Review Article

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Dentistry Section

Biomarkers of Periodontal Disease and the Biologic Matrix Conundrum: A Narrative Review

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ABSTRACT

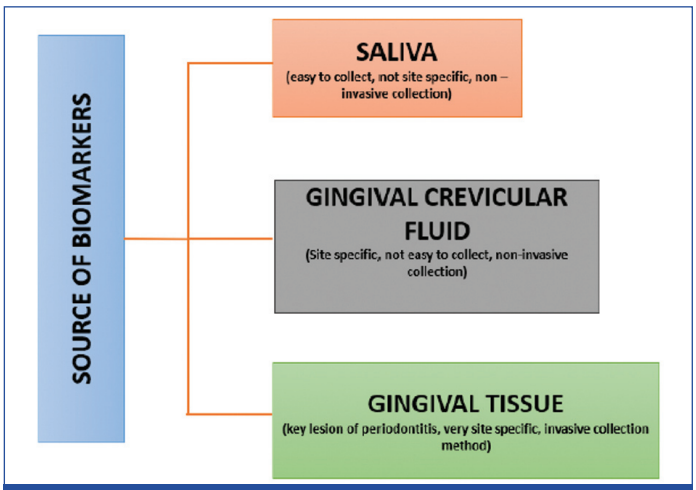
Monitoring periodontal disease using clinical parameters is time-consuming and reflects past destruction only. The clinical measurements are also prone to error with possible inter-examiner variability. Within the scope of current knowledge and understanding of periodontal pathogenesis, biomarkers play a crucial role in revealing ongoing periodontal disease activity. These biomarkers are identified from various biological matrices such as saliva, Gingival Crevicular Fluid (GCF), serum, gingival tissue, or even plaque samples. Most studies have utilised saliva and GCF due to their ease of collection and non invasive sampling to identify biomarkers of periodontal disease activity; consequently, most hypotheses regarding the initiation and progression of periodontal disease have been derived from such studies. Most proteins are secreted by tissues and then seep into GCF and saliva. Therefore, protein expression occurs in the tissues and their amounts in GCF or saliva represent only a small part of the total. The sensitivity of detecting and monitoring a given biomarker in GCF, saliva, or gingival tissue is debatable. The appropriateness of the biological matrix from which the biomarkers have been isolated and identified has not been validated. The present review highlights the need to address the choice of biological matrix suitable for studying biomarkers involved in periodontal disease pathogenesis and summarises promising biomarkers of periodontal disease activity in saliva, GCF and gingival tissues.

Keywords: Gingival crevicular fluid, Gingival tissue, Pathogenesis, Periodontitis, Proteomics, Saliva

INTRODUCTION

Periodontal disease has a multifactorial aetiology and is a cumulative influence of genetic, environmental, microbial and host immune response factors. Studies indicate the host response as a key factor in the initiation and progression of periodontal tissue destruction [1,2]. Early detection of periodontal disease plays a key role in successful therapy. However, monitoring periodontal disease progression is highly demanding and tedious, as it involves documenting repeated clinical measures at multiple sites. Clinical parameters such as bleeding on probing, probing pocket depth, clinical attachment loss and radiographic bone loss represent past disease only. Moreover, they are also error-prone measurements with inter-individual variability [3]. Furthermore, a significant amount of damage to the tooth's supporting structures must have taken place before diagnostic parameters are able to assess the amount or severity of disease [4]. Biomarkers of disease activity, on the other hand, are appropriate for measuring current disease activity and are pivotal in understanding cellular and molecular responses during host–microbe interactions [5]. Hence, researchers have devoted themselves to identifying diagnostic and prognostic biomarkers. Available literature on biomarkers of periodontal activity has largely utilised saliva and GCF as biological matrices. The predominant reason for this is the ease of availability and non invasive collection of samples. Serum has also been used to detect biomarkers in a very small number of studies, though serum is not specific for periodontal disease monitoring [6,7].

Gingival tissue samples to analyse biomarkers of periodontal disease activity have been reported [8,9]. The gingival/periodontal pocket tissue is more directly indicative of inflammatory events than GCF and saliva, which reflect inflammatory events of the underlying pathological processes in the periodontal pocket tissue [10]. However, tissue collection is invasive and sampling is possible only during extraction of hopeless teeth or during periodontal surgical procedures. The choice of biological matrix from which a particular marker is sampled is crucial, as the matrix should be the most appropriate source for that biomarker. The sensitivity of detection of the biomarker depends on where the molecule is secreted and which matrices the molecule passes through physiologically [11]. Changes in biomarker concentration can be expected depending on the matrix from which it is collected. This biological-matrix conundrum remains unresolved and is not adequately addressed in the literature. The present review summarises promising biomarkers of periodontal disease activity in saliva, GCF and gingival tissues and highlights the need to address the choice of biological matrix suitable for studying biomarkers involved in periodontal disease pathogenesis. Biomarkers in serum have not been discussed, as serum is not specific to the oral cavity. The review also provides an account of how proteomic approaches offer greater promise for biomarker identification. Biomarkers of periodontal disease activity have been identified in various biological matrices such as saliva, GCF and gingival tissues, as summarised in [Table/Fig-1]. Saliva and GCF have the advantages of ease of availability



[Table/Fig-1]: Various biologic matrices as source of biomarkers for periodontal disease. (Image hand-drawn by authors).

