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GCF As A Diagnostic Biomarker Of Periodontal Diseases- A Review Article.

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Abstract:
The existence of Gingival crevicular fluid (GCF), which emerges between the surface of the tooth and the epithelial integument, has been recognized for over 100years. Since it can be easily collected and contains locally and systemically derived markers of periodontal disease, they may offer the basis for a patient-specific biomarker assessment for periodontitis and other systemic diseases. This article reviews about the GCF and the various biomarkers in GCF used for assessing periodontal disease activity.

Keywords: *Biomarkers, Exudate, GCF*

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I. INTRODUCTION

Periodontal diseases encompass everything from the earliest stage of marginal gingivitis to the most advanced destructive periodontitis, with loss of connective tissue, loss of attachment, loss of bone and resultant tooth mobility and exfoliation of teeth, if not treated. They are characterized by periods of disease activity followed by periods of remission (inactivity). The diagnosis of active phases of periodontal disease, and the identification of patients at risk for active disease, represents a challenge for both clinical investigators and clinicians.

Various inflammatory mediators and tissue destructive molecules have been detected in the gingival tissues, Gingival crevicular fluid (GCF) and saliva of patients affected by periodontitis, and qualitative changes in the composition of these biomarkers could have diagnostic and therapeutic significance. Gingival crevicular fluid [Fig-1] has been particularly attracted as a source of marker of the periodontal disease activity and are highly beneficial in the determination of current periodontal status. These substances known as biomarkers help in determination of inflammatory mediator levels, as they are good indicators of inflammatory activity.[1]

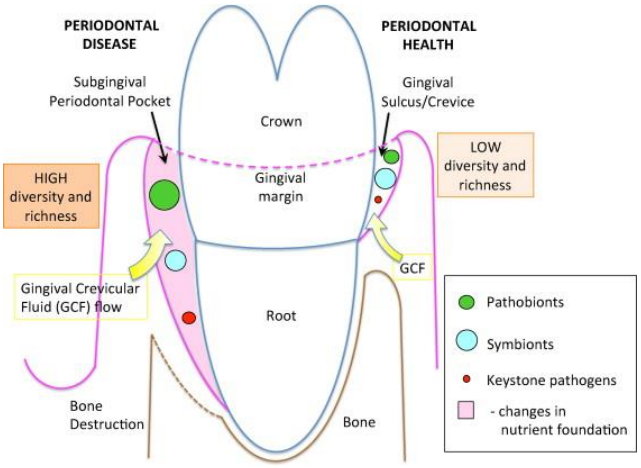

FIG 1: SHOWING THE GCF

Saliva vs. GCF:

Since GCF has the chance of being closely approximated to the periodontal tissues where periodontal disease begins, it seems to provide more information than markers in the saliva. The molecules in saliva can also be originated from salivary glands where cellular and biochemical mediators in saliva may reflect the diseases and metabolic status of glands instead of periodontal diseases.

Rationale of biomarker:

Traditional methods of assessing the periodontal status involve probing measurements and reactions to probing, supplemented by radiography. The strengths of these traditional tools are their ease of use, their cost effectiveness, and that they are relatively non-invasive. But these diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. So, there is a need for the development of new diagnostic tests that can detect the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients.

BIOMARKERS:

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[2]

Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases, are tell-tale molecules that could be used to monitor health status, disease onset, treatment response and outcome. Hence, allowing earlier detection of disease evolution and more robust therapy efficacy measurements.[3]

IDEAL PROPERTIES OF BIOMARKER:

- ❖ Should indicate the presence of disease process before extensive clinical damage.
- ❖ Should have high specificity and sensitivity.
- ❖ Should be able to use it easily at the chair side.
- ❖ Should be useful at patient level.

II. GINGIVAL CREVICULAR FLUID:

Although the existence of Gingival crevicular fluid (GCF), a fluid that emerges between the surface of the tooth and the epithelial integument, has been recognized for over 100years, the exact nature of the fluid, its origins and composition, has been the subject of controversy. This may be a result of variations in the amount and/or nature of the fluid produced under different clinical conditions and the use of a wide variety of sampling methods. Gingival Crevicular Fluid (GCF) is regarded as a window for non-invasive analysis of periodontal conditions, including markers of connective tissue and bone destruction. [Fig:2]

