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GCF A Pioneer Biomarker: A Review

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Abstract:- GCF is a potential biomarker which can be a perio diagnostic tool which will be non-invasive and efficient. In this review article we will discuss in detail about the role of GCF as a diagnostic aid, a potential indicator tool for periodontitis and also various changes associated with the markers in periodontitis. There are many ways to assess the amount of GCF collected with help of various quantitative and qualitative analysis. With the advancement in technology, newer biochemical test kits are being introduced in periodontics which utilizes the gingival crevicular fluid for early diagnosis and detection of periodontal diseases. Overall, GCF has broadened our vision of periodontal pathogenesis.

I. INTRODUCTION

Gingival crevicular fluid (GCF) is an inflammatory exudate which is derived from the periodontal tissues. It is mainly composed of serum and locally generated materials such as tissue breakdown products, inflammatory mediators, and antibodies functioning against the bacteria which causes dental plaque. These elements are derived from a number of sources, including serum, the connective tissue, and epithelium through which GCF passes through the crevice.[1]

GCF helps in ruling out various diseases associated with periodontium such as gingivitis, periodontitis and also for the detection of presence of certain drugs in the periodontal pockets which has passed through the systematic pathway . At present it is widely being used for proteomic analysis. It is composed of a sequence of arranged cellular and biochemical factors which depicts the metabolic status of various tissue segments of the periodontal structure. This article will give an insight on how GCF can act as a diagnostic and screening tool with advanced outcomes and results.

- *GCF as A Diagnostic Aid :*

The utilisation of gingival crevicular fluid flow measurements for the assessment of gingival health and the effects of various constituents on the gingival tissues has become widely accepted, based upon the hypothesis that the fluid is an objective and quantitative predictor of gingival inflammation.[2]

- *Conventional Diagnostic Measure Versus GCF:*

The monotonous practice involves biased trends that rely on three concepts . Firstly, the clinical signs and symptoms such as bleeding on probing, change in the gingival color, contour, texture and size plays a significant role in the establishment of the gingival inflammation, Second conception is based on evaluation of PPD (periodontal pocket depth) with the aid of a periodontal probe. Lastly, to determinte the grade of periodontitis with the radiological assessment of alveolar bone loss .

The physiopathology of diseases associated with periodontium can be assessed by the measurement of the protein composition of GCF , since during a inflammatory state various cells and inflammatory biomarkers such as enzymes, cytokines, and local tissue degradation products are released into the crevicular fluid. GCF can be collected easily, is economic and biocompatible and has no associated complications post collection , which esatablishes GCF as an ideal specimen for both the practitioner and the patients. GCF helps reduce the patient's anxiety , fear and time at chairside . Hence they are ideal for early diagnosis of periodontal health [3].

- *GCF- Markers of Disease :*

GCF mirrors can predict the health status periodontal tissues. An substantial research has been done for GCF components as they might become potential prognostic markers in future for predicting disease activity . Utilizing GCF constituents for analysis and as a diagnostic tool for ruling out periodontal disease has distinct advantages as well as challenges. Firstly, from a patient multitudinous samples can be collected. As teeth are not uniformly affected by periodontal disease, collection of samples can be targeted to specific teeth or areas of concern. A quantified number of fluid samples can be retrieved , in case entire mouth has to be evaluated. Secondly the sample collection procedure is non-invasive or minimally invasive. The crevicular tissues are not permanently altered by sampling and at times temporary disruption of the subgingival plaque can occur which results in transient microscopic alteration in the gingival vasculature . Bleeding is rarely seen when sampling is done deep within the gingival crevice. Since sampling can occur longitudinally, it even allows the verification of minute fluid chemistry over defined time intervals.[4]Lasty, with the aid of various sensitive biochemical and immunochemical assays developed recently helped in the analysis of various constituents in picogram range with minimal amount of GCF collected.

- *GCF As an Indicator of Physiologic Changes in Bone Cell Activity*

GCF, in addition to becoming a forefront prognostic tool in the identification of periodontal diseases can boost in determining the progression of the disease. Early diagnosis of progression of periodontitis can provide degree of disease activity clinically and also enhance patient monitoring.

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Hence, sampling of GCF from sites can aid in detecting sites with increased chances of disease susceptibility, which can help in early management of targeted risk site and helps in preventing further progression of the disease.

So many research studies were conducted to evaluate the changes seen within GCF during orthodontic tooth movement . In the initial stages, when orthodontic exertion is applied for tooth movement to occur, changes happens within the periodontium metabolically .This includes the process of bone remodelling such as osteoblastic and osteoclastic activity. Within the periodontal space, these biological and physiological process results in acute inflammatory response .A vital enzyme Lactate dehydrogenase(LDH) liberated after cell death, is reported as a potential marker, as its levels were found to be elevated within GCF during orthodontic tooth movement . Serra et al. demonstrated the changes seen in LDH level in GCF during the early stages of orthodontic treatment. He also proposed that when orthodontic force is applied, the periodontium either goes under tension or compression, resulting in cell death. Bone cells are deposited at the site of tension and resorbed at the site of compression. Enzyme levels were more at site of tension when compared to the area of compression. These enzymes represent the forefront novel markers and are associated with gingival inflammation caused during forces applied by orthodontic appliances[5]. Hence, any diagnostic tool that is non-invasive, has minimal after effects and on which the clinician can rely on to determine the growth spurt is in great demand nowadays .

