A systematic review of biomarkers of gingival crevicular fluid: Their predictive role in diagnosis of periodontal disease status

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ABSTRACT

The objective of the study was to conduct a systematic review of the literature so as to evaluate and summarize the diagnostic and prognostic potential of GCF. Included studies were systematically analyzed based on PRISMA (Preferred Reporting Items For Systematic Reviews and Meta Analyses) and studies were identified based on the PICO (Glossary of evidence based terms 2007):

- 1) Patients with chronic periodontitis.
- 2) Intervention- NSPT (Non-Surgical Periodontal therapy); NSPT + Chemotherapeutics.
- 3) Comparison between treated v/s non treated sites.
- 4) Outcomes measured: Analysis of variation in constituents of GCF.

Electronic database search of Pubmed, Medline, Google Scholar and Scopus was performed using (MeSH) terms- Gingival Crevicular fluid and Cytokines, MMP's, NE, PGE-2, A2M, B2M, ALP, AST, Osteocalcin and Calprotectin. Articles published between year 2000-2016 were reviewed and were included based on inclusion and exclusion criteria.

Based on this systematic review of literature, it can be concluded that analysis of constituents of GCF can be used as an effective and efficient diagnostic tool of periodontal diseases. These biomarkers in turn with their prognostic significance could act as a valuable tool in the combat of periodontal disease.

1. Introduction

Gingival crevicular fluid (GCF) is considered 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.¹

The potential diagnostic importance of gingival fluid was recognized more than six decades ago and serious investigations of the dynamics of GCF production began with the epoch making reports of Brill and co-workers in 1950's.^{2,3}

The gingival tissue is subjected to a continuous mechanical and bacterial onslaught. Saliva, sulcular fluid, epithelial surface keratinisation and initial stages of inflammation provide resistance to these constant irritations.⁴

The sulcular fluid is produced in minute quantities from a completely healthy periodontium, being nearly similar in composition to blood plasma, GCF became a source for non-invasive testing for periodontal disease presence. Its various constituents have been assayed to evaluate its efficacy as a prognostic marker before and after conventional periodontal therapy. Thus, GCF has been a much researched fluid

and holds prominence in published periodontal literature (Fig. 1).

Gingival crevicular fluid (GCF) has been employed in the analysis of periodontitis, taking into account indicators and markers of connective tissue and bone destruction therefore has often been used as a useful indicator in determining the severity of periodontal diseases.⁵ The action of inflammatory mediators results in an increased volume of fluid coming out of the pocket which could be partly attributed to increased vascular wall permeability. Thus the composition of GCF alters with the status of inflammation, therefore, has often been considered as a reliable indicator of underlying tissue changes.

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 may be assessed by the constituents of gingival fluid. Bacterial enzymes, bacterial and connective tissue degradation products, host mediated enzymes, inflammatory mediators, extracellular matrix proteins can be detected in gingival crevicular fluid during the active phase of periodontal disease. It has also been re-researched as a prognostic indicator, to assess its role as an indicator of successful therapeutic outcomes.

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Fig. 1. Schematic diagram for procedural methodology.

2. Objectives

To conduct a systematic review of the literature so as to evaluate and summarize the diagnostic and prognostic potential of GCF. Also, included studies were systematically analyzed to detect the variation of GCF constituents at baseline and post-treatment of periodontal disease entities.

3. Methodology

This systematic review is based on PRISMA (Preferred Reporting Items For Systematic Reviews and Meta Analyses).

4. Focused question

Is GCF an effective and efficient diagnostic and prognostic tool by means of analysis of constituents and increase in levels with disease and reduction on treatment?

5. Search strategy

Literature was searched systematically and studies were identified based on the-PICO (Glossary of evidence based terms 2007):

- 1) Patients with chronic periodontitis as per (AAP 1999 classification)
- 2) Intervention-NSPT; NSPT + Amoxycillin; NSPT + NSAID'S;
NSPT + LDD; NSPT + Mucogingival surgeries
- 3) Comparison between healthy and diseased sites.