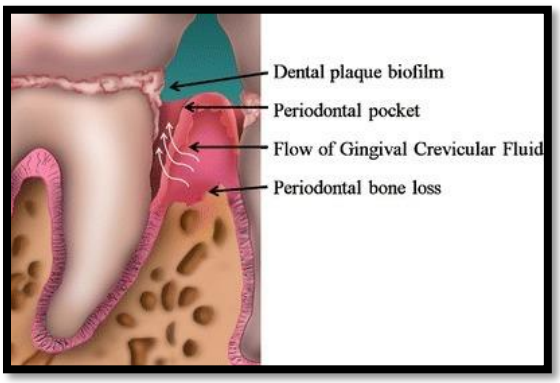

FIG 2: SHOWING THE FLUX OF GCF THROUGH EPITHELIAL CELL IN THE PRESENCE OF BIOFILM.

HISTORICAL BACKGROUND:

The study of GCF can be traced back more than 70 years.[4] The pioneer research of Waerhaug in the early 1950s was focused on the anatomy of the sulcus and its transformation into a gingival pocket during the course of periodontitis. In the late 1950s and early 1960s a series of ground-breaking studies by Brill and co-workers laid the foundation for understanding the physiology of GCF formation and its composition.[5, 6]

RESEARCHERS	STUDIES
WAERHAUG (1952)	Anatomy of sulcus: Sulcus → pocket
BRILL ET AL (1962)	Physiology of GCF formation and composition
LÖE AND HOLM-PEDERSON (1965)	GCF as an Indicator of periodontal diseases
EGELBERG (1966)	Gingival vasculature and permeability
SCHROEDER (1969), LISTGARTEN (1966)	Electron microscopic studies of Dentogingival structure
SUEDA, BANG AND CIMASONI (1966)	First to explore presence and functions of proteins esp. enzymes in GCF
ATTSTROM (1970)	Migration of neutrophils and their function in GCF

RATIONALE FOR THE USE OF GINGIVAL CREVICULAR FLUID AS A DIAGNOSTIC TEST:

- ✓ GCF is easily and non-invasively collected and contains products of the host, the plaque and their interactions.
- ✓ Prior to the appearance of GCF in the gingival crevice, the exudates from the gingival micro circulation traverses the inflamed periodontal tissues and is thought to collect molecules of potential interest in route that may reflect the underlying disease or health status of the tissues.
- ✓ As GCF is derived from the periodontal tissues, analysis of its constituents may provide on early indicator of changes in the tissue.

LIMITATIONS OF GCF AS A DIAGNOSTIC MEDIUM:

- According to Listgarten 1986; Beck 1995, one of the major limitations, in the interpretation of information obtained from a diagnostic test is the lack of a gold standard against which the test can be evaluated.
- Unfortunately, a serious drawback of most of the potential markers studied in GCF is that they reflect processes occurring in the gingival connective tissues and tell us little about, attachment loss or bone loss.
- There is no uniform consensus on choice of collection device, its placement and collection time.
- Potential contamination by serum components and loss of sample from the collection device.

CATEGORIES OF MARKERS IN GINGIVAL CREVICULAR FLUID:

It is generally recognized that the constituents of GCF arise from a variety of sources, including microbial plaque, host inflammatory cells, host tissue, and serum-derived factors.
Curtis et al. stated that **"markers of disease"** might encompass three separate categories: [7]
1) Indicators of current disease activity;
2) Predictors of future disease progression;
3) Predictors of future disease initiation at currently healthy sites.

CLASSIFICATION OF BIOMARKERS FROM GCF:

Over 65 GCF components have been preliminarily examined as possible markers for the progression of periodontitis. Biomarkers that are released into GCF can be broadly grouped according to their sources: [8]

1. Inflammatory Mediators and Host Response Modifiers:

a. **Cytokines:** Interleukin-1α, Interleukin 1-β, Interleukin 1ra, Interleukin-2, Interleukin-6, Interleukin-8, TNF-α, Interferon α.
b. **RANTES:** Chemo-attractant and activator of macrophages and lymphocytes.

c. Prostaglandin E_2, Leukotriene B_4.
d. Acute Phase Proteins: Lactoferrin, Transferrin, α2-Macroglobulin, α-1Proteinase Inhibitor, C-reactive protein.
e. Autoantibodies- Anti-desmosomal antibody
f. Antibacterial antibodies- IgG1, IgG2, IgG3, IgG4, IgM, IgA.
g. Plasminogen Activator, PA inhibitor-2 (PAI-2)
h. Substance P, Vasoactive Intestinal Peptide, Neurokinin A, Neopterin
i. Platelet-Activating Factor, CD-14, Cystatin, Calgranulin A(MRP-8)