Saliva as a biologic matrix:

and non invasive collection techniques. Saliva is secreted by the parotid, submandibular and sublingual glands and other minor salivary glands. The fluid is readily accessible and can be collected non invasively. It is also abundant and can be sampled in a much larger volume without the need for clinical facilities or complex skills, compared with GCF. Saliva contains components that reflect the activity of all periodontal sites and therefore its composition mirrors the overall, whole-mouth inflammatory status rather than activity at individual sites, in contrast to GCF [12]. There are two types of saliva samples that can be collected: whole unstimulated saliva and stimulated saliva. Whole unstimulated saliva is reported to be suitable for biomarker analysis.

The composition of saliva includes locally produced host mediators and microbial products, among other enzymes and minerals. Investigators over the past two decades have used saliva for the detection of risk of dental caries, periodontal disease, oral cancer, breast cancer; detection of hormones and drugs; therapeutic monitoring of drugs such as digoxin and methadone; and many other systemic disorders [10,13].

Biomarkers in saliva [Table/Fig-2]: Matrix Metalloproteinase-8 (MMP-8) is reported in studies as the most promising biomarker candidate for diagnosing and predicting the progression of periodontal disease in saliva. MMP-8 levels decrease after periodontal therapy, demonstrating its role in disease pathogenesis. MMP-8 also correlates well with periodontal severity [14,15]. Diagnostic power has also been demonstrated for certain proinflammatory markers such as Interleukin (IL)-1β, IL-6 and TNF-α [14,16]. Gursoy UK et al., reported that IL-1β and MMP-8 together were able to detect periodontitis more accurately than each marker alone [16]. Oxidative stress markers have also been extensively studied and found to be biomarkers of disease activity [17]. A few other salivary biomarkers elevated in periodontal destruction include alkaline phosphatase, aspartate aminotransferase, Receptor Activator of NF-κB Ligand (RANKL)/Osteoprotegerin (OPG), visfatin, chemerin and soluble CD44 (a cell-surface adhesion molecule that mediates neutrophil adhesion and transendothelial migration) [18,19].

Biologic matrix	Promising biomarkers identified
Saliva	Inflammatory biomarkers, such as IL-1β, IL-6 and IL-8, MMP-8, TIMP-1 and TNF-α, Enzymes: α-glucosidase, alkaline phosphatase, aminopeptidases, β-galactosidase, β-glucosidase, caprylate esteraselipase, collagenase, elastase, esterase, gelatinase, kallikrein, kininase, lysozyme, myeloperoxidase, trypsin, Immunoglobulins: IgA, IgG. IgM, sIgA, Proteins: C-reactive protein, cystatins, epidermal growth factor, Fibronectin, Lactoderrin, Platelet activating factor, Vascular endothelial factor, Phenotypic markers: Epithelial keratins, Host cells: Leucocytes, Ions: Calcium, Hormone: Melatonin, insulin, epidermal growth factor, leptin, Steroids: Cortisol, androgens, (testosterone), estriol, estrogen, progesterone, aldosterone, DHEA, Growth Factors: EGF, NGF, VEGF, IGF, Nucleic Acids: Human Deoxyribonucleic Acid (DNA), microbial DNA, mRNA, siRNA, microRNA (miR-125a and miR-200a) and salivary proteomic biomarkers from [Table/Fig-4]
GCF	Inflammatory mediators- interleukin-1beta (IL-1β), IL-2, IL-6, IL-8, IL-17 and tumour Necrosis Factor-alpha (TNF-α), Monocyte Chemoattractant Protein-1 (MCP-1), Pentraxin-3, periodontal disease-specific biomarkers including; visfatin, leptin, adiponectin and resistin, Host-derived enzymes: MMP-3, MMP-13 and TIMP-1, Markers of oxidative stress: melatonin, Markers of bone homeostasis: Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen, receptor activator of nuclear factor-κB-ligand (RANK-L), OPG and osteopontin, markers for monitoring periodontal wound healing: Cell adhesion molecules, periostin, progress of periodontal repair and regeneration: transforming growth factor-beta, Normal turnover of periodontal ligament, root cement formation and maintenance and bone homeostasis: Alkaline phosphatase, Periodontal disease activity markers: beta-glucuronidase, cathepsin B, Collagenase-2 (MMP-8) and gelatinase (MMP-9), Dipeptidyl peptidases II and IV, Elastase and biomarkers from proteomic profiling of GCF in [Table/Fig-4]
Gingival tissue	IL-1β, TNF-α, IL-2 and IFN-γ, IL-11, IL-17, IL-4, IL-10, IL-6 and biomarkers from proteomic profiling in [Table/Fig-4]

[Table/Fig-2]: Biomarkers of periodontal disease activity identified in various biologic matrices [7].
DHEA: Dehydroepiandrosterone, EGF: Epidermal growth factor, NGF: Nerve growth factor, DNA: Deoxyribonucleic acid, mRNA: messenger Ribonucleic acid; VEGF: Vascular endothelial growth factor; IGF: Insulin-like growth factor

Gingival Crevicular Fluid (GCF) as a biological matrix: GCF is an inflammatory exudate. Griffith GS, in his review, states that the fluid originates from the blood vessels of gingival connective tissue and permeates through the diseased soft tissue of the periodontal pocket [20]. GCF is assumed to reflect the ongoing response generated by cells and tissues in the periodontium and is therefore considered a promising source of biomarkers of periodontal disease activity. GCF is collected by filter-paper strips, braided threads, micropipettes, etc., by either the intra-crevicular or the extra-crevicular approaches. The intra-crevicular approach is most commonly used [21]. The volume of GCF thus collected needs to be weighed immediately using a Periotron to reduce evaporation. The volume of GCF collected is an indicator of the extent of inflammation and reporting GCF volume is also useful for calculating biomarker concentrations in the GCF sample. Very low volumes of GCF collected have a dramatic effect on the biomarker concentration calculated [22]. GCF collection is non invasive and its site-specific presence makes it a valuable sample for deciphering biomarkers and their role in periodontal pathogenesis. Collecting GCF, however, is not without challenges despite the advantages. GCF analysis is time-consuming and requires sampling from multiple tooth sites to obtain an adequate amount of GCF. The procedure is labour-intensive and somewhat technically demanding, requiring equipment for calibrating and measuring fluid volumes [23].