- 4) Comparison between treated v/s non treated sites.
5) Outcomes measured: Analysis of variation in constituents of GCF:

IL-1 β , IL-1 α , IL-6, IL-8, IL-10, MMP-3, MMP-1, MMP-13, MMP-8, MMP-9, Osteocalcin, Calprotectin, Aspartate Aminotransferase, Elastase, β -2Microglobulin, α -2 Macroglobulin, Alkaline Phosphatase

Electronic database search of Pubmed, Medline, Google Scholar and Scopus was performed using (MESH) terms- Gingival Crevicular fluid and Cytokines, MMP's, NE, PGE-2, A2M, B2M, ALP, AST, Osteocalcin and Calprotectin. Articles published between year 2000-2016 were reviewed. The selected titles were reviewed by two authors – Gupta S & Chhina S and were included based on inclusion and exclusion criteria.

6. Inclusion criteria

1. Retrospective and Prospective studies
2. Case Series
3. Cohort studies
4. Randomised controlled trials

7. Exclusion criteria

1. Case Reports
2. Systematic Reviews
3. In Vitro studies
4. Animal Studies
5. Publications in language other than English

8. Results

Search results and excluded trials:

Database searching yielded 612 papers relevant to the present review. Additional search yielded 10 more papers; 42 articles were excluded based on duplicate data presented. Papers published from year 2000 to 2016 were included in the present review, thus excluding 92 papers. Only papers published in English language were reviewed and 7 animal studies and 5 in vitro studies were excluded.

Based on review of titles and abstracts 366 studies were further evaluated. 260 studies that included patients with conditions other than chronic periodontitis were considered ineligible for inclusion. Based on inclusion and exclusion criteria only 17 papers were considered eligible for the present systemic review.

9. Discussion

The following biological markers were assessed in GCF pre and post periodontal therapy in Chronic Periodontitis patients.

9.1. Prostaglandin E2

Arachidonic acid metabolites like PGE2 play a key role in the progression of periodontal destruction. PGE2, a metabolite of cyclooxygenase pathway, is considered to induce fibroblasts and osteoblasts activity for the synthesis of MMPs, IL-1b and other cytokines. PGE2 levels are enhanced and have been correlated with the severity of periodontal disease.⁶ According to Table 1 PGE2 levels (214.5 ng/ml) showed positive correlations with probing depth (209.2 ng/ml), attachment loss (206.6 ng/ml), bleeding on probing (209.7 ng/ml).⁷ PGE2 levels showed significant decrease from baseline (326.62 ± 78.43 ng/ml) to (107.92 ± 35.54 ng/ml) post 8 weeks after treatment with NSPT in patients suffering from chronic periodontitis.⁸ PGE2 levels showed no significant changes when NSPT combined with Meloxicam was done in patients with chronic periodontitis, Baseline (17.81 ± 6.5) and 4 weeks post treatment (17.73 ± 6.9).⁶ PGE2 levels show lower levels of inhibition when gene expression is done after treatment with NSPT and NSAID's together.⁹

9.2. Aspartate aminotransferase

Aspartate aminotransferase (AST), a cytoplasmic enzyme predominantly distributed in heart, liver and skeletal muscles, extracellular release of AST is associated with cellular damage and cell death. Elevated levels are identified at sites undergoing periodontal disease progression. From Table 1, it can be inferred that; AST levels were found to be positively correlated with clinical parameters such as PD, CAL, BOP; AST levels were significantly reduced 12 months post treatment in patients with chronic periodontitis from ≥ 800 μ l to 200 μ l.¹⁰