2. Host Derived Enzymes and their Inhibitors:

Hydrolytic enzymes

i. Aspartate Aminotransferase,
ii. Alkaline phosphatase,
iii. Acid phosphatase,
iv. β-glucuronidase,
v. Aryl sulfatase
vi. Myeloperoxidase
vii. Lysozyme
viii. Lactoferrin

Proteolytic enzymes

I. Elastase,
II. Elastase Inhibitors- α2-Macroglobulin, α1-proteinase Inhibitor,
ix. Trypsin-like enzymes,
x. Immunoglobulin-degrading enzymes,
xi. Glycosidases,
xii. Dipeptidyl peptidases,
xiii. Non-specific neutral proteinases,
xiv. Collagenases- MMP-1, MMP-3, MMP-8, MMP-13,
xv. Gelatinases- MMP-2, MMP-9,
xvi. TIMP-1,
xvii. Stromyelysins,
xviii. Lactate dehydrogenase
xix. Creatinine kinase
xx. Cathepsin- Cysteine proteinases, Serine proteinase, Cathepsin-D,
xxi. B-N-acetyl-hexosaminidase

3. Tissue Breakdown Products:

a) Glycosaminoglycans- Hyaluronic acid, Chondroitin-4-sulphate, Chondroitin-6-sulphate, Dermatan sulphate.
b) Hydroxyproline
c) Fibronectin fragments
d) Connective tissue and bone proteins- Osteonectin, Osteocalcin, Type-I collagen peptides, Osteopontin.
e) Laminin
f) Calprotectin
g) Haemoglobin β-chain peptides
h) Pyridinoline crosslinks

WHICH OF THE GCF MARKERS HAVE THE MOST PROMISE?
AMONG THE INFLAMMATORY MEDIATORS

1. Studies claim that in GCF, PGE2 is predictive for periodontal disease activity. Levels greater than 66 ng/mL were found to be predictive of further possible loss of attachment with a sensitivity of 0.76 and specificity of 0.96 with an overall predictive value of 0.92 to 0.95, But it cannot clearly differentiate between gingivitis and periodontitis nor between active and inactive sites.[9]

2. Specific antibody or total immunoglobulin in GCF appears to be of no use in distinguishing between stable and progressive sites because —

- The total immunoglobulin in GCF does not correlate with disease severity or progression and indeed may be lower at progressive sites than non-progressive sites.[10]

- Reduction in specific antibody in serum and consequently GCF in patients with existing disease can place them at risk for further disease progression.

Thus, specific antibodies in gingival tissues and serum are important in modulating the pathology of periodontal diseases; but with the present level of knowledge, they do not offer a means of either identifying patients at risk for active disease or of predicting active sites within particular patients.[11]

3. Among the cytokines, studies have shown association of elevated levels of IL-1B with gingival inflammation and severities of periodontitis. However, within a group of patients with similar levels of disease, differences were detected in GCF interleukin levels between groups of patients with different IL-1 gene polymorphisms.

Thus, the most likely diagnostic marker of the inflammatory and immune factors is GCF PGE2.

AMONG THE HOST-DERIVED ENZYMES

1. Alkaline phosphatase, beta-glucuronidase, cathepsin-B, collagenase-2 (MMP-8), gelatinase (MMP-9), elastase and dipeptidyl peptidases II and IV may have potential diagnostic utility for treatment planning and for monitoring treated patients.[12]

2. In addition, cathepsin-B, collagenase-2 (MMP-8), dipeptidyl peptidases II and IV, and elastase seem promising for distinguishing periodontitis from gingivitis.[12]

3. No factor in GCF has been identified which can distinguish between aggressive and chronic periodontitis, although a multitude of these markers have been studied in both types of disease.

AMONG TISSUE BREAKDOWN PRODUCT

1. Chondriotin-4-sulfate and bone-specific GAGs are the most promising potential markers as they reflect the degradation of bone. However, currently there are no longitudinal studies to evaluate the diagnostic and prognostic value of these markers.

