Gingival Crevicular Fluid-An Eos of Biomarkers

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Abstract

GCF is a serum transudate or inflammatory exudate that is derived from the periodontal tissues and can be collected at the orifice or from within the gingival crevice. The potential diagnostic importance of gingival fluid was recognized more than 60 years ago and serious investigation of the dynamics of GCF production did not begin until the late 1950s with the reports of Brill and co-workers. The fluid component of GCF derives primarily from microvascular leakage. Additions to the fluid volume are made from the intercellular fluid and cellular cytoplasm. The gingival tissue is constantly subjected to mechanical and bacterial aggregation. Resistance to these actions is provided by saliva, sulcular fluid, epithelial surface keratinisation and initial stages of inflammation. The origin, composition and clinical significance of gingival crevicular fluid have significantly helped us in understanding the pathogenesis of periodontal disease.

The need to find a non-invasive test method in periodontitis drew attention to the sulcus fluid that is produced in small quantities even in a completely healthy periodontium, and its composition is near similar to that of blood plasma.

KEYWORDS: GCF, PGE2, AST, NE, ALT, PMN.

INTRODUCTION

Gingival crevicular fluid (GCF) is treated as a diagnostic marker for non invasive analysis of periodontitis, taking into account indicators and markers of connective tissue and bone destruction so it could be a useful indicator in determining the severity of gum disease. According to researchers, GCF protein level obtained from the sulcus with clinical symptoms of inflammation is much higher and has a concentration similar to the concentration of proteins in blood serum. Thus, the fluid produced with no clinical signs of gingival inflammation is a physiological infiltrative material. The volume of fluid coming out of the pocket increases together with raising vascular wall permeability caused by the action of inflammatory mediators. Its composition changes during the development of inflammation. The biochemical analysis of the fluid offers a non invasive means of assessing the host response in periodontal disease. Active phase of periodontal disease process can be measured or assessed by the constituents of gingival fluid. Bacterial enzymes, bacterial degradation products, connective tissue degradation products, host mediated enzymes, inflammatory mediators, extracellular matrix proteins either together or individually can be detected in higher levels in gingival crevicular fluid during active phase of periodontitis.
CLINICAL SIGNIFICANCE OF GCF AS A DIAGNOSTIC TOOL

Gingival crevicular fluid (GCF) is a serum transudate or inflammatory exudate that can be collected from the gingival crevice. The biochemical analysis of the fluid offers a noninvasive means of assessing the host response in periodontal disease. The potential diagnostic importance of gingival fluid was recognized more than 60 years ago. (6, 7)

The fluid component of GCF derives primarily from microvascular leakage. Additions to the fluid volume are made from the intercellular fluid and cellular cytoplasm. The constituents of GCF are derived from a number of sources, including serum, the connective tissue and epithelium through which GCF passes on its way to the crevice, and inflammatory cells and bacteria present in the tissues and crevice. The collection and analysis of GCF offers a noninvasive means of evaluating the host response in periodontal disease.

There are a number of distinct advantages and challenges in utilizing GCF constituents as a diagnostic test for periodontal disease:

1. Multiple samples can be collected from a patient. Since teeth are variably affected by periodontal disease, collection of samples can be targeted to specific teeth or areas of concern. If the entire mouth is being evaluated, a representative set of samples can be collected.

2. Sample collection is a non-invasive or minimally invasive procedure. The most commonly utilized sampling device is a precut methylcellulose filter strip which can be placed at the crevicular orifice or within the crevice. The crevicular tissues are not permanently altered by sampling and, while some temporary disruption of the subgingival plaque may occur and a transient microscopic alteration in the gingival vasculature may be observed.

3. The amount of "native" GCF present at a site is minimal, with an average of 0.5 ul of fluid collected from Periodontitis patients following a 30-second insertion of a filter strip into the crevice.

The rationale for developing a diagnostic test for periodontal disease based on analysis of one or more components of the host response in GCF derives from evidence that has accumulated during the past 30 years suggesting that various aspects of the host response participate in the pathological processes that result in loss of periodontium.