- *Role of GCF as A Potential Diagnostic Marker In Periodontitis:*

Biomarkers are indicator markers that could be used for determining the health status, disease onset, treatment response and even assess treatment outcomes. Informative biomarkers can further serve as early sentinels of disease. Around 65 GCF constituents have been recognized as potential diagnostic markers of periodontal disease progression , according to Armitage (2004)[6].These markers are of 3 types : host-derived enzymes and their inhibitors, inflammatory mediators and host-response modifiers, and byproducts of tissue breakdown.

DIAGNOSTIC MARKERS	CHANGES ASSOCIATED WITH PERIODONTITIS
INFLAMMATORY MEDIATORS	
Aspartate aminotransferase[7] (AST)	Its characterized by increased levels of AST in GCF ,especially in areas of severe gingival inflammation and progressive attachment loss
Cytokines and chemokines[8]	• IL1β, IL-2, IL-6, IL-8, IL-17 and TNF-α in GCF are elevated during periodontitis and decreases following SRP. • The levels of anti-inflammatory cytokines (IL-4, IL-9, IL-10, and IL-13)are also elevated during periodontitis in GCF
Adipokines[8]	Visfatin, leptin, Adiponectin and Resistin which are periodontal disease-specific biomarkers which are elevated in GCF
HOST DERIVED ENZYMES[9]	
Alkaline phosphatase	The levels of alkaline phosphatase is associated with the depth of periodontal pocket and the percentage of bone loss. During periodontitis,the levels of alkaline phosphatise is about 20 times in GCF.
Acid phosphatase	The levels of acid phosphatase in Gingival fluid is around 10-20 times more than that present in serum, however these levels don't depict severity of the disease nor its activity.
β –Glucuronidase	Its levels are directly correlated with the subgingival periodontal pathogens and increases proportionally disease severity in GCF .
Elastase	In periodontitis , the levels of elastase in GCFis high
Cathepsins	In cases of periodontal disease, concentrations of cathepsin B in GCF are high. But their values where decreased in cases of gingivitis.
Trypsin-like enzymes	In periodontitis, GCF levels of trypsin like enzymes were higher as these enzymes increases the potential for periodontal tissue destruction.
Immunoglobulin-degrading proteases	GCF IgG antibodies levels in periodontal disease activity are significantly high
Dipeptidyl Peptidases (DPP)	GCF levels of DPP were elevated in regions with rapid and gradual attachment loss , hence higher among patients with periodontitis.
Non-specific neutral proteases	Proteases levels are elevated during periodontitis .
Matrix Metalloproteinases	Its levels in GCF are increased directly with the complexity of inflammation ,depth of pocket and alveolar bone loss.
TIMPs	TIMP levels in GCF is significantly higher in gingivitis and periodontitis.
Myeloperoxidase	Their amount in GCF are increased for patients with progressive chronic periodontitis

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Lactate dehydrogenase(LDH)	Changes in the levels of LDH in crevicular fluid has no impact on the periodontal disease activity , ie. no correlation exists .
Arylsulfatase	Its level in GCF are increased in gingivitis and periodontitis
β-N-acetyl-hexosaminidase (β-NAH)	Its levels are increased in GCF during periodontitis
TISSUE BREAKDOWN PRODUCTS[9]	
Glycosaminoglycans	In periodontitis, the levels of glycosaminiglycans are increased in GCF
Hydroxyproline	Levels of Hydroxyproline ,a collagen breakdown product present in the GCF are increased in periodontitis.
Fibronectin fragments	In GCF fibronectin is invariably found in a degraded form therefore is inactive. Its prescence in GCF indicated tissue destruction .
Connective tissue and Bone proteins Osteonectin[9]	
Osteonectin	Its levels are increased during inflammation , periodontitis .
Osteocalcin	The link between GCF osteocalcin levels and periodontal disease are propotional.
Type I collagen peptides	Their levels in GCF of patients with periodontitis were detected but increased post scaling and root planning(SRP)
Osteopontin (OPN)	OPN concentrations in GCF increased proportionally with the progression of periodontal disease.