Biologic markers in GCF [Table/Fig-2]: Chapple ILC et al., in a review, reported that host-derived molecules such as cathepsin B, alkaline phosphatase and beta-glucuronidase are promising biomarkers and showed greater than 77% diagnostic accuracy in predicting future periodontal disease activity [24]. Some biomarkers reported to be associated with disease activity are MMP-8, MMP-9 and dipeptidyl peptidases [25,26]. Reports in the literature suggest that inflammatory cytokines such as IL-1β, TNF-α, IL-17, IL-2 and IL-6 are potential biomarkers that are reliable inflammatory markers [27]. The level of these markers is reportedly reduced after periodontal therapy, indicating their involvement in the disease process. One of the most studied cytokines is IL-1β, which is also a potent bone-resorbing factor [21]. Monocyte Chemoattractant Protein (MCP)-1 and MCP-4, chemokines, in saliva and GCF were reported to increase with disease progression and decrease after treatment and hence are proposed as potential biomarkers [28]. Adipokines associated with higher levels in periodontitis show a decrease after therapy and therefore have been suggested as potential biomarkers [29]. MMP-8 is considered a robust biomarker, very specific for the early detection and progression of periodontal disease [27]. Other host-derived enzymes such as alkaline phosphatase and myeloperoxidase have also been investigated for biomarker potential [30]. Oxidative-stress markers in GCF have been studied, given that current knowledge of periodontal pathogenesis places emphasis on the balance between reactive oxygen species and antioxidants underlying tissue destruction [31].

Bone homeostasis markers in GCF that have been investigated for biomarker potential are pyridinoline cross-linked carboxyterminal telopeptide of type I collagen, RANKL, OPG and osteopontin [32]. Certain tissue breakdown products such as soluble CD40 ligand have been reported in studies. Calprotectin and periostin are other molecules that have been evaluated for biomarker potential, with suggested protective roles in periodontitis [31,33].

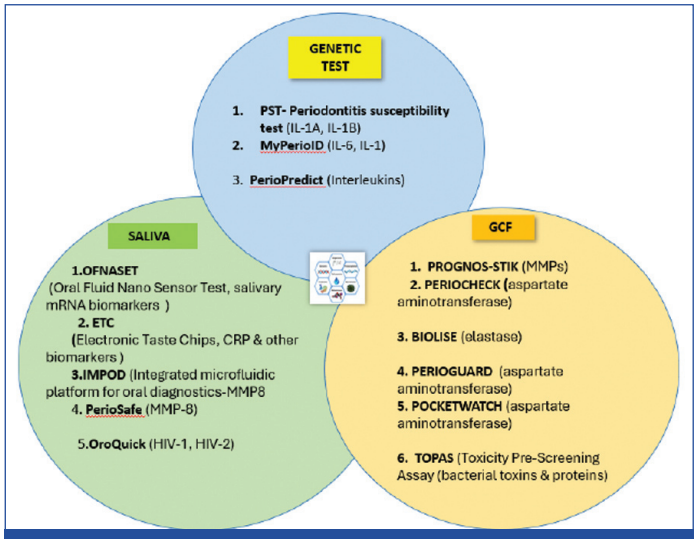
Gingival/pocket tissue as biologic matrix: The gingival/pocket tissue is the key anatomo-pathological lesion in periodontitis. It is from this tissue that all molecules released or secreted during periodontal destruction reach GCF or saliva by microleakage from capillaries and venules. However, collecting gingival tissue is

invasive and can only be performed in certain situations. Only a handful of studies have analysed potential biomarkers in gingival tissue/pocket tissues. In the early 2000s and in the 1990s, a few authors published reports on cytokines detected in gingival tissues [8,34]. For proteomic profiling, gingival tissue samples are claimed to be more suitable compared to saliva and GCF [12].

Biologic markers in gingival tissue: Gorska R et al., analysed and correlated key cytokines in gingival tissue with clinical parameters and reported that high absolute levels of IL-1β, TNF-α, IL-2 and IFN-γ and, especially, their high ratios to IL-4 and IL-10 found in inflamed tissue biopsies- were closely associated with periodontal disease severity [35]. Other biomarkers analysed in tissue samples included IL-11, IL-17, IL-1β, IL-6 and IL-8 by McGee JM et al., and IL-1α, IL-1β and TNF-α by Stashenko P et al., [8,32]. After 2005, authors predominantly worked on GCF and saliva. Later, two papers by Monari E et al., and Bertoldo C et al., in 2015 and 2012 respectively, on proteomic profiling using mass spectrometry (MS) identified unique markers in gingival tissue [9,12].