The acute inflammatory response, humoral immune response, and cellular immune response have also been implicated as contributing to loss of periodontal tissue.

Development of a diagnostic test for periodontal disease based on analysis of a host mediator in GCF begins with identification of a potentially important mediator. The greatest interest has been focused on PGE2, an arachidonic acid metabolite; β glucuronidase and neutrophil elastase, markers of lysosomal enzyme release from neutrophils; and aspartate aminotransferase, a cytoplasmic enzyme indicative of cellular necrosis. (8, 9, 10)

Prostaglandin E2

Prostaglandin E2 (PGE2) is formed as a result of the metabolism of arachidonic acid. The action of the enzyme cyclooxygenase upon arachidonic acid yields a series of fatty acids that participate in the inflammatory response. PGE2 has been shown to have many proinflammatory effects, including increased vasodilation, enhanced responsiveness of receptors to painful stimuli, release of collagenase by inflammatory cells, and activation of osteoclasts.

Offenbacher et al described measurement of PGE2 in GCF collected from humans using a sensitive radio-immunoassay (RIA). This study demonstrated differences between PGE2 concentration in GCF collected from Periodontitis patients compared to gingivitis patients. In addition, relatively small variation in the PGE2 concentration was observed when studying gingivitis patients, and considerable variability was seen in the PGE2 concentration in GCF from the Periodontitis patients. (11)

β-Glucuronidase
ß-glucuronidase (ßG) is a lysosomal enzyme involved in the degradation of the connective tissue ground substance. This acid glycohydrolase has been used as a marker for primary granule release from polymorphonuclear leukocytes (PMN).

ßG in GCF has been shown to be correlated with the number of PMN in the crevice. The basis for evaluating ßG as a diagnostic test for periodontal disease derives from a number of lines of evidence.(12)

1. PMN are the predominant leukocytes in the gingival crevice (< 90%) and are observed in the crevice during all stages of periodontal disease.

2. There is an historical association between abscess formation and suppuration and tissue destruction, and suppuration represents a large accumulation of neutrophils and bacteria that have lysed due to failure of the neutrophil influx to control the infection.

3. An exuberant PMN response is associated with tissue alterations observed in a number of non-dental diseases, including emphysema.

Harper et al. observed that total ßG activity in GCF was significantly correlated with the occurrence of subgingival periodontal pathogens associated with more severe periodontal disease. (13)

Neutrophil Elastase

The assessment of neutrophil elastase (NE) in GCF provides another marker of intracrevicular PMN activity. NE is a serine endopeptidase found in primary granules, and is a powerful proteolytic enzyme that can attack many substrates. The relationship of NE to the severity of periodontal disease has been studied during the past 10 years. The preliminary studies suggested that NE activity is higher in GCF from Periodontitis patients compared to gingivitis patients. Bergstrom et al reported that NE in GCF increases with the development of experimental gingivitis.

Palcanis and co-workers examined the relationship between total NE in GCF and disease progression and concluded increased chances of bone loss with significant levels of NE in GCF.(14,15)

Aspartate Aminotransferase

Aspartate aminotransferase (AST) is a cytoplasmic enzyme present in many body tissues with pronounced distribution in heart, liver, and skeletal muscle. The extracellular release of AST and other cytoplasmic enzymes is associated with cell damage and cell death. Chambers et al evaluated the changes in the AST level in GCF during the development of experimental Periodontitis in beagle dogs. They observed a pronounced period of attachment loss in the 3 weeks following ligation, and a peak in the AST concentration in GCF, 2 weeks following ligation. In a cross-sectional study of the relationship of AST in GCF to clinical parameters, a positive association of total AST in a 30-second GCF sample to disease severity was observed, but considerable variation was present in this relationship. Also, parameters of sampling of GCF for AST activity have been studied, and the results have suggested that a shorter sampling period (5 to 10 seconds) would be better than a longer sampling period (20 to 30 seconds) to discriminate between sites.(16)

Analysis of the data allows a number of conclusions to be drawn concerning the potential diagnostic significance of GCF:

1) The relationship of test results to the development of Periodontitis in patients with gingivitis;