Table 1

➢ *Qualitative & Quantitative Analysis of GCF :*

There are mainly two ways to analyze the amount of GCF collected. These include the qualitative and the quantitative analysis. The quantitative analysis mainly used in GCF include[11]:

- Electronic Method: It measures the amount of fluid collected on a "blotter" (Periopaper), employing an electronic transducer. The dampness of the paper strip interrupts the flow of electric current and gives a digital read-out.
- Periotron:. It is an electronic instrument which measures the effect of wetness of filter paper strips on the capacitance between the 'jaws' of the device between which the filter paper is placed following the sample collection.
- Bradford method: This assay is used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue.
- Electrochemiluminescence technique (ECL) : It merges the unique advantages of highly specific immunoreaction and convenient ECL biosensors which makes it an outstanding method for protein analysis .
- Flow cytometry:It enhances lasers as light sources to produce both scattered and fluorescent light signals that are read by detectors such as photodiodes or photomultiplier tubes. These signals are converted into electronic signals that were analyzed by a computer and upgraded to a standardized format data file. Cell populations can be analyzed and/or purified based on their fluorescent or light scattering characteristics.
- Spectrophotometer: It is used to estimate the level of an analyte in solution and is a supreme option for quick analysis of small quantities of materials.
- Capillary zone electrophoresis coupled with laser induced fluorescence detection (CZE-LIFD): It gives off the analysis of complex mixtures with remarkably high efficiency. CZE coupled with laser-induced fluorescence detection (LIFD) helps in detection of various molecules such as amino acids,5 peptides,s proteins and Carbohydrates .
- The Enzyme Immunoassay (EIA): An in vitro quantitative assay kit for detecting biological peptides or proteins based on the competitive enzyme immunoassay principle.

The various qualitative analysis include immunoblotting or 'sandwich' ELISA, polymerase chain reaction and colorimetric assay (automatic colorimetric method and Erels colorimetric method).

II. ROLE OF GCF AS A CHAIRSIDE DAGNOSTIC KIT IN PERIODONTICS

Significance of early diagnosis helps in controlling the disease with minimally invasive and economic therapeutic modalities. Introducing novel diagnostic kits rules out disease status, predict future disease progression and evaluate response to various periodontal therapy. Biochemical test kits used in periodontics utilizes the gingival crevicular fluid (GCF) for analysis and this fluid helps in the early detection of periodontal diseases .

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DIAGNOSTIC KIT	TYPE OF TEST	MECHANISM OF ACTION	FUNCTIONS
PROGNOS -STIK[12]	Quantitative	Once the GCF is collected onto the filter paper strip loaded with a known amount of buffered elastase substrate labeled with a fluorescent indicator, the Elastase on the test strip will cleave the substrate during the reaction time of 4–6 min .On cleaving , it emits the indicator which is visible under fluorescent light. *Elastase will then be accumulated at sites of gingival inflammation which will be released from lysosomes of polymorhonuclear leukocytes.	*It detects (Matrix metalloproteinases)MMPs present in the gingival crevicular fluid such as the elastases and their elevated levels indicate active disease sites.
PERIOCHECK[13]	Qualitative	*The sample strip with GCF is placed on a gel containing insoluble dye-labeled collagen fibrils and incubated. * This compound is digested to release soluble dye-labeled fragments in the presence of insoluble collagen dye complex, which is then diffused back into the strip, turning its color blue.	* A novo quick chairside test which detects the presence of neutral proteases like elastases, proteinases and collagenases, all of which have been associated with collagen breakdown .
PERIOGUARD[12,13]	Qualitative	* The filter paper strip following collection of GCF is kept in tromethamine hydrochloride buffer. A substrate reaction mixture consisting of 1-aspartic and α-ketoglutaric acid is then added to the sample and allowed to react for ten minutes. * The aspartate, and α-ketoglutaric acid are catalyzed to oxaloacetate and glutamate in the prescence of AST. * On adding dye such as fast red results in a color product, the intensity of which is parallelly proportional to the AST activity in the GCF sample	*It test to detect an enzyme called aspartate aminotransferase (AST) and act as potential indicator for early periodontal tissue destruction.
POCKETWATCH[14]	Qualitative	*In the presence of pyridoxal phosphate, AST catalyzes the transfer of an amino group of cysteine sulfuric acid by α-ketoglutaric acid to yield β-sulfinyl pyruvate. *Glutamate β-sulfinyl pyruvate spontaneously and rapidly breakesdown and releases inorganic sulfite. The sulfite ion instantaneously reacts with malachite green (MG), concurrently changing its color from a green dye to its colorless form, thereby allowing the pink–colored rhodamine B dye to show through. This colour change is direct proportion with the levels of AST.	*A simple chairside test for analyzing Asparate amino Transferase (AST), which helps in assessment of extent of destruction of pocket.
TOPAS (Toxicity Pre Screening Assay)[14,15]	Qualitative	*The mechanism of this test relies on the detection of rapidly dividing and growing pathogens which can be assessed through the metabolic activity of these organisms in the crevicular fluid. *The metabolic activity is in direct proportion with the concentration of toxins..	*It helps in determination of gingival infection by indirect assessment of bacterial toxins and proteins.
BIOLISE[16]	Qualitative	*To detect the prescence of elastase activity in GCF	

Table 2

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III. CONCLUSION

The analysis of gingival crevicular fluid has colossally refined our perception of periodontal pathogenesis.The various biomarkers in GCF holds a promising future of it being used as an periodontal diagnostic tool that is non-invasive and efficient. The outstanding characteristic feature of GCF as a diagnostic marker is its site-specificity. This allows laboratory investigations of GCF constituents to be linked with the clinical assessments at the sample site. Hence it can be used as a patient-specific diagnostic test for periodontal disease. Most importantly, the simplicity of its use along with a level of reliability and minimal cost favours it over other aids.

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