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Clinical Significance Of Gcf In Periodontics

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Abstract
Gingival tissue is constantly subjected to mechanical and bacterial aggression. Gingival crevicular fluid (GCF) is an inflammatory exudate derived from the leaky venules adjacent to the sulcus and junctional epithelium. It is also called as sulcular fluid which passes from the blood stream through the tissues, and exiting into the gingival sulcus. Its composition and possible role in host defense mechanism were elucidated by Waerhaug et al. GCF is a complex mixture of substances derived from serum, leukocytes, structural cells of the periodontium, and oral bacteria. These substances posses a great potential to serve as indicators of periodontal disease and healing after therapy. In short, GCF acts as a window to the condition of the periodontium. This article addresses the role of GCF in and the important clinical changes that occur in periodontal diagnosis.

Key Words
Gingival crevicular fluid, biomarker, treatment planning

Introduction

Periodontitis is a chronic disease characterized by gingival inflammation, alveolar resorption, and destruction of periodontal ligament.[1] The goal of periodontal diagnostic procedures is to supply useful information on the type, location, and severity of the disease.[1] However, current diagnostic parameters only reveal the damage caused by the previous destructive episodes and not the present disease status.[1],[3] Enzymes and proteins that are released in health and disease differ significantly in their amount and type and their analysis can provide valuable information about tissues.[2],[5] Gingival crevicular fluid (GCF) is an important tool that makes such analysis possible.[6]

Gingival Crevicular Fluid

GCF is an inflammatory exudate that plays a major role in antimicrobial defence of the periodontium.[7] Formation of GCF depends upon the permeability of the junctional epithelium and sulcular epithelium. An important characteristic of the flow of GCF is its flushing action, which washes away bacteria, bacterial products, and foreign bodies.[6] GCF production is influenced by gingival stimulation, tooth brushing, bacterial insult, inflammation, IV injection of histamines. In inflammation, GCF flow rate increases and washes away the microbes and their metabolites from the gingival crevice and thereby controls their penetration into the tissues, and also facilitates the passage of more immunoglobulins into the sulcus and thereby enhances host defense.[8],[6] The flow might increase about 30-fold in periodontitis as compared to healthy sulcus and quantitative measurement of GCF may better indicate early inflammation as compared to subjective measures like colour change, bleeding, and gingival Index.[7],[6],[9] The negative side of increase in GCF flow is that, nitrogen, carbon, growth factors, vitamins, and minerals present in the GCF serve as a nutritional source for the microbes in the periodontal pocket.[10],[11]

GCF also plays a significant role in defense by transporting antibacterial substances of host origin & by introducing systemic antibiotics to the gingival crevice.[6],[12] GCF presents an efficient means to sample biomarkers of disease and provides a unique window to analyse the current condition of the periodontium.[7],[1](biomarker is a substance that is objectively measured that indicates the normal biologic processes, pathogenic processes, or responses to therapeutic intervention.[13],[1],[14]

In 1965, Löe et al started to explore the use of GCF to indicate periodontal diseases.[7] In 1971, Sueda, Bang and Cimasoni researched the proteins and enzymes present in the GCF and soon it was understood that the enzymes released from the damaged periodontal tissues had an enormous potential for periodontal diagnosis.[7]

Mechanism of GCF production

Alteration in microcirculation
Causing momentary blood vessel contraction
Followed by arteriolar vasodilatation
Increased vascular permeability
Seepage of fluids in sulcus
Exudate
Formation of GCF

Contents of GCF

The constituents of GCF are derived from serum, epithelium, connective tissue, host derived enzymes, electrolytes, proteins, tissue breakdown products, desquamated cells, inflammatory cells, inflammatory mediators, immune components, antibodies, bacteria and their metabolites.

Bacterial products in GCF

Bacterial products include endotoxin, collagenase, amines, butyrate, trypsin-like proteases. It also contains specific antibodies, organic compounds, lipids, drugs, etc.

Lipopolysaccharides (endotoxin) are molecules that are found in the outer membrane of the cell wall of gram negative bacteria. It is highly toxic to gingival tissues and is a potent stimulator of bone resorption. It also induces

production of collagenase and plasminogen activator and their levels positively correlate with gingival inflammation. Thus it is an important diagnostic factor in the GCF.