9.3. Cytokines

Cytokines are cellular immune response indicators involved in a variety of biological responses. IL-1b is generated by monocytes, macrophages, fibroblasts and bone cells. Microbial products and antigens induce its synthesis. IL-1, IL-6, IL-8 and TNF is repressed by IL-10, which suppresses the production of MMP's, while increasing the synthesis of TIMP's in macrophages.¹¹ Studies reveal that Interleukins level vary according to severity of periodontal disease.¹² IL-1 β is decreased from baseline (0.88 ± 1.24) to 4 weeks (0.45 ± 0.44) post treatment with NSPT in conjugation with NSAID's.⁶ IL-1 β (136.8 ng/ml) showed positive correlation with clinical parameters-CAL (145.8 ng/ml), BOP (159.2 ng/ml), PD (155.0 ng/ml).⁷ IL-6 levels found to be higher in non-diseased sites (0.033 ± 0.012 μ l) and

increased significantly following treatment at diseased sites (0.036 ± 0.013) as compared to diseased sites (0.186 ± 0.042 μ l).¹¹ IL-8 levels found to be significantly increased post NSPT combined with surgical periodontal therapy (Modified Widman's Flap) from baseline (1103.76 ± 498.22 ng/ml) to (6290.47 ± 609.77 ng/ml) post 32 weeks.¹¹ Gene expression of IL-1 β showed lower levels of IL-1 β inhibition when patients of chronic periodontitis are treated with NSPT and NSAID's.⁹ IL-8 levels significantly reduced when treated with NSPT from baseline (48.3 ± 40.6) to 6 months post treatment (25.87 ± 38) in ng/ml.¹³ IL-10 levels differed significantly when compared at diseased and non diseased sites. Baseline levels in diseased patients were (0.186 ± 0.112) ng/ml, at non diseased sites (0.033 ± 0.017) ng/ml which reduced to, at diseased (0.036 ± 0.019) ng/ml and at non diseased (0.013 ± 0.009) ng/ml post NSPT.¹⁴

9.4. Neutrophil elastase

Neutrophil Elastase (NE) is a serine endopeptidase of primary granules, and is a potent proteolytic enzyme. Preliminary studies have shown that NE level is higher in GCF from Periodontitis patients as compared to gingivitis patients. Bergstrom et al. study revealed that Elastases levels were significantly different in patients treated with NSPT in healthy and chronic periodontitis patients.¹³ In chronic periodontitis patients, levels of Elastases reduced from (4.62 ± 3.56 ng/ml) to (2.34 ± 3.15) ng/ml post NSPT.¹³

9.5. Osteocalcin and calprotectin

Osteocalcin (OC), is considered a specific vitamin-K dependent, calcium binding protein, produced by osteoblasts. Due to its tissue-specific expression, has a role in normal bone homeostasis.¹⁵ Calprotectin complex, has several function in inflammatory reactions. It coordinates adhesion and migration of neutrophils or monocytes. Its levels in GCF are higher in periodontitis patients.¹⁵ According to Table 1 Osteocalcin levels were found to be significantly lesser in patients with chronic periodontitis (0.19 ± 0.1 ng/ml) as compared to patients with healthy periodontium (0.77 ± 0.1 ng/ml). Calprotectin levels were found to be increased as the periodontal disease progresses from health to disease exhibiting that calprotectin is a significant contributor to host-defense against infection.¹⁵

9.6. Alkaline phosphatase

ALP is a membrane bound glycoprotein derived from leukocytes, osteoblasts, macrophages and fibroblasts. Resident bacteria of the sulcus or pocket also produce ALP. Abundance of PMNL's at the site of periodontal inflammation serve as a primary source for GCF ALP. Early detection of periodontal changes, prognosis and efficacy of treatment can be monitored by ALP levels.¹⁶ ALP values showed a significant decrease after NSPT post 60 days from (17.81 ± 6.5) ng/ml to (11.32 ± 5.2) ng/ml.¹⁶ Reduction in values lead to a conclusion that the effect of mechanical plaque control on the progression and regression of the disease can be evaluated precisely by corresponding GCF levels. Thus, ALP level is not only a biomarker for the pathology but also an indicator of prognosis of periodontitis.¹⁶

9.7. Macroglobulins (Alpha 2, Beta 2)

Macroglobulins play a major role in the immune system. They have the ability to inactivate varied microorganisms, thereby protecting oral tissues. A2M has been associated with tissue destruction where as B2M plays a role in T and B lymphocytes response. Inflamed tissues release A2M and B2M to stall inflammation and negate microorganisms proliferation.¹⁷

Table 1
Profile of biomarkers assessed in ggf.