Biologic-matrix conundrum [36]: The biomarkers identified are mostly proteins and are secreted by cells in tissues. Therefore, it is reasonable to assume that most of their expression occurs in gingival/pocket tissue and that via microleakage from capillaries a small volume of the secreted molecules reaches GCF or saliva. GCF/saliva represents only a small portion of it [17]. In light of the above, the extent to which cytokines detected in various biologic matrices correspond with and the sensitivity of detecting both the mRNA and protein levels of a particular cytokine in the same biologic matrix, is questionable according to some authors. IL-6 detection in tissues is reportedly higher than its detection in GCF in a study [37]. Another study compared GCF cytokine expression with the corresponding values from the same tissue sites for expression of interferon-gamma, IL-1β, IL-6, IL-17A and IL-17F and stated that GCF may not express all the markers in the same proportion as at the corresponding tissue site [38]. Therefore, the choice of biologic matrix from which a particular marker is sampled is crucial for it to be a potential biomarker. Thus, the appropriateness of the matrix for the potential biomarker molecule identified should be clarified prior to its assessment. This conundrum remains unresolved.

Biomarkers utilised in chairside diagnostics: Chairside diagnostic kits [Table/Fig-3] using saliva and GCF have been developed and are documented in the literature. However, their efficacy in day-to-day practice appears limited and therefore they are not routinely used in practice [39,40,41].



[Table/Fig-3]: Chair-side diagnostic kits for periodontal disease diagnosis [40,41].

Methods used for analysis of biomarkers in literature: Some of the most commonly used assays are Enzyme-Linked Immunosorbent Assay (ELISA), microarrays and radioimmunoassays for biomarker identification in research. Recently, proteomic profiling has emerged as the best approach for preliminary unbiased biomarker research [42].

The ELISA is a technique that has been extensively used and documented in periodontal literature, as it is sensitive, well-established and a widely available biochemical diagnostic tool. ELISA-based techniques are relatively cost-effective, simple, highly reproducible and inexpensive to perform. However, one key disadvantage of using ELISA is that its antibodies identify only pretargeted proteins. Without prior knowledge of the protein involved in pathogenesis, we cannot use ELISA to identify it. Microarray is another method similar to ELISA, with the advantage of targeting multiple proteins simultaneously and requiring minimal sample volume. However, it shares the same disadvantage as ELISA in that it is specific and identifies only targeted proteins with prior knowledge [43]. Radioimmunoassay is a very sensitive and specific technique to identify even trace amounts of labeled antigen, but it again detects only targeted antigens [44]. Proteomics is the study of the proteome, the complete set of proteins expressed by an organism in a biological matrix. Proteomic approaches look at the entire proteome, not just a handful of individual proteins and therefore offer an unbiased, non targeted means of protein discovery [45].

Proteomic approaches have been utilised in recent years in periodontology to understand the proteome, the complete set of proteins expressed in periodontal tissue at a given time. Many novel biomarkers have been unravelled using this powerful technology. Proteomics is a powerful means to study differentially regulated proteins in disease and health, along with associated pathways and protein-protein interactions. The proteomic tools used in investigations include Polyacrylamide Gel Electrophoresis (PAGE), High-Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), Matrix-Assisted Laser Desorption Ionisation (MALDI) and Surface-Enhanced Laser Desorption/Ionisation–Mass Spectrometry (SELDI-MS) and label-free proteomic technology for high-throughput outcomes [46]. It is believed to uncover newer and novel biomarkers that were not investigated previously and are probably crucial to periodontal pathogenesis [Table/Fig-4] [9,12,47-57].

Limitations and challenges in proteomic approaches: In the majority of studies, due to heterogeneity of sampling sites, a pooled approach is used [9,57]. It is reported that a number of individual proteins are lost in the approach [45]. Moreover, infrequent detection of low-abundance proteins, such as cytokines, is also reported as an issue with the current proteomic approaches. Since more abundant proteins interfere with MS detection of less abundant proteins, lately shotgun proteomic strategies based on digesting proteins into peptides and sequencing them using tandem MS and automated database searching have become the method of choice. Despite the latest advances in proteomic technology, the salivary or GCF proteome is far from fully mapped, as stated by Bostanci N and Bao K [42].

Challenges in biomarker identification in periodontal disease: Due to the complex and multifactorial nature of periodontal disease, the identification of a single specific biomarker is considered illusory [58]. It is said that a combination of biomarkers may provide more accurate information on disease progression and diagnosis. The biomarkers and the few chairside kits that were developed are reported to have not resulted in major conceptual changes in the field of periodontal diagnostics, as they fall short of providing clinically useful information that can modify treatment planning [59]. Biomarker research reported in the literature is largely cross-sectional, with small sample sizes and very few longitudinal studies. Evidence of associations of molecules from such studies with periodontal pathogenesis is therefore not reliable. Even the handful of promising biomarkers identified from studies have not been