Trypsin-like enzymes are virulence factors produced by certain microbes that destroy the periodontium and their levels in the GCF provide useful information on the condition of the periodontium.[15]

Host Derived Enzymes

Host enzymes include collagenase, elastase, lactate dehydrogenase (LDH), acid phosphatase, aspartate aminotransferase(AST), alkaline phosphatase(ALP), lactoferrin, lysozyme, prostaglandins, β-glucuronidase, serum proteinase inhibitors, cycloperoxidase, pyrophosphatase, hyaluronidase.[4]

Neutrophil elastase(NE) is a neutral serine proteinase released by the neutrophils. It cleaves elastin and many other components of the gingival tissue and it has a positive relationship with inflammation and attachment loss.[4],[2] NE is the only enzyme to increase in gingivitis and a longitudinal study by Cox SW et al has shown that NE may predict future attachment loss.[4] Collagenase, another enzyme derived from neutrophils is also correlated with gingival inflammation.[7]

Cathepsin isa cysteine proteinase chiefly produced by macrophages. Cathepsin G converts inactive angiotensin-I to biologically active angiotensin-II and thereby regulates vascular permeability and monocyte chemotaxis. Cathepsin G and elastase and are capable or activating epithelial cells to produce IL-8, IL-6, PgE2, which increases chemotaxis and tissue degradation. Cathepsin levels are elevated in GCF in periodontitis but lower in gingivitis.[15]

Intracytoplasmic enzymes like lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) are highly active biochemical markers.[4] Lactate dehydrogenase levels in GCF change at mild, moderate, and severe periodontitis and patients with increased probing depths show higher activity of LDH. Aspartate aminotransferase is a cytoplasmic enzyme which is released on cell death and its levels become higher in severe periodontitis.[4],[15]

β-glucuronidase is a hydrolysing enzyme produced by PMNs, macrophages, endothelial cells, and fibroblasts. Elevated levels of β-glucoronidase in GCF is associated with an increased risk for probing attachment loss and it is found to be increased six fold in periodontal disease.[15]

Myeloperoxidase(MPO) is an enzyme found in the primary granules of PMN. MPO levels in GCF increase propotional to the presence of plaque & severity of periodontitis and it has a good correlation with inflammatory response.[16]

Arylsulfatase activity in GCF is higher in gingivitis and periodontitis and their levels decrease following treatment.[15] β-N-acetyl-hexosaminidase (β-NAH) is an acid lysosomal hydrolase that is released during neutrophil phagocytosis and its levels are increased in periodontitis.[15]

Alkaline phosphatase (ALP) is a membrane-bound glycoprotein that is produced by polymorphonuclear leukocytes (PMNLs), macrophages, fibroblasts, osteoblasts, and gram-negative anaerobes.[1] It is involved in inflammation and also in regeneration and is a phenotypic marker for osteoblasts and is an indicator of bone formation.[11] However, the presence of ALP in the GCF usually indicates inflammation and/or destruction of periodontal tissues and it is an indicator of future periodontal breakdown.[1] ALP levels in GCF are detectable before the increase in gingival indices and hence it is a better marker of gingival inflammation.[1] Increased ALP activity in GCF has predictive value in terms of loss of attachment which is more accurate than clinical parameters and their levels are 20 times greater in diseased sites.[15],[1] Moreover, there is sustained, statistically significant decrease in ALP values after treatment.[2] Thus, the ALP levels precisely depict progression or regression of the disease and hence used in treatment planning and monitoring.[2]

Matrix metalloproteinases (MMP) are zinc and calcium-dependent endopeptidases (neutral proteinase) derived mainly from PMN and fibroblasts and they play an important role in initiation and progression of periodontal destruction.[17],[18] GCF levels of MMP-1 (collagen I), MMP-2 (gelatinase-2) and MMP3 (stromlysin). MMP8 and MMP-9 are higher in periodontitis patients.[17] Neutrophils are the major cells that produce MMP-8 & MMP-9. MMP-8, otherwise known as collegenase-2 potentially degrades interstitial collagen and rapid chair side test on detection of elevated MMP-8 in GCF helps in diagnosis and monitor the course and treatment of periodontitis. MMP-9, also known as gelatinase B degrades extracellular matrix proteins. MMP-9 too serves as a guide in periodontal treatment monitoring. In 1992, Tere showed a world ofincrease in mean MMP-9 levels in patients with recurrent attachment loss. MMP-13 is collagenase-3 that is expressed during bone formation and gingival wound healing. MMP-13 may be useful in monitoring periodontal attachment loss and in tracking the efficacy of therapy. MMP-2 is secreted by fibroblasts and its levels are lower in gingivitis and periodontitis compared to health.[17] Detection method for GCF MMPs include immunoblot, Elisa and Time involved immunofluorescence technique.