2) The level of test accuracy needed to justify use of these tests;

3) The unit of observation (patient, site) that is being evaluated by the test. 4) The need for such tests as perceived.
GCF FLUID FLOW

Gingival crevice fluid flow is an important determinant in the ecology of the periodontal pocket or sulcus. It can be defined as the process of fluid moving into and out of the gingival crevice or pocket. It creates a flushing action and an isolation effect. In addition, it probably determines the growth level of subgingival microorganisms and is a potential marker for periodontal disease activity. Substances put into the periodontal pocket are rapidly washed out due to its flushing action. Intensive studies on GCF proved that even microorganisms were rapidly removed from the periodontal pocket due to its flushing action.

In fact, this has been the primary rationale for the use of intrapocket controlled drug delivery systems for the administration of medication to periodontal tissues. The second important characteristic associated with GCF flow is the isolation effect. Substances from outside environment cannot easily penetrate the periodontal pocket. It is now widely appreciated that GCF is formed as a blood ultrafiltrate but accumulates elements of the metabolism from both bacterial and host cells from the gingival crevice environment. Because of this characteristic of accumulation, GCF composition has become a logical focus of methods to diagnose disease in the periodontal tissues. The volume of a GCF sample is most commonly measured by placing a calibrated filter paper strip at the opening of the gingival crevice or periodontal pocket and allowing fluid to accumulate for a time period of 30 seconds. The volume can be measured by Periotron based on the dielectric change of the wetted filter paper. These strips cannot be used to collect volumes greater than 1 μl. (17)

According to GCF sampling protocol of a study done by Lamster et al., a filter paper strip was placed in the sulcus for 30 s and removed for volume determination - (V1). Thirty seconds were allowed to elapse and then second strip was introduced into the site for 30 seconds - V2. The actual flow is the collected volume divided by the collection interval (f1 = V2/33s). The actual resting volume, however, is the measured sample volume minus the amount of fluid that entered the sulcus during the sampling period (V2 = V1 - 30x f1). (43)

According to recent data, GCF flow measurements are closely associated to disease-related spectrum of values. Shallow sulci in healthy subjects have GCF flow rates of 3-8 μl/h. Pockets with intermediate periodontal disease have GCF flow rates of approximately 20 μl/h. GCF flow at sites with advanced periodontal disease is found to be around 137 μl/h. Such evaluations indicate that GCF flow could be used to evaluate treatment response. (16, 17)

CIRCADIAN PERIODICITY

Gingival crevicular fluid is defined as a specific serum originating biologic fluid found in periodontal microenvironment and can be harvested from the gingival sulcus of natural teeth. The flow of GCF is widely accepted as one of the indicators of periodontal health or disease status, as it has the capacity to reflect the cellular response within the periodontium created by both serum and gingival sulcus components. Such features are likely to make GCF a reliable tool for understanding the pathogenesis of periodontal diseases and also for developing susceptible and specific tests for definitive periodontal diagnosis. (18)

It has been well demonstrated that GCF volume is affected by an array of factors, including mechanical stimulation, smoking, sex hormones, periodontal therapy, drugs, and circadian periodicity. Smoking has been demonstrated to cause a transient but remarkable increase in GCF flow. (18)

During the periods of puberty, ovulation, and pregnancy, an increase in the production of sex steroid hormones and a subsequent increase in GCF flow was observed in women.

GCF production was shown to increase during the healing period after periodontal surgery. Circadian periodicity is an important factor with the potential to determine the volume or flow of GCF. Bissada et al. suggested that GCF actually exhibits a clear circadian periodicity. Bergmann and Deinzer in 2008 reported daytime variations of interleukin-1b levels in GCF. (19)

Further, Deinzer et al. in 2000 claimed that circadian periodicity was associated with the irritation from GCF sampling.
That is the reason that in extracrevicular sampling methods, the paper strip is placed at or over the entrance of the gingival sulcus to minimize the mechanical irritation that may alter the actual flow of the fluid. In a number of studies, inflammatory status of periodontal tissues was suggested as an important factor affecting GCF volume or flow.