III. COMMERCIALY AVAILABLE DIAGNOSTIC KIT:

Molecular analysis of GCF elution can be time consuming while most of the GCF analytic assays are laboratory based and usually cannot be performed in a chair side manner. These procedures, as well as GCF sampling, are technically demanding and the GCF volume can be very small (1-5 µl). Despite these apparent diagnostic and technical disadvantages, GCF is still considered as a potential oral fluid for the development of adjunctive non-invasive chair side diagnostic technology. A number of test kits for the detection of GCF biomarkers by different methods or for the detection of certain periodontopathogens by an enzymatic reaction have been developed for commercial use or as a prototype test.

COMMERCIALLY AVAILABLE CHAIRSIDE DIAGNOSTIC KITS

ASSAY	KIT	MANUFACTURER/ SUPPLIER	FUNCTION
BACTERIAL ENZYMES & HOST ENZYMES	BANA periodontal test	Ora Tec Corporation Manassas (USA)	It utilizes the BANA test for bacterial trypsin like proteases.
	Periocheck (ASTech)	CollaGenex Pharmaceuticals, Newtown, PA	Detects presence of neutral proteinases i.e. Collagenase
	Perioscan	Oral-B Laboratories	Detects enzymatic activity of Aggregatibacter actinomycetemcomitans, T forsythias,
IMMUNOLOGICAL DETECTION	Evalusite	Kodak Eastman Company (Switzerland)	Immunological detection of antigens of Aggregatibacter actinomycetemcomitans, P intermedia, P gingivalis using antibodies (ELISA)
BIOCHEMICAL IDENTIFICATION	Prognostic	DENTSPLY	Aids in detection of serine proteinases and elastase
	Biolise	SLT-Lab instruments, Crailsheim, Ger-many	Aids in detection of elastase
	Periogard	Colgate	Detects the presence of AST
	Pocket watch	SteriOss®, San Diego, CA, USA	Detects aspartate aminotransferase through colorimetric detection
	TOPAS	Affinity Labelling Technologies (USA)	Detects toxins derived from anaerobic metabolism and measures GCF protein level

IV.RECENT RESEARCH ON GCF:

PROTEOMIC ANALYSIS OF GINGIVAL CREVICULAR FLUID IN SEARCH FOR PERIODONTAL DISEASE MARKERS:

Currently, proteomic analysis of GCF is still in its infancy with limited data available in the literature. With the understanding that the protein composition of GCF may reflect the pathophysiology in monitoring the periodontal diseases, establishment and progression, protein profiles of GCF obtained from apparently healthy individuals are starting to be explored as standard GCF proteomic patterns to potentially serve as a reference to identify biomarkers of periodontal diseases by proteome analyses.

Zelko and collaborators analysed GCF proteins in the GCF of periodontally healthy individuals using a gel free method and described proteins broken down into small peptide fragments and analysed directly by LC-MS/MS analysis.[13]. The authors found a total 327 GCF proteins and reported eight protein spots found to be significantly more intense in GCF including superoxide dismutase 1 (SOD1), apolipoprotein A-I; (ApoA-I), and dermcidin (DCD). The superoxide dismutase's (SOD) are the most important line of antioxidant enzyme defence system against reactive oxygen species (ROS).

ApoA-I is the major protein component of high-density lipoprotein (HDL) in plasma. ApoA-I has a specific role in lipid metabolism.[14]

In search of markers to predict health or disease, Bostanci and investigators used quantitative proteomic analysis to analyse 10 GCF samples 5 healthy subjects and 5 patients with aggressive periodontitis. They reported that GCF proteins Cystatin-B and defensins were detected only in the healthy control samples and also Annexin-1 was detected in 5-fold higher levels in samples from periodontally health individuals. L-plastin was detected only in aggressive periodontitis [15]. This protein has been shown to be elevated also in chronic periodontitis. Its value as a marker for periodontal disease still needs to be further evaluated.[16]

Baliban et al. aimed to use biomarker combinations to predict health or disease from GCF samples using proteomic analysis, described G3P_HUMAN, TYPH_HUMAN and KV101_HUMAN selected as human protein biomarkers for periodontally healthy patients. G3P_HUMAN (Glyceraldehyde 3-phosphate dehydrogenase) is an enzyme that participates in glycolysis and serves to break down glucose for energy and carbon molecules. TYPH_HUMAN (Thymidine phosphorylase) is an enzyme that participates in purine metabolism pathway and pyrimidine metabolism pathway and is only found in periodontally healthy GCF samples tested. The protein KV101_HUMAN (Ig kappa chain V-I region AG) was observed in most of periodontally healthy samples but few of the periodontitis samples. The authors recommend further investigation in the functional role of these proteins.[17]

Hence, the combination of human protein biomarkers and bacterial protein biomarkers needs to be further validated in experiments exploring different periodontal conditions, as well as dynamic changes during and after periodontal treatment.