Non-specific neutral proteases are non-specific metalloproteaseswhich cleave fibronectin and collagen and their elevated level shows an active phase of periodontitis.[15]

Dipeptidyl Peptidases (DPP) are derived from macrophages, lymphocytes, and fibroblasts. They activate proforms of enzymes and cytokines and also degrade collagen and their levels are higher in GCF in diseased sites.[15]

Extracellular matrix components in GCF

Tissue destruction products of GCF include fibronectin(FN), osteonectin, hydroxyproline, glycosaminoglycans, collagen peptides, bone-specific markers like osteocalcin and collagen telopeptide fragments.[7] Collagen is the most important structural protein of the periodontium and the presence of greater amounts of collagenous products in the GCF represent a measure of periodontal destruction.[19] Collagen levels in GCF are usually measured by hydroxyproline assay.[19] FN is a component of the extracellular matrix (ECM) and its presence in GCF indicates tissue destruction.[15] Elastin and proteoglycan levels are also increased in periodontitis.

Laminin is a glycoprotein which is found in the basement membrane.[15] During inflammation, activated neutrophils cause an extensive destruction of basement membrane and higher amounts of laminin is released into the GCF.(35) Thus presence of laminin in GCF suggests the presence of hyperactive neutrophils.[15]

When periodontal tissues are degraded, glycosaminoglycans (GAG) are released

and considerable amount of sufated-GAGs are found in GCF in advanced periodontal disease.[15] Chondroitin-4-sulfate is the major GAG in periodontium. It is a potential marker for bone breakdownand its levels are increased in periodontitis.[15] Chondroitin-6 sulfate levels are also increased in periodontitis. Overall, the presence of increased levels of GAG reflects active destruction of alveolar bone and early preclinical changes in the periodontium can be analysed by the presence of GAG in GCF.

Calprotectin is a protein produced by PMNs, macrophages, and epithelial cells.[15] It has antimicrobial and antifungal properties and it is a proinflammatory protein for neutrophil recruitment and activation. It also plays a role in immunoregulation and its levels in GCF are higher in periodontitis.[15]

Osteocalcin is a non collagenous protein of bone chiefly synthesized by osteoblasts. It plays a role both in bone mineralization and resorption. It is chemoattractant to osteoclasts progenitors and monocytes and their levels in GCF are correlated significantly with pocket depths and gingival index scores.[14],[15] Also, Nakashimaet al has reported that osteocalcin levels significantly correlated with GCF levels of ALP.

Osteopontin is produced both by osteoblasts and osteoclasts and it functions in anchoring the osteoclasts onto the bone surface. It exhibits chemoattractive activity to osteoblast progenitor cells and monocytes. With progression of periodontal disease, GCF osteopontin concentration increased and their levels significantly reduced after nonsurgical therapy.[15],[20] Hence, it may be considered as a marker of progression of periodontitis.[20]

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is the carboxyterminal telopeptide of type I collagen and it is released subsequent to osteoclastic resorption and collagen degradation.[15] These cross-linked telopeptides are produced as a result of post-translational modification of collagen and they could not be reused for collagen synthesis.[21] Their levels are increased in active bone destruction and they are good predictors of future bone loss and attachment loss.[15],[21].

Biochemical mediators and products of inflammation

In response to chemoattractant effects of bacteria, macrophages/neutrophils migrate to the sulcus, get activated and produce important mediators like TNF-alpha, IL-1, IL-6, which are related to host response and tissue destruction. Levels of IL-1alpha, IL-1beta, and IL-1Ra were significantly higher in GCF from diseased site compared to healthy sites.

Large proportion of B lymphocytes and plasma cells were present in advanced stages of periodontal disease. Interferon-inducible protein IP-10 and IL-1β are found to be much higher in sites that have BOP with IL-1β levels directly propotional to BOP, probing depth, and presence of P. gingivalis in the subgingival plaque.[22] Also, there is increase in levels of IL-1 alpha, IL-2, IL-6, IL-8, TNF alpha, interferon-gamma in periodontal disease. Interleukin-23 plays a role in the initiation and progression of periodontitis and its concentration in the GCF increases directly proportional to the periodontal damage.[23] RANTES is a proinflammatory cytokine present in GCF in periodontitis, which is undetectable in health.[24]

Other immune components in the GCF include complement, immunoglobulins, cytokines, eicosanoids, acute phase proteins, colony stimulating factor, leukotrienes, transferrin.[25],[19] At least 40 different cytokines and chemokines are present in the GCF.[22]

Complements in the GCF kill microbes directly or by teaming up with antibodies or by signaling the PMNs to migrate to the gingival sulcus and phagocytose the microbes. C3 and C4 are found in GCF, with C3 at higher levels in chronic periodontitis. When osteoclast like cells are stimulated by C5a, significant amounts of bone resorbing IL-6 are released, suggesting a potential tissue destructive contributor fromthis type of host response.