Suppipat et al reported higher GCF flow and volume in the presence of gingival inflammation and suggested that the degree of gingival inflammation has more influence on GCF flow than probing depth.(19,20)

**COLLECTION OF GINGIVAL CREVICULAR FLUID (19,20,21)**

Gingival Crevicular Fluid can be collected in various ways, and its ultimate fate is the use of its diagnostic potential in the diagnosis of periodontal disease. GCF harbors a rich array of cellular and biochemical molecules that have association with disease activity. This has led to extensive research of GCF components that might serve as potential diagnostic and prognostic markers for determining progression of periodontitis.

Once the Gingival Crevicular Fluid has been collected, the biomarkers and disease indicators in the sample are identified, quantified and analysed using various techniques which vary with the component to be studied. These include Enzyme Linked Immunosorbent Assay (ELISA), two – dimensional Polyacrylamide Gel Electrophoresis, using chromogenic or fluorogenic substrates or immunoassays with mass spectrometry.

Over 65 GCF components have been preliminarily examined as possible markers for the progression of periodontitis. These components fall under five different categories:

1. Host Derived Enzymes and their Inhibitors
   a) Proteolytic enzymes:
   Matrix metalloproteinases (MMPs) e.g. Collagenase (MMP 1, 8,13), Elastase, Cathepsin B, Cathepsin G, Cathepsin D, Dipeptidyl peptidases, Tryptase b)Hydrolitic enzymes: Aryl sulfatase, β – glucuronidase, Alkaline phosphatase, Acid Phosphatase, Myeloperoxidase, Lysozyme, Lactoferrin

2. Microbiological Markers:
   Bacteria and their Products

3. Inflammatory Mediators and Host Response Modifiers:
   a) Immune Response: Antibody (Total Immunoglobulin and IgG subgroups, complement) b) Inflammatory Response: Arachidonic Acid derivatives e.g. Prostaglandin E, Cytokines e.g. Interleukin 1, Interleukin 2, Interleukin 4, Interleukin 6, Tumor Necrosis Factor α.

4. Tissue Breakdown Products:
   Fibronectin, Hydroxyproline, Collagen Cross link peptides, Terminal peptides, Glycosaminoglycans, Heparin sulphate, Chondroitin-4-sulphate, Chondroitin-6- sulphate. 5. Products of Bone Resorption:
   Osteonectin, Bone phosphoprotein, Osteocalcin and Telopeptides of type 1 collagen. In terms of application of diagnostic tests, sampling of crevice fluid provides advantages that are analogous to drawing of blood:

1. It is non invasive.
2. It is site specific for teeth.
3. Comparatively easy to obtain.
4. Offers one of the most accessible entries to any tissue in the body as means of assessing the disease state.

**METHODS OF COLLECTION OF GCF (20)**

Several techniques have been employed for the collection of GCF and the technique chosen will depend upon the objectives of the study as each technique has advantages and disadvantages. The techniques can be divided into three basic strategies, subject to various modifications in their application by different authors.

1. **CAPILLARY TUBING OR MICROPIPETTE**

In this technique the site is dried and isolated and capillary tubes of known internal diameter are inserted into the entrance of the gingival crevice. Gingival Crevicular Fluid from the crevice migrates into the tube by capillary action and because the internal diameter is known the volume of the fluid collected can be accurately determined by measuring the distance which the GCF has migrated.

The contents of the capillaries were collected by centrifuging at 3000 rpm for 5 minutes, and the plastic tube was weighed before and after the collection to determine the total amount of fluid. In a patient with an average PMA index of 3 and an average vestibular pocket depth of 5mm on the upper anterior teeth, 40 mg of fluid could be collected in 15 minutes.

The GCF was collected using 1–3 μL calibrated micro capillary pipettes and immediately transferred to aliquots and stored at -70°C; till the time of the assay. Hirschmann micropipette was placed extra sulcularly parallel to tooth surface and GCF was collected up to first marking of micropipette corresponding to 1μl of GCF volume.

