C-TELOPEPTIDE AS DIAGNOSTIC MARKER FOR
ACTIVE PERIODONTAL DESTRUCTION- A REVIEW

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ABSTRACT

As classical periodontal disease manifests itself by alternating between periods of active tissue destruction and quiescent intervals it becomes essential to differentiate the two to formulate an adequate treatment plan at the earliest possible time. As current methods of periodontal diagnosis based on clinical parameters like probing depth and clinical attachment loss and radiological diagnostic methods prove inadequate for accurate diagnosis of active destruction areas, newer modalities which involve using biomarkers from oral fluids like saliva and gingival crevicular fluid are being advocated to supplement clinical diagnostics. As the predominant connective tissue component of periodontal tissues is collagen, the use of collagen degradation products like C-Telopeptides as disease specific biomarkers to identify collagen degradation and bone turnover is gaining current relevance. Hence use of C-TP as a proteome biomarker to identify active periodontal and peri-implant bone destruction from latent disease sites may be useful for predicting disease progression and earlier