Medium / biologic matrix	Authors/ year	Proteomic technology used	Sample size	Potential biomarker molecules differentially expressed in periodontitis
GCF	1.Bostanci N et al., [47]	Label-free absolute quantitative proteomics in human Gingival Crevicular Fluid (GCF) by LC/MSE	Sample size: 10 subjects (5 healthy and 5 generalised aggressive periodontitis)	Proportion of bacterial, viral and yeast protein was increased in periodontal disease. Cystatin-B and defensins and Annexin1 molecules were reported only in health while L-plastin was detected in disease only.
	2. Choi Y-J et al., [48]	LC- ESI-MS/MS coupled to nano-LC	Sample size:11 healthy, 12 moderate periodontitis	Azurocidin could be a potential biomarker candidate for the early detection of inflammatory periodontal destruction
	3.Baliban RC et al., [49]	High-performance liquid chromatography and fragmented using tandem Mass Spectrometry (MS/MS)	Sample size: 12 chronic periodontitis and 12 healthy	Angiotensinogen, clusterin and thymidine phosphorylase identified in health. 33 kDa chaperonin, iron uptake protein A2 and phosphoenolpyruvate carboxylase (health-associated) and ribulose biphosphate carboxylase, a probable succinyl-CoA:3-ketoacid-coenzyme A transferase, or DNA-directed RNA polymerase subunit beta (CP associated)
	4. Kido J et al., [50]	Nano liquid chromatography coupled with tandem MS	Sample size: 9 subjects- 8 periodontitis and 1 healthy	Mostly blood, cytoskeleton, immunity-inflammation and lipid related proteins and enzymes were identified. Ceruloplasmin, glycogen phosphorylase, glutathione S-transferase, phosphoglycerate mutase, psoriasin, S100A11 and resistin, were identified for the first time in GCF
	5. Ngo LH et al., [51]	Matrix-Assisted Laser Desorption/ Ionisation Time-of-Flight (MALDI-TOF) MS	Sample size: 41 periodontal maintenances were followed and GCF collected at each visit	Models generated from the GCF mass spectra could predict attachment loss at a site with a high specificity (97% recognition capability and 67% cross-validation).
	6.Guzman YA et al., [52]	Samples were analysed using an online liquid chromatography-nanoelectrospray-hybrid ion trap-Orbitrap mass spectrometer. Spectra were processed with the PILOT_PROTEIN proteomics software suite	10 chronic periodontitis patients were analysed	Azurocidin, lysozyme C and myosin-9 as candidate biomarkers prominent at baseline and alpha-smooth muscle actin as prominent 13 weeks after treatment.
Saliva	1. Wu Y et al [53]	Electrospray ionisation tandem MS	Sample size: 5 healthy and 5 generalised aggressive periodontitis participants	Proteins increased in generalised aggressive periodontitis were levels of serum albumin, Immunoglobuling (Ig) gamma2 chain C region, Ig alpha2 chain C region, vitamin D-binding protein, salivary alpha-amylase and zinc-alpha2 glycoprotein were increased in whole unstimulated saliva.
	2. Haigh BJ et al., [54]	(MALDI-TOF) MS	Sample size: 9 participants evaluated before and after periodontal therapy	Predominant observation was increase in the abundance of the S100 proteins S100A8/ A9/A6. Other altered proteins in saliva were haptoglobin, prolactin inducible protein and parotid secretory protein.
	3. Goncalves LDR et al., [55]	Liquid chromatography on line to electrospray ionisation Quadrupole Time-of-flight MS		Study reported that gingival inflammation was associated with increased amounts of blood proteins (serum albumin and haemoglobin), immunoglobulin peptides and keratins. In the control group, salivary cystatins, which were detected.
	4. Salazar MG et al., [56]	Label free LC-MS/MS	Sample size: 20 healthy and 20 periodontitis participants	Of the 344 proteins identified, 20 proteins showed 1.5-fold difference in abundance between controls and patients. Majority of them were linked with acute phase response and inflammatory processes
	5. Hartenbach FARR et al., [57]	LC-MS/MS	Sample size:5 healthy and 15 diseased pools were sampled	Author stated that salivary acidic proline-rich phosphoprotein, submaxillary gland androgen-regulated protein, histatin-1, fatty acid binding protein, thioredoxin and cystatin-SA were predominant in diseased patients.
Gingival tissue	1. Bertoldi C et al., [12]	LC-MS/MS analysis	Sample size: 25 periodontitis patients compared with healthy sites in the same patient	Proteins under expressed in periodontitis were annexin A2, actin cytoplasmic 1, carbonic anhydrase 1 & 2; Ig kappa chain C region (two spots) and Flavin reductase were overexpressed, whereas 14-3-3 protein sigma and zeta/delta, heat-shock protein beta -1 (two spots), triosephosphateisomerase, peroxiredoxin-1, fatty acid-binding protein-epidermal and galectin-7.
	2. Monari E et al., [9]	LC-MS/MS	Sample size: 15 periodontally healthy and equal number of periodontally diseased samples were taken	A total of 32 proteins were identified out of which S100A9, HSPB1, LEG7 and 14-3-3 proteins were validated by western blot.

[Table/Fig-4]: Proteomic profiling studies in GCF, saliva and gingival tissues in periodontitis [9,12,47-57].

widely validated in populations worldwide to establish their practical relevance in periodontal practice [60]. Proteomic approaches have identified newer molecules not detected earlier in disease pathogenesis; however, they still require validation [61].

CONCLUSION(S)

The present review provides an understanding of available markers involved in periodontal disease pathogenesis. Despite the availability of various matrices as sources for biomarker identification, the need for choosing an appropriate matrix for biomarker isolation is established in this review. In the current scenario of biomarker research, before embarking on the quest for biomarkers one has to give deep thought to the choice of the biological matrix chosen as a sample, as this may greatly affect the quality, quantity and sensitivity of the molecule in question.

REFERENCES

[1] Van Dyke TE, Bartold PM, Reynolds EC. The nexus between periodontal inflammation and dysbiosis. Front Immunol. 2020;11:511. Available from: https://doi.org/10.3389/fimmu.2020.00511.