IV. DETECTION METHODS FOR GCF BIOMARKERS

Laboratory methods for GCF biomarker detection

The presence of proteolytic enzymes in GCF has been measured by immunological assays such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting, both of these using high affinity antibodies to recognise the given enzymes. Immunoblotting used with chemo luminescence is a sensitive method for the estimation of molecular forms and mass of the enzyme of interest, but it is not very useful for quantitative analysis.[18]

ELISA/IFMA-methods can be used to test multiple markers and their levels in single samples. Two different antibodies are used: one for capturing the soluble enzyme and the other to Substrate assays measure primarily the ability of the enzyme to degrade the substrate, and the enzyme activity will be estimated by different methods like gelatine enzymography, release of radioactivity from radiolabelled collagen fibrils, and fluorogenic assays based on synthetic peptides that mimic the cleavage site of collagenase. All of these methods are sensitive and specific and suitable to quantify collagen lytic enzymes/MMPs in microliter samples but all of them are time-consuming and not practical for routine clinical use.

METHODS OF GCF COLLECTION [*Fig-3]*

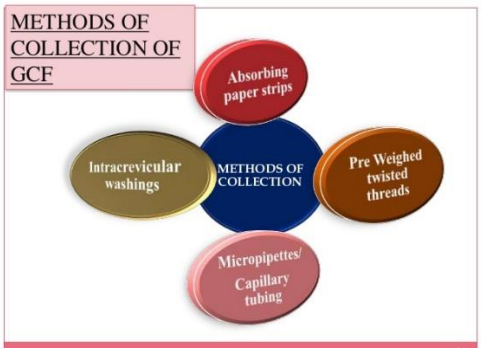

FIG 3: COLLECTION OF GCF

V. FUTURE STRATEGIES: [Fig-4]

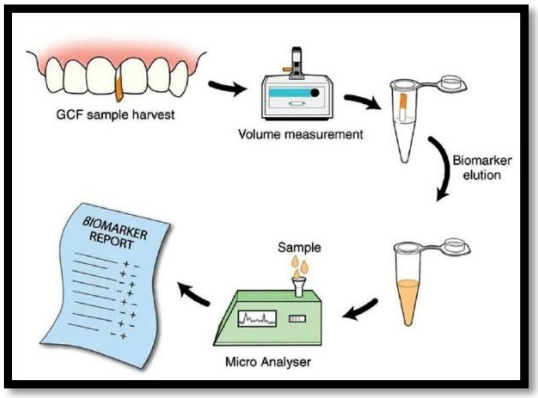

FIG 4: FUTURISTIC CHAIRSIDE DIAGNOSTIC TEST BASED ON GCF SAMPLING. CONSIDERING THE GCF FLUID AS A POTENTIAL ANALYTE FOR THE SCREENING OF MULTIPLE BIOMARKERS.

There are huge possibilities for the future use of oral fluids in biotechnology and health care applications, especially in the field of diagnostics. A tremendous amount of research activity is currently under way to explore the role of oral fluids as a possible medium in a variety of applications.

- ✓ Should be useful primarily at the patient level.
- ✓ Self-performed test such as home use dip stick test which assess the risk of periodontitis.

Through the biomarker discovery process, new therapeutics have been designed, linking therapeutic and diagnostic approaches together, especially in the area of host modulatory drugs for periodontal disease treatment. Moreover, new diagnostic technologies, such as microarray and microfluidics, are now currently available for risk assessment and comprehensive screening of biomarkers.

VI. CONCLUSION:

Up to now, blood has been the gold standard for detection of disease markers and diagnostics of diseases. The search for markers of periodontal disease activity will progress with the refinement and application of specific detection techniques for selected factors. GCF will continue to be a vehicle for monitoring tissue and cell products showing potential benefit, particularly those directly from specific regions of the periodontium which give a clue as to which tissue components are at risk. Today, no single GCF marker or combination of GCF markers are available to determine whether periodontal treatment is sufficient and⁄or necessary to prevent further periodontal breakdown. Till now, no factor in the GCF which can distinguish between chronic and aggressive periodontitis. The development of a wide spectrum of marker factors will be the primary goal of periodontal research.

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