Study Designs and authors.	Constituents	Level of evidence ²⁹	Population (F/M)	Mean Age (years)	Treatment done	Mean Values (ng/ml)	Parameters Checked	Methods of collection	Inference
Oringer et al., 2001 ¹⁰	AST	I	56 (41-Healthy) (41-Chronic Periodontitis)	48 years	NSPT	Reduced from $\geq 800 \mu\text{l}$ to $200 \mu\text{l}$.	PD,RAL, BOP	Methylcellulose strip; intravascularly for 30 sec; AST analysis using pocket watch	AST levels were significantly reduced 12 months post treatment in patients with chronic periodontitis
Engelbreton et al., 2002 ²⁵	IL-1 β	II-2	29	≥ 18 YEARS	NSPT	CP-58.0 $\pm$ 51.6 6 weeks-47.1 $\pm$ 47.9;	PD,CAL,BOP	Methylcellulose strip;intravascularly for 30 sec. Storage at -20°C Periotron 6000for analysis of IL-1 β	IL-1 β reduced after NSPT; High GCF IL-1 β levels is in part of a host trait
Goutoudi et al., 2004 ¹⁴	IL-10	I	12	35–65 Years	NSPT	NON-DISEASED Baseline-0.186 $\pm$ 0.112 32weeks-0.036 $\pm$ 0.019 DISEASED Baseline-0.033 $\pm$ 0.017 32 weeks-0.013 $\pm$ 0.009 ELASTASE CP-4.62 $\pm$ 3.56 H-2.34 $\pm$ 3.15 IL-8 CP-48.3 $\pm$ 40.6; H-25.87 $\pm$ 38	PD,CAL,PI,GI	Paperstrip inserted intravascularly for 30 sec, 250 μl PBS used, stored at 4°C for 2 hrs and then frozen at -70°C degrees. Periotron 6000 used for quantification. DURAPHORE filter membrane(0.22 μm pore size,entered into sulcus for 15 seconds, Stored at -20°C .	Total amount in GCF rather than the concentration of IL-10 positively correlated with periodontal disease and reduced post treatment
Isabelle et al., 2006 ¹³	Elastase IL-8	II-2	18	35–68 Years	NSPT	ELASTASE CP-4.62 $\pm$ 3.56 H-2.34 $\pm$ 3.15 IL-8 CP-48.3 $\pm$ 40.6; H-25.87 $\pm$ 38	PPD,BOP,PI	DURAPHORE filter membrane(0.22 μm pore size,entered into sulcus for 15 seconds, Stored at -20°C .	Improved clinical parameters with reduced elastase levels and IL-8 levels higher in CP patients which reduced after NSPT
Zhong et al., 2007 ⁷	IL-1 β PGE-2	II-2	6277	62.5 YRS	NSPT	PGE2 (214.5 ng/ml) PPD (209.2 ng/ml), CAL (206.6 ng/ml) BOP (209.7 ng/ml) IL-1 β (136.8 ng/ml) CAL (145.8 ng/ml),m BOP (159.2 ng/ml, PPD (155.0 ng/ml).	PPD,CAL,BOP	Paperstrips were placed gently and left until visibly dampened. Harco periotron was used. This volume was then further used to compute the final mediator concentration using elisa kit	GCF IL-1 β , PGE-2 levels positively correlated to PPD, CAL, BOP
Buduneli et al., 2010 ⁶	PGE2 IL-1 β	I	40 (20 + 20)	Control gp-47.2 Test Gp-48.4	NSPT + MELO-XICAM	PGE-2 Baseline- (17.81 $\pm$ 6.5) 4 weeks- (17.73 $\pm$ 6.9) IL-1 β Baseline- (0.88 $\pm$ 1.24) 4weeks- (0.45 $\pm$ 0.44)	PBI,PD,CAL,BOP	Filter paper strips (Periopaper) into 1 mm for 30 sec; sample was analysed using Periotron 8000	Clinical parameters reduced, PGE-2 values non-significant, IL-1 β -reduced
Popova, Mlachkova, 2010 ⁹	IL-1 β PGE-2	II-2	30(20 + 10)	≥ 18	- NSPT + COX-2 INHIBITOR (AULIN)	Gene expression levels of PGE-2 and IL-1 β	PI,GI	Tagman RT-PCR for evaluation of gene expressions levels of IL-1 β and PGE2 in gingival tissue of periodontal patients.	PGE-2 and IL-1 β show lower levels of inhibition when gene expression is done post treatment with NSPT and NSAID's together.
Becerik et al., 2011 ¹²	Calprotectin Osteocalcin	II-1	80 F-39 M-41	43.1 $\pm$ 4.2 Years	NSPT	Osteocalcin CP (0.19 $\pm$ 0.1 ng/m) H- (0.77 $\pm$ 0.1 ng/ml)	PD,CAL,PI,PGI	Paperstrips (periopaper),inserted until resistance felt for 30 sec,stored at -40° Periotron 8000 used for analysis of constituents. Samples collected in forenoon-pipettes of 5 μl by Brill technique for 20 minutes,storage -20°C Paperstrip inserted into crevice until mild resistance	Calprotectin increased as the periodontal disease progressed. Osteocalcin lesser in patients with CP as compared to patients with healthy periodontium All parameters reduced,Positive correlation between PD and ALP IL-6 and IL-8 levels Increased post treatment (continued on next page)
Kunjappu et al., 2012 ¹⁶	Alkaline Phosphatase	II-2	(20)	39.3 $\pm$ 3.4 29.0 $\pm$ 7.8	NSPT	Baseline-(17.81 $\pm$ 6.5) ng/ml to 60 days- (11.32 $\pm$ 5.2) ng/ml.	PI,PD		
	IL-6 IL-8	II-2	12 F-7 M-5	45.4 years			PI,GI,PD,CAL		