[2] Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64:57-80. Available from: https://doi.org/10.1111/prd.12002.

[3] Ryan ME. Clinical attachment level change as an outcome measure for therapies that slow the progression of periodontal disease. J Int Acad Periodontol. 2005;7:162-71; discussion 172-174.

[4] Needleman I, McGrath C, Floyd P, Biddle A. Impact of oral health on the life quality of periodontal patients. J Clin Periodontol 2004;31:454-57. Available from: https://doi.org/10.1111/j.1600-051X.2004.00498.x.

[5] Miller CS, King CP, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: A cross-sectional study. J Am Dent Assoc. 2006;137:322-29. Available from: https://doi.org/10.14219/jada.archive.2006.0181.

[6] Jaedicke KM, Preshaw PM, Taylor JJ. Salivary cytokines as biomarkers of periodontal diseases. Periodontol 2000. 2016;70:164-83. Available from: https://doi.org/10.1111/prd.12117.

[7] Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000. 2016;70:53-64. Available from: https://doi.org/10.1111/prd.12107.

[8] McGee JM, Tucci MA, Edmundson TP, Serio CL, Johnson RB. The relationship between concentrations of proinflammatory cytokines within gingiva and the adjacent sulcular depth. J Periodontol. 1998;69:865-71. Available from: https://doi.org/10.1902/jop.1998.69.8.865.

[9] Monari E, Cuoghi A, Bellei E, Bergamini S, Lucchi A, Tomasi A, et al. Analysis of protein expression in periodontal pocket tissue: A preliminary study. Proteome Sci 2015;13:33. Available from: https://doi.org/10.1186/s12953-015-0089-y.

[10] Malamud D, Rodriguez-Chavez IR. Saliva as a diagnostic fluid. Dent Clin North Am. 2011;55:159-78. Available from: https://doi.org/10.1016/j.cden.2010.08.004.

[11] Zhang L, Henson BS, Camargo PM, Wong DT. The clinical value of salivary biomarkers for periodontal disease. Periodontology 2000. 2009;51:25-37. Available from: https://doi.org/10.1111/j.1600-0757.2009.00315.x.

[12] Bertoldi C, Bellei E, Pellacani C, Ferrari D, Lucchi A, Cuoghi A, et al. Non-bacterial protein expression in periodontal pockets by proteome analysis. J Clin Periodontol 2013;40:573-82. Available from: https://doi.org/10.1111/jcpe.12050.

[13] Rathnayake N, Gieselmann D-R, Heikkinen AM, Tervahartiala T, Sorsa T. Salivary Diagnostics-Point-of-Care diagnostics of MMP-8 in dentistry and medicine. Diagnostics (Basel) 2017;7:E7. Available from: https://doi.org/10.3390/diagnostics7010007.

[14] Costa PP, Trevisan GL, Macedo GO, Palioto DB, Souza SLS, Grisi MFM, et al. Salivary interleukin-6, matrix metalloproteinase-8, and osteoprotegerin in patients with periodontitis and diabetes. J Periodontol. 2010;81:384-91. Available from: https://doi.org/10.1902/jop.2009.090510.

[15] Kinney JS, Ramseier CA, Giannobile WV. Oral fluid–based biomarkers of alveolar bone loss in periodontitis. Annals of the New York Academy of Sciences. 2007;1098:230-51. Available from: https://doi.org/10.1196/annals.1384.028.

[16] Gursoy UK, Könönen E, Pradhan-Palikhe P, Tervahartiala T, Pussinen PJ, Suominen-Taipale L, et al. Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. J Clin Periodontol. 2010;37:487-93. Available from: https://doi.org/10.1111/j.1600-051X.2010.01563.x.

[17] Loos BG, Tjoa S. Host-derived diagnostic markers for periodontitis: Do they exist in gingival crevice fluid? Periodontology 2000. 2005;39:53-72. Available from: https://doi.org/10.1111/j.1600-0757.2005.00129.x.

[18] Villa-Correa YA, Isaza-Guzmán DM, Tobón-Arroyave SI. Prognostic value of 8-Hydroxy-2'-Deoxyguanosine and human neutrophil elastase/α1-proteinase inhibitor complex as salivary biomarkers of oxidative stress in chronic periodontitis. J Periodontol. 2015;86:1260-67. Available from: https://doi.org/10.1902/jop.2015.150293.

[19] Nomura Y, Shimada Y, Hanada N, Numabe Y, Kamoi K, Sato T, et al. Salivary biomarkers for predicting the progression of chronic periodontitis. Arch Oral Biol. 2012;57:413-20. Available from: https://doi.org/10.1016/j.archoralbio.2011.09.011.

[20] Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontol 2000. 2003;31:32-42. Available from: https://doi.org/10.1034/j.1600-0757.2003.03103.x.

[21] Nazar Majeed Z, Philip K, Alabsi AM, Pushparajan S, Swaminathan D. Identification of gingival crevicular fluid sampling, analytical methods, and oral biomarkers for the diagnosis and monitoring of periodontal diseases: A systematic review. Dis Markers. 2016;2016:1804727. Available from: https://doi.org/10.1155/2016/1804727.

[22] Guentsch A, Kramesberger M, Sroka A, Pfister W, Potempa J, Eick S. Comparison of gingival crevicular fluid sampling methods in patients with severe chronic periodontitis. J Periodontol. 2011;82:1051-60. Available from: https://doi.org/10.1902/jop.2011.100565.