Advantages and Disadvantages of capillary tubes and micropipettes:

**Advantages:**
1. Apparent ideal volume can be accurately assessed. 2. Undiluted samples can be collected.

**Disadvantages:**
1. It is difficult to collect an adequate volume of GCF in a short period unless the site is inflamed and contains large volume of GCF. To collect reasonable amount collection time exceed 30 min. 2. An adequate sample from healthy crevice may be impossible to obtain.
3. Holding the capillary at the entrance for such a long time makes it a traumatic technique.
4. It is difficult to move the entire sample from the tubing. 3 methods are generally employed: -Forcing out GCF in a jet of air.

- Forcing out GCF by pumping larger volume of diluting solution through the capillary.

- By centrifugation of the tube

2. **GINGIVAL WASHING METHOD:**

The method of washing of crevicular contents is said to be very useful because it enables the collection of both cellular and humoral components of the gingival crevice. In this technique the gingival crevice is perfused with an isotonic solution, such as Hanks’ balanced salt solution, usually of fixed volume.

There are two techniques of gingival washing.
a) Syringe Method: It is said to be the simpler of the two techniques. The method was first demonstrated in 1976 by Skapski H and Lehner T. Crevicular washings were carried out within 3-4 hours of eating and oral hygiene measures. Within 10 minutes of collection of the crevicular washings, the viability of leucocytes was determined in the paired serial specimens by the Trypan blue dye exclusion test in a Neubauer chamber.

b) Acrylic Stents: The second method is more complicated and involves use of acrylic stents which isolate the gingival tissues from the rest of the mouth. The tissues are then irrigated for 15 minutes, with a saline solution, using a peristaltic pump, and the diluted GCF is removed. Advantages and disadvantages of Gingival Washing

Advantages:

1. The syringe method is simple and mechanically less demanding.

2. Valuable for harvesting cells from the gingival crevice region.

Disadvantages:

1. Production of customized acrylic stents is complicated and technically demanding.

2. The second technique can be applied only to maxillary arch due to it being technically challenging to make stents for mandibular arch.

3. GCF from individual sites cannot be analysed.

4. All fluid may not be recovered during the aspiration and re-aspiration procedure.

5. Accurate composition of GCF cannot be determined.

6. Accurate identification of GCF volume is not possible.

3. ABSORBENT FILTER PAPER STRIPS: It is quick and easy to use, can be applied to individual sites and is possibly the least traumatic one when correctly used. The method of collection may be divided into intracrevicular and extracrevicular techniques. Extracrevicular technique involves the strips being overlaid on the gingival crevice; this technique is used to minimize trauma. The strip is placed just at the entrance or over the entrance. Intracrevicular technique depends on the strip being inserted into the gingival crevice and is the most frequently used method.

The form of the GCF collected can be classified into two types:

1. Gingival Crevicular Fluid at rest (i.e. That present inside the sulcus or pocket)

2. Gingival Crevicular Fluid flow (i.e. That newly formed after the collection of the resting gcf ) Advantages of Filter Paper Strips

1) Quick technique

2) Easy to use

3) Can be applied to individual sites

4) Least traumatic of all techniques.
The amount of GCF collected on a strip was assessed by the distance the fluid had migrated up the strip. A more accurate value was achieved by staining the strips with ninhydrin to produce a purple color in the area where GCF had accumulated. When 2g fluorescein was given systemically to each patient 2 hours prior to the collection of GCF, following which the strips were examined under ultraviolet light. Although the techniques were used to determine presence or absence of fluid, rather than to quantify the volume collected, it was found that fluorescein labelling was 100 times more sensitive than ninhydrin for staining protein.

Disadvantages of the staining techniques:

a) They are not easily applied at the chair side. The inevitable delay in measuring the strip may result in increased variation in the reported volume as a result of evaporation.

b) The staining of the strips for protein labelling prevents further laboratory investigations of the components of GCF, effectively limiting the technique to that of volume determination.

2. Weighing Of Strips: An alternative approach involves the weighing of strips before and after sample collection and it has been adopted by some workers. This has been successful but requires a very sensitive balance to estimate the very small amounts of fluid which may be collected from a healthy crevice. Moreover like the other methods, evaporative losses because of delays in determining the volume may distort the volumes obtained.