Table 1 (continued)

Study Designs and authors.	Constituents	Level of evidence ²⁹	Population (F/M)	Mean Age (years)	Treatment done	Mean Values (ng/ml)	Parameters Checked	Methods of collection	Inference
Gontoudi, Diza, Arvanitidou, 2012 ¹¹					NSPT NSPT + Modified Widman Flap	IL-6 Baseline- 11.38 ± 4.73 32 weeks- 38.72 ± 11.77 IL-8 Baseline- 1103.76 ± 498.22 32 weeks- 6290.47 ± 609.77	Baseline- 326.62 ± 78.4- 3 8weeks- (107.92 ± 35.- 54)	was felt for 30 sec. Samples stored at -70 °C Periotron 6000 was used calibrated with 1:5 diluted serum., PI,GI,PPD,CAL	GCF collected using 1–3 ml calibrated volumetric pipettes,placed extracrevicularly (unstimulated) for 5–20 min; 3 ml GCF was collected. PGE2 was analysed using Elisa kit.
Kumar et al., 2013 ⁸	PGE2	I	25 (20–60 YRS)	23.5 Years	NSPT				
Positive correlation between PGE2 and PI, GI, PPD, CAL Reduced after treatment									
Reddy et al., 2013 ²⁴	MMP-3 TIMP-1	I	30 (20–50 yrs) 3 groups for periodontal health,disease and treatment	23.5 years	NSPT	MMP-3: Baseline- 0.344 ± 0.131 CP- 2.129 ± 1.101 TIMP-1 Baseline- 8.781 ± 0.584 CP- 6.408 ± 0.386 B2 M CP-(87.32 ± 8.1) H- (32.4 ± 2) ng/ml. A2 M CP- (382.5 ± 23.5) ng/ml H-153.6 ± 41.3) ng/ml.	GI,PD,CAL	GCF collected by 1–3 µl calibrated volumetric micro-capillary pipettes,placing the tip of the pipette extra-crevicularly (unstimulated) for 30 seconds.	MMP-3 levels increase and TIMP levels decrease with periodontal disease progression
Ertugrul et al., 2013 ¹⁷	B2M A2M	I	80	31.3 ± 1.98	NSPT		PI,GI,PD,CAL,B-OP	GCF samples taken from four Rmaford teeth (16,24,36,44). Samples placed in eppendrof tubes with 500 µl of buffer. (hcl,nacl,cacil2)Sample collected with prefabricated paper strip,Peritron 8000 was used.	Decreased in patients post NSPT
Kinney et al., 2014 ²⁰	MMP-8 MMP-9	II-2	(100)	29.3 ± 4.4 33.0 ± 5.8	NSPT	MEDIAN (pg/ml) MMP-8- Baseline-9328 6months-10,9321 MMP-9 Baseline-8378 6months-9323	CAL,BOP,PI,PD	A methylcellulose strip was inserted into the sulcus until light resistance was felt for 30 seconds. GCF strips were stored in cryovials at -80 °C. GCF volume was immediately determined using a calibrated Periotron 6000.	Levels Increased with periodontal disease progression.