[23] Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. Periodontol 2000. 2016;70:65-79. Available from: https://doi.org/10.1111/prd.12109.

[24] Chapple ILC. Periodontal diagnosis and treatment- where does the future lie? Periodontol 2000. 2009;51:09-24. Available from: https://doi.org/10.1111/j.1600-0757.2009.00319.x.

[25] Chapple ILC, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000. 2007;43:160-232. Available from: https://doi.org/10.1111/j.1600-0757.2006.00178.x.

[26] Luchian I, Goriuc A, Sandu D, Covasa M. The Role of Matrix Metalloproteinases (MMP-8, MMP-9, MMP-13) in Periodontal and Peri-Implant Pathological Processes. Int J Mol Sci. 2022;23(3):1806. Doi: 10.3390/ijms23031806.

[27] Sorsa T, Gursoy UK, Nwhator S, Hernandez M, Tervahartiala T, Leppilahti J, et al. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontol 2000. 2016;70:142-63. Available from: https://doi.org/10.1111/prd.12101.

[28] Stadler AF, Angst PDM, Arce RM, Gomes SC, Oppermann RV, Susin C. Gingival crevicular fluid levels of cytokines/chemokines in chronic periodontitis: A meta-analysis. J Clin Periodontol. 2016;43:727-45. Available from: https://doi.org/10.1111/jcpe.12557.

[29] Selvarajan S, Perumalsamy R, Emmadi P, Thiagarajan R, Namasivayam A. Association between gingival crevicular fluid leptin levels and periodontal status-a biochemical study on Indian patients. J Clin Diagn Res. 2015;9:ZC48-ZC53. Available from: https://doi.org/10.7860/JCDR/2015/12335.5941.

[30] Kunjappu JJ, Mathew VB, Hegde S, Kashyap R, Hosadurga R. Assessment of the alkaline phosphatase level in gingival crevicular fluid, as a biomarker to evaluate the effect of scaling and root planing on chronic periodontitis: An in vivo study. J Oral Maxillofac Pathol 2012;16:54-57. Available from: https://doi.org/10.4103/0973-029X.92974.

[31] Chaturvedi R, Gupta M, Jain A, Das T, Prashar S. Soluble CD40 ligand: A novel biomarker in the pathogenesis of periodontal disease. Clinical Oral Investigations. 2015;19:45-52. Available from: https://doi.org/10.1007/s00784-014-1216-3.

[32] Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. J Periodontol.1991;62:504-09. Available from: https://doi.org/10.1902/jop.1991.62.8.504.

[33] Kumaresan D, Balasundaram A, Naik VK, Appukuttan DP. Gingival crevicular fluid periostin levels in chronic periodontitis patients following nonsurgical periodontal treatment with low-level laser therapy. Eur J Dent. 2016;10(4):546-50. Available from: https://doi.org/10.4103/1305-7456.195179.

[34] Johnson RB, Wood N, Serio FG. Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. J Periodontol. 2004;75:37-43. Available from: https://doi.org/10.1902/jop.2004.75.1.37.

[35] Górska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madaliński K. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. J Clin Periodontol. 2003 Dec;30(12):1046-52. Doi: 10.1046/j.0303-6979.2003.00425.x. PMID: 15002890.

[36] Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DTW. Salivary biomarkers: Toward future clinical and diagnostic utilities. Clin Microbiol Rev. 2013;26:781-91. https://doi.org/10.1128/CMR.00021-13.

[37] Duarte PM, De Lorenzo Abreu L, Vilela A, Feres M, Giro G, Miranda TS. Protein and mRNA detection of classic cytokines in corresponding samples of serum, gingival tissue and gingival crevicular fluid from subjects with periodontitis. J of Periodontal Research 2019;54:174-79. Available from: https://doi.org/10.1111/jre.12617.

[38] Lomba KSB, Beiler TFC de SB, Sete MRC, Pires FR, Figueredo CM da S. Use of minimally invasive gingival biopsies in the study of inflammatory mediators expression and their correlation with gingival fluid in patients with chronic periodontitis. Indian J Dent Res. 2015;26:126-30. Available from: https://doi.org/10.4103/0970-9290.159134.

[39] Miranda TS, Heluy SL, Cruz DF, da Silva HDP, Feres M, Figueiredo LC, et al. The ratios of pro-inflammatory to anti-inflammatory cytokines in the serum of chronic periodontitis patients with and without type 2 diabetes and/or smoking habit. Clin Oral Investig. 2019;23:641-50. Available from: https://doi.org/10.1007/s00784-018-2471-5.

[40] Ko T-J, Byrd KM, Kim SA. The chairside periodontal diagnostic toolkit: Past, present, and future. Diagnostics (Basel). 2021;11:932. Available from: https://doi.org/10.3390/diagnostics11060932.

[41] Mani A, Anarthe R, Marawar PP, Mustilwar RG, Bhosale A. Diagnostic kits: An aid to periodontal diagnosis. Journal of Dental Research and Review. 2016;3:107. Available from: https://doi.org/10.4103/2348-2915.194837.

[42] Bostanci N, Bao K. Contribution of proteomics to our understanding of periodontal inflammation. Proteomics. 2017;17:1500518. Available from: https://doi.org/10.1002/pmic.201500518.