3. PERIOTRON:

Periotron is an electronic instrument that measures the affect on the electrical current flow of the wetted paper strips. The introduction of the Periotron, has allowed accurate determination of the GCF volume and subsequent laboratory investigation of the sample composition. The PERIOTRON has two metal ‘jaws’ which act as the plates of an electrical condenser. If a dry strip is placed between the jaws, the capacitance is translated via the electrical circuitry and registers ‘zero’ on the digital readout. A wet strip will increase the capacitance in proportion to the volume of the fluid and this can be measured as an increased value in the read out. Advantages of the Periotron:

1) The technique is rapid.

2) Has no discernible effect upon the GCF sample.

3) An electronic measuring device, which allows accuracy in measurement. Three models of Periotron have been produced, the 600, 6000 and now the 8000. Each one has been shown to be an efficient means of measuring the volume of fluid collected on the filter paper strips. PERIOTRON 6000: It is a fluid analyser that is widely employed in the analysis of the gingival crevicular fluid (GCF) volume and its use has also been advocated in the quantification of tear drop volume to determine the secretion rates of minor salivary glands and more recently to measure dentine wetness and permeability. It is an improvement of the earlier model of the Periotron 600 which was invented by Kleinberg.

PERIOTRON 8000: The Periotron 6000 has now been super ceded by the Periotron 8000, a machine that has bi-modal function such that it may be converted from a sialometer to a periotron, or vice versa, by a flick of the switch at the back of the device. Several other refinements include an apparently wider dynamic range of volume measurement and software to interface the machine with a PC for automatic data recording and output. A more sophisticated calibration of the Periotron 8000 has been made possible due to the introduction of a software program, which accompanies the device called “MLCONVERT”. The program will automatically convert inputted data to volumes (μl).

The Periotron 8000 appears to measure volumes on Periopaper Strips up to 1.2 μl. Periotron 8000 measures the electrical capacitance of a wet filter paper strip placed between the jaws of the instrument. The electric field created by opposing charges on the jaws induces polarity of the molecules which reduces the potential difference between the plates and increases the capacitance. Thus the higher the number of polar molecules between the jaws of the Periotron, the larger the capacitance and higher the Periotron score.

CONCLUSION

Periodontitis is characterized by the destruction of connective tissue, loss of periodontal attachment and resorption of alveolar bone. The tissue destruction in periodontal disease appears as a result from the interplay between the pathogenic bacteria and the host’s immune and inflammatory responses. The immune system is activated in order to protect against local microbial attack and their damaging products from spreading or invading the gingival tissues. Diagnosis of the
diseases affecting the periodontium and assessing its outcomes are based on clinical signs such as tissue colour, presence or absence of bleeding on probing, gingival recession, pocket depths, attachment levels, suppuration and tooth mobility. Radiographs are used as an additional diagnostic tool to visualize the loss of periodontal tissue.

However, these methods are only useful to assess the past disease activity. Reliable diagnostic methods are essential to assess the active disease status and for monitoring the response to periodontal therapy.

GCF, an exudate, harnessed from the sulcus or periodontal pocket, has been regarded as a promising medium for the detection of periodontal disease activity. The composition of this fluid resembles that of serum, and the intensity of its flow has been shown to vary as a function of gingival inflammation. Based on this review of literature,

It can be concluded that various constituents of GCF—such as cytokines, matrix metalloproteinases, PGE2, aspartate aminotransferase, neutrophil elastase, alkaline phosphatase, osteocalcin, calprotectin, alpha-2 macroglobulins, beta-2 microglobulins, beta-2 Macroglobulins, and beta-2 Macroglobulins can be used as effective and efficient diagnostic tools for diagnosis and prognosis of periodontal diseases. The analysis of these components of GCF can reflect the disease status of individual sites and thus, identify potential biomarkers of periodontal disease status and its application in prognostic significance and response to therapy thereby proving to be a valuable tool to combat periodontal disease.

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