Chhina S, Rathore AS, Juneja S, 2015 ¹⁸	A2M	II-2	(30)	25.3 ± 1.6 32.0 ± 6.8	-NSPT + LD (TTC) -NSPT Alone	Baseline-(37.81 ± 6.5) 90Days-(21.32 ± 5.2)	PPD,CAL,PI,GI	1 µl collected by color coded microcapillary pipettes (1–5 µl) unstimulated,extra-crevicular Samples were analysed by ELISA kit	Clinical parameters and A2 M levels reduced in patients treated with NSPT + LDD
Pawar et al., 2015 ²¹	MMP-3 MMP-13	II-2	(55) Group1-11/ 9 Group 2-19/16	29.3 ± 3.4 39.0 ± 7.8	NSPT	MMP-3 Baseline- 4.49 ± 1.15 6 weeks- 3.20 ± 0.31 MMP-13- Baseline- 1513.8 ± 2380.6 6 weeks-1243.3 ± 2014.2	PI,GI,PD,CAL	GCF collected by placing white color-coded 1-µl calibrated volumetric microcapillary pipettes by extra-crevicular (unstimulated) method,stored at 70 °C MMP levels determined by ELISA GCF collected with sterile paper strips for 1–2 mm for	Clinical Parameters showed reduction MMP 3,13 reduced after Phase 1 Therapy
Skurska et al., 2015 ²²	MMP-8 MMP-9	I	(36) F-24,M-12	39.3 ± 2.4 29.0 ± 5.8	NSPT		PI,GI,PPD,CAL		(continued on next page)

Table 1 (continued)

Study Designs and authors.	Constituents	Level of evidence ²⁹	Population (F/M)	Mean Age (years)	Treatment done	Mean Values (ng/ml)	Parameters Checked	Methods of collection	Inference
						MMP-8 Baseline- 61.30 ± 63.43 6 months-30.32 ± 29.77 MMP-9 Baseline- 352.92 ± 73.72 6months- 198.42 ± 107.64		30 seconds. Samples placed in eppendorf tubes containing 20ul PBS and frozen at -20°C. Periotron 8000 used for analysis for MMP.	MMP-8 and MMP-9 levels found to be reduced post NSPT.

Studies have demonstrated that levels of B2 M were higher in patients suffering from chronic periodontitis (87.32 ± 8.1) as compared to healthy individuals (32.4 ± 2) in ng/ml.¹⁷ Levels of A2 M were also significantly higher in patients suffering from chronic periodontitis (382.5 ± 23.5) ng/ml when compared to healthy individuals (153.6 ± 41.3) ng/ml.¹⁷

9.8. Matrix metalloproteinases

MMP'S are a family of zinc and calcium dependent endopeptidases. They play a key role in physiologic and pathologic periodontal extracellular matrix degradation and remodeling. Tissue Inhibitors Of MMP's restrict extracellular matrix breakdown.¹⁴ Detection of MMP'S in healthy periodontal tissues is difficult and can be detected in accordance to inflammatory status.

