activity is also increased.  

**Lactate dehydrogenase**

Lactate dehydrogenase (LDH) is a ubiquitous enzyme present in the cytoplasm of the cell, which catalyzes the conversion of the pyruvate to lactate. LDH is released extracellularly only after cellular death. Thus, LDH represents a marker to cell death and tissue breakdown and its level in GCF raised during gingivitis and periodontitis.

**Aryl sulfatase**

Aryl sulfatase is a lysosomal enzyme involved in connective-tissue ground substance degradation. It is involved in the degradation of the proteoglycans through hydrolysis of sulphate esters. Its level is elevated in gingivitis and periodontitis patients.

**β-N-acetyl-hexosaminidase (β-NAH)**

It is an acid lysosomal hydrolase that is released into GCF during neutrophilic phagocytosis and cellular lysis. Its level is increased in periodontitis patients.

2. **Tissue breakdown products:**

**Glycosaminoglycans**

Glycosaminoglycans are the polysaccharides composed of uronic acid with hexosamine, and they are combined with various proteins in various organs and the connective tissue. Significant amounts of sulfated glycosaminoglycan (S-GAG) especially chondroitin sulfate with non-sulfated hyaluronan, detected in the GCF of sites with periodontitis.  

**Hydroxyproline**

It is a characteristic amino acid of collagen that allows the sharp twisting of the collagen helix. After degradation of collagen it appears in the GCF. Hence, it acts as a biomarker for periodontal destruction.

**Fibronectin fragments**

Fibronectin is an important component of the extracellular matrix (ECM) of periodontal tissue. It has a main role in cell attachment and proliferation, which explains its potential use in regenerative strategies. Therefore, the presence of fibronectin fragments in GCF indicate tissue destruction and their concentration is elevated in periodontal disease.

**Osteonectin**

Osteonectin is a non-collagenous calcium binding protein and mainly associated with the extracellular matrix of bone. It has a role in remodeling and repair and its level are elevated in GCF at sites with severe periodontitis.

**Osteocalcin (OC)**

Osteocalcin is a non-collagenous matrix protein, produced by osteoblasts and associated with bone formation. Its levels are elevated in GCF of patients with untreated periodontitis.

**Type I collagen peptides**

Collagen is synthesized in a pro-form containing a terminal propeptide. After cleavage, these peptides are removed through the gingival sulcus where they can be measured. These peptides are detected in the GCF of patients with periodontitis.

**Osteopontin**

It is an extracellular-matrix cell-adhesion protein, synthesized by preosteoblasts, osteoblasts, and osteoclastic cells. Its level in GCF increased with the progression of periodontal disease, and therefore it is considered as a marker of alveolar bone destruction.

**Laminin**

It is a 900-kDa glycoprotein seen in all basement membranes. Periodontal disease causes extensive destruction of the basement membrane. Therefore, higher amounts of laminin is detected in GCF from patients with periodontitis.

**Calprotectin**

It acts as a proinflammatory protein for neutrophil recruitment and activation. Kido *et al.* identified calprotectin in GCF and found that GCF concentration levels in patients with periodontitis were higher than those in GCF from healthy subjects.

**Hemoglobin β-chain peptides**

Decapeptide and dodecapeptide are the two derivatives of hemoglobin. They act as inflammatory mediators and also as substrates of proline-specific peptidases. The levels of these peptides will decrease after successful periodontal therapy in GCF.

3. **Inflammatory mediators and products**

**Interleukins**

It is a potent bone resorbing cytokine and found in two active forms IL1α and IL1β. Once secreted, IL1 may activate lymphocytes, stimulate macrophage chemotaxis, prostaglandin production, and stimulate osteoclastic resorption of bone. IL1, IL6, and TNFα are found in significant concentrations in GCF from periodontally diseased sites.

**RANTES**

It is a member of a superfamily of pro-inflammatory cytokines and it is involved in the development of the gingival inflammatory response by mediating the recruitment and activation of leukocytes. Its level is increased in periodontitis.

**Prostaglandin E2**

Prostaglandins are synthesized by most mammalian cells. They produce vasodilatation, bone resorption and inhibition
of collagen synthesis. Its level is elevated in GCF collected from periodontally diseased sites.

**Leukotriene B4 (LTB4)**

It is a membrane derived lipid mediator formed from arachidonic acid via the 5-lipoxygenase enzymatic pathway, possesses a variety of biological actions during inflammatory response. Increased levels of LTB4 are noted in GCF of periodontally diseased sites.

**Substance P (SP)**

Substance P is localized in sensory nerves that innervate blood vessels. Its level in GCF is correlated with the degree of periodontal inflammation.

**Monocyte chemo-attractant protein (MCP)**

MCP-1 acts as a potent mediator for monocyte recruitment and activation. It is expressed by different type of cells like monocytes, endothelial cells, fibroblasts, and T-cells, primarily on the basal layer of epithelial tissues. The MCP activity in GCF increases with the progression of periodontitis.

**Commercially available chair side kits**

Commercially available chair side kits are summarized in Table 2.

<table>
<thead>
<tr>
<th>Test kits</th>
<th>Evaluation</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periocheck</td>
<td>Neutral proteins such as collagenases, elastases, and proteases.</td>
<td>Most rapid chairside diagnostic test</td>
<td>Not a specific test for PMNL, collagenase</td>
</tr>
<tr>
<td>Prognos-Stik</td>
<td>Serine proteinase elastase.</td>
<td>Indicate active disease sites.</td>
<td>More clinical trials are to be conducted to validate the relationship between elastase levels in GCF and periodontal disease activity</td>
</tr>
<tr>
<td>Perio-Gard</td>
<td>Aspartate amino transferase (AST).</td>
<td>Indicate active disease sites.</td>
<td>More clinical trials are to be conducted to validate the relationship between elastase levels in GCF and periodontal disease activity</td>
</tr>
<tr>
<td>Pocket Watch</td>
<td>Aspartate transaminase</td>
<td>Indicate active disease sites.</td>
<td>Complete procedure involving multiple steps</td>
</tr>
<tr>
<td>MMP Dip Stick Test</td>
<td>Matrix metalloproteinase-8 (MMP-8)</td>
<td>Can differentiate healthy gingiva and gingivitis sites from periodontitis sites.</td>
<td>Needs to be confirmed with existing methods of disease evaluation</td>
</tr>
<tr>
<td>Toxicity Prescreening Assay</td>
<td>Detect indirectly the presence of bacterial toxins and bacterial proteins</td>
<td>Need to be confirmed and benchmarked with existing methods of disease evaluation</td>
<td>Needs to be confirmed and benchmarked with existing methods of disease evaluation</td>
</tr>
</tbody>
</table>

**Table 2: Chair side diagnostic kit.**

**Conclusion**

GCF as a diagnostic and prognostic tool has been explored since the initial studies on GCF which aimed to demonstrate that the flow of gingival fluid was sufficiently indicative of the inflammatory state of the periodontal tissues. Research methods have evolved to enable the assessment of the transition phase between health and inflammation at the gingival level to disease progression. Thus GCF considered as vehicle for monitoring tissue and cell products and permits a degree of non-invasive entry to the periodontium.

**References**


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