IgG predominate in GCF in advanced periodontal lesions and the levels of IgG1 and IgG4 are significantly elevated in the active sites relative to serum.[26],[25] Particularly, IgG4 was found to be almost 25 times that of serum level.[27],[25]. IgA levels were significantly higher in GCF of gingivitis sites compared that of periodontitis sites, which suggests that IgA can have a locally protective function.[25],[28]

Prostaglandin E2 (PgE2) is an arachidonic acid metabolite and the primary cells that produce PgE2 in the periodontium are macrophages and fibroblasts.[24] PgE2 causes bone resorption and inhibits collagen synthesis and is present in elevated levels in active phases of periodontitis.[24] Leukotriene B4 (LTB4) is another arachidonic acid metabolite produced by PMNs, macrophages, eosinophils.[24] LTB4 contributes to inflammation and bone destruction and their levels are increased in disease.[24]

The levels of the acute phase proteins C-reactive protein (CRP), lactoferrin, and transferrin increase in periodontitis.[24] Rapid and dramatic rise in blood levels of CRP is produced by inflammatory cells and its levels rise dramatically in inflammation in response to stimulation by IL-1. CRP binds to phosphocholine expressed on dead or dying cells and some bacteria and activates the complement system.[24] Lactoferrin (Lf) is an iron-bindingglycoprotein which is antibacterial against Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Prevotella intermedia.[36] Lactoferrin levels are found to be increased two fold in the GCF in chronic and localised aggressive periodontitis and their levels are useful to assess of periodontal inflammation. Transferrin- is iron binding glycoprotein.[24] In the GCF, it limits the amount of iron and thus functions as an antibacterial agent.[24] Its levels are increased in both gingivitis and periodontitis and hence it does not distinguish between gingivitis and periodontitis.

α2- macroglobulin is a protease inhibitor produced by the tissues and its concentration in the GCF increases in inflammation.[15] TIMPs arelocally produced enzymes which defends the connective tissues against degradation by metalloproteinases and their levels are significantly greater in periodontitis.[15]

Lysozyme levels in GCF are found to be increased in patients with aggressive periodontitis and experimental gingivitis

Proteins in GCF include histones, keratins, beta 2-microglobulin, histones, lipoproteins, fibrinogen, and albumin, ALB protein, apolipoprotein A-I, etc. Proteins like annexin A3, myosin 9, L-plastin (plastin-2/LCP1), profilin-1, S100A8, S100A9, S100-P, cystatin-B , azurocidin, actin, myosin, vitamin D-binding protein, serotransferrin were present at higher levels in periodontitis

cases compared to healthy individuals. Protein Annexin-1 is at 5-fold greater concentration in GCF in healthy individuals compared to the diseased and the level of Cornifin-A is downregulated in periodontitis.[29] L-plastin (plastin-2/LCP1) and dermcidin are detected in the GCF only in periodontitis.[29] Azurocidin is a cationic antimicrobial protein that prevents alveolar bone loss by inhibiting the differentiation of macrophages to osteoclasts. It also exhibits antimicrobial activity against Gram-positive & Gram-negative bacteria, and fungi and its levels are highly elevated in the GCF in periodontitis.[29]

The levels of electrolytes sodium, potassium, and calcium in the GCF were higher in sites with gingivitis and periodontitis than their corresponding levels in serum.[30] Sodium is present in large amounts in the bone and with its destruction, more of sodium is present in the GCF.[30] The increased amount of potassium in periodontitis is probably due to its release by the disrupted epithelial cells, connective tissue cells, and blood cells in the periodontal pocket.[30]

Total oxidant status (TOS), receptor activator of nuclear factor-κB ligand (RANKL), and RANKL/OPG values are increased in the GCF in periodontitis, with the increase more evident in aggressive periodontitis than chronic periodontitis.[31]

Desmosomes establish and maintain the cell-cell contact.[36] In periodontitis, autoantibodies against desmosomes may be produced, which inhibit cell-cell contact and cause apical migration of the junctional epithelium.[24] Increased titers of anti-desmosomal antibodies in the GCF distinguish periodontitis from the unaffected sites.[24]

Melatonin is a hormone which has powerful antioxidant properties and is believed to play a protective role against periodontal disease.[32] GCF melatonin levels drop to the lowest concentrations in periodontitis compared to health.[32]

CD14 is a pattern recognition receptor and a key component of the innate immune system that is expressed on monocytes, macrophages, neutrophils, and fibroblasts.[33] Higher levels of mCD14 is found in clinically healthy tissues and low levels of sCD14 are associated with greater probing depths.[33] This implies that sCD14 might play a protective role against disease.[33]

Soluble cell adhesion molecules

Cell adhesion molecules (CAM) are cell surface proteins that facilitate cells to bind to each other, to endothelial cells or to the extracellular matrix. The soluble forms of CAMs are produced by proteolytic cleavage from the cell surface and are shed into the GCF. The CAM in GCF are s1CAM-1, sVCAM-1, and sE selectin. These potential markers may offer greater diagnostic sensitivity and specificity.