[43] Bostanci N, Heywood W, Mills K, Parkar M, Nibali L, Donos N. Application of label-free absolute quantitative proteomics in human gingival crevicular fluid by LC/MS E (gingival exudatome). J Proteome Res. 2010;9:2191-99. Available from: https://doi.org/10.1021/pr900941z.

[44] Shimada Y, Tabeta K, Sugita N, Yoshie H. Profiling biomarkers in gingival crevicular fluid using multiplex bead immunoassay. Arch Oral Biol. 2013;58:724-30. Available from: https://doi.org/10.1016/j.archoralbio.2012.11.012.
[45] Guzman YA, Sakellari D, Arsenakis M, Floudas CA. Proteomics for the discovery of biomarkers and diagnosis of periodontitis: A critical review. Expert Review of Proteomics 2014;11:31-41. Available from: https://doi.org/10.1586/14789450.2014.864953.
[46] Khurshid Z, Mali M, Naseem M, Najeeb S, Zafar MS. Human Gingival Crevicular Fluids (GCF) proteomics: An overview. Dent J (Basel) 2017;5:E12. Available from: https://doi.org/10.3390/dj5010012.
[47] Bostanci N, Ramberg P, Wahlander Å, Grossman J, Jönsson D, Barnes VM, et al. Label-free quantitative proteomics reveals differentially regulated proteins in experimental gingivitis. J Proteome Res. 2013;12:657-78. Available from: https://doi.org/10.1021/pr300761e.
[48] Choi Y-J, Heo S-H, Lee J-M, Cho J-Y. Identification of azurocidin as a potential periodontitis biomarker by a proteomic analysis of gingival crevicular fluid. Proteome Sci 2011;9:42. Available from: https://doi.org/10.1186/1477-5956-9-42.
[49] Baliban RC, Sakellari D, Li Z, DiMaggio PA, Garcia BA, Floudas CA. Novel protein identification methods for biomarker discovery via a proteomic analysis of periodontally healthy and diseased gingival crevicular fluid samples. J Clin Periodontol. 2012;39:203-12. Available from: https://doi.org/10.1111/j.1600-051X.2011.01805.x.
[50] Kido J, Bando M, Hiroshima Y, Iwasaka H, Yamada K, Ohgami N, et al. Analysis of proteins in human gingival crevicular fluid by mass spectrometry. J Periodontal Res. 2012;47:488-99. Available from: https://doi.org/10.1111/j.1600-0765.2011.01458.x.
[51] Ngo LH, Darby IB, Veith PD, Locke AG, Reynolds EC. Mass spectrometric analysis of gingival crevicular fluid biomarkers can predict periodontal disease progression. J Periodontal Res. 2013;48:331-41. Available from: https://doi.org/10.1111/jre.12012.
[52] Guzman YA, Sakellari D, Papadimitriou K, Floudas CA. High-throughput proteomic analysis of candidate biomarker changes in gingival crevicular fluid after treatment of chronic periodontitis. J Periodont Res. 2018;53:853-60. Available from: https://doi.org/10.1111/jre.12575.
[53] Wu Y, Shu R, Luo L-J, Ge L-H, Xie Y-F. Initial comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients and healthy control subjects. J Periodontal Res. 2009;44:636-44. Available from: https://doi.org/10.1111/j.1600-0765.2008.01172.x.
[54] Haigh BJ, Stewart KW, Whelan JRK, Barnett MPG, Smolenski GA, Wheeler TT. Alterations in the salivary proteome associated with periodontitis. J Clin Periodontol. 2010;37:241-47. Available from: https://doi.org/10.1111/j.1600-051X.2009.01525.x.
[55] Gonçalves LDR, Soares MR, Nogueira FCS, Garcia C, Camisasca DR, Domont G, et al. Comparative proteomic analysis of whole saliva from chronic periodontitis patients. J Proteomics. 2010;73:1334-41. Available from: https://doi.org/10.1016/j.jprot.2010.02.018.
[56] Salazar MG, Jehmlich N, Murr A, Dhople VM, Holtfreter B, Hammer E, et al. Identification of periodontitis associated changes in the proteome of whole human saliva by mass spectrometric analysis. J Clin Periodontol. 2013;40:825-32. Available from: https://doi.org/10.1111/jcpe.12130.
[57] Hartenbach FARR, Velasquez É, Nogueira FCS, Domont GB, Ferreira E, Colombo APV. Proteomic analysis of whole saliva in chronic periodontitis. Journal of Proteomics. 2020;213:103602. Available from: https://doi.org/10.1016/j.jprot.2019.103602.
[58] Ghallab NA. Diagnostic potential and future directions of biomarkers in gingival crevicular fluid and saliva of periodontal diseases: Review of the current evidence. Archives of Oral Biology. 2018;87:115-24. Available from: https://doi.org/10.1016/j.archoralbio.2017.12.022.
[59] Khiste SV, Ranganath V, Nichani AS, Rajani V. Critical analysis of biomarkers in the current periodontal practice. J Indian Soc Periodontol. 2011;15:104-10. Available from: https://doi.org/10.4103/0972-124X.84376.
[60] Garbis S, Lubec G, Fountoulakis M. Limitations of current proteomics technologies. Journal of Chromatography A. 2005;1077:01-18. Available from: https://doi.org/10.1016/j.chroma.2005.04.059.
[61] Ou FS, Michiels S, Shyr Y, Adjei AA, Oberg AL. Biomarker discovery and validation: Statistical considerations. J Thorac Oncol. 2021;16:537-45. Available from: https://doi.org/10.1016/j.jtho.2021.01.1616.

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