The activities of most MMPs are low in normally healthy periodontium, whereas more MMP concentrations can be detected in diseased and inflammatory conditions of periodontal tissues.¹⁹ MMP-3 levels reduced from baseline (4.49 ± 1.15) ng/ml to (3.20 ± 0.31) ng/ml, 6 weeks post NSPT.¹⁶ MMP-3 levels were found to be significantly and proportionally increased with the progression of periodontal disease (2.129 ± 1.101 ng/ml) and decreases after treatment with NSPT (0.344 ± 0.131).¹⁹ TIMP levels in GCF decreases proportionally with progression of periodontal disease in chronic periodontitis patients (6.408 ± 0.386) ng/ml and increases after treatment with NSPT (8.781 ± 0.584) ng/ml.¹⁹ MMP-8 and MMP-9 levels were found to be increased with periodontal disease progression when compared at baseline (Median-9328 pg/ml) and 6 months (10,931 pg/ml) and at baseline (8378 pg/ml) to (9323 pg/ml) at 6 months, respectively.²⁰ MMP-13 levels were reduced from (1513.8 ± 2380.6) ng/ml to (1243 ± 2014.2) ng/ml at 6 weeks post NSPT.²¹ MMP-8 levels were found to be decreased from baseline (61.30 ± 63.43 ng/ml) to 6 months (30.32 ± 29.77 ng/ml) post NSPT.²² MMP-9 levels were found to be reduced from baseline (352.92 ± 73.72 ng/ml) to (198.42 ± 107.64 ng/ml) 6 months post NSPT.²²

Inflammatory processes of the periodontal tissues trigger release of biomarkers such as-Prostaglandins, Cytokines, Matrixmetalloproteinases, osteocalcinin, osteoprotegrin, Aspartate aminotransferase, elastase, Alpha-2macroglobulin, Beta2microglobulin, Alkalinephosphatase. Based on current evidence GCF of healthy compared to Chronic Periodontitis sites has been the subject for numerous research. Present Systematic review of 17 original studies within the confines of pre-established inclusion and exclusion criteria was carried out. Also the review compared the GCF level of these biomarkers in response totherapy. NSPT; NSPT + Local drug delivery NSPT + Antibiotics (Amoxicillin, Metronidazole) NSPT + Surgical therapy (Modified Widman's Flap);NSPT + NSAID'S (Cox-2 inhibitors).

Based on the observation of included studies, it can be outlined that GCF is an effective and efficient prognostic tool by means of analysis of variations in constituents before and after periodontal therapy.

Meta Analysis of the included studies was not possible due to heterogeneity in study designs, cohort, and sampling techniques. It is suggested that future studies should follow standardized and coherent GCF sampling and analysis.

9.9. Controversies

Though GCF analysis can provide site-specific status of inflammation without necessitating histopathological evaluation, it is still rife with controversy. Firstly, GCF quantity outflow along with inflammatory status could also be affected by extent of sulcular epithelium ulceration.

There is a wide variation in the methods of collection, storage and analysis along with elution protocols making the values alter based on this variation. Also, timing of fluid sample collection could alter results. Another important consideration is dry or buffer based storage of

samples. Though recommended temperature of sample storage is –80 degrees centigrade, whether, all samples collected for various studies strictly adhere to this protocol is questionable.

9.10. Limitations

1. This analysis included studies where different components of GCF were evaluated biochemically and therefore could not be used to detect differences in prognostic value of GCF.
2. Verification of bias not carried out

10. 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 exudates, 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, followed by a systematic review of studies conducted in the past fifteen years, it can be concluded that various constituents of GCF such as Cytokines, Matrix Metalloproteinases, PGE-2, Aspartate Aminotransferase, Neutrophil-Elastase, Alkaline-Phosphatase, Osteocalcin, Calprotectin, Alpha-2 Microglobulins, Beta-2 Macroglobulins can be used as effective and efficient diagnostic tool 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.

Conflict of interest

None.

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