GCF as tool to diagnose systemic diseases

GCF can be analysed to determine the presence of specific markers for systemic diseases.

Diabetes is one of the most frequent metabolic disorder of which nearly half the cases are undiagnosed. As metabolic control of diabetes mellitus decreases, levels of beta glucoronidase increase. Beta glucoronidase is a periodontal disease activity marker and this reveals that diabetics with poor metabolic control are at a high risk for periodontitis. At the same time, no relationship was found between the amount of LDH and diabetic control. PgE2 and IL-1 beta levels are higher in insulin dependent cases, while IL-6 levels are higher in noninsulin dependent cases. Also, VEGF levels are found to be increased in periodontally inflamed tissue with diabetes mellitus.

The relationship of CVS/CNS & periodontal disease is of special interest. Leukotriene levels are found to be increased in periodontitis. The increased levels of cysteinyl leukotriens in GCF may be an important inflammatory marker for increase in risk of atherosclerosis associated periodontal disease.

Oxidative modification of low density lipoproteins (LDL) occur in diseased tissues and local inflammation sites.[34] The ratio of anti-oxidized low density lipoprotien (ox LDL) to LDL in GCF is found to be significantly greater in chronic periodontitis as compared to health.[34] A spike in ox LDL levels in systemically healthy patients may increase the patient's risk to develop atherosclerosis.[34]

GCF may be a significant source of hepatitis virus in saliva. Anti HCV antibodies are detected in GCF of HCV positive patients. Greater levels of HCV rna are present in GCF in hepatitis patient and hence analysis of GCF concentration of HCV rna reveal a better detection rate.

Increased levels of IL-1 beta, IL-6, TNF-alpha, interferon-gamma in GCF are found to be associated with periodontal disease in HIV-1 infected patients.

GCF can also be used to screen for the presence of hepatopathy. In hepatopathy, alanine aminotransferase (ALT) levels are increased in the GCF.

Smokers demonstrate higher levels of IL-6 &IL-8 but low levels of IL-4 & IL-1alpha. FGF-beta1 levels in GCF are greater than nonsmokers.

GCF and periimplantitis

As per Boutrous et al (1996), neutrophil protein levels are higher at moderate to severely inflamed implant sites. In osseointegrated implants, there is less activity of elastase and collagenase in GCF. But in failing implants, neutrophil elastase, myeloperoxidase, and beta glucoronidase levels are significantly higher.

Longitudinal studies have been conducted to evaluate the effects scaling and root planing (SRP) on GCF components and it was found that generally there was a decline of markers for progressive periodontitis after SRP.[35],[36] However, the markers stayed at an elevated level at sites unresponsive to nonsurgical therapy.[35],[37] This enables the clinician know if a particular site is at a risk of disease progression and thereby plan treatment strategy accordingly.[35]

Limitations

1. Multiple GCF samples are needed.
2. Selection of teeth and site to check for disease progression is difficult.
3. Lab diagnostic tests for multiple patient samples are difficult to adopt.

Conclusion

GCF is an inflammatory exudate that has wide clinical significance in assessing the severity of periodontal disease and effectiveness of periodontal therapy. It is an easier and noninvasive collection medium to assess changes in periodontal tissues and is one of the most reliable "predictor of periodontal health".

Researches in medicine and biotechnology are continuously investigating the use of oral fluids like saliva and GCF to diagnose oral and systemic diseases and to determine their response to therapy.[21] GCF and saliva are also under research to be used to detect toxins and to screen for metabolites of

drugs of abuse.[21] In the field of oral diagnosis, substantial improvements have been made in the detection of biomarkers in GCF and saliva with latest diagnostic techniques like protein and nucleic acid microarrays and microfluidics.[21],[38] GCF is closer to periodontal tissues compared to saliva, and hence it can provide more information about the periodontal condition than markers in saliva.[38] Also, the molecules in saliva might reflect the metabolic status and diseases of salivary glands instead of the periodontal condition.[38] As no single biomarker will fulfil all the criteria necessary for assessment of health of the periodontium, simultaneous screening of multiple biomarkers in the GCF may prove a valid clinical diagnostic tool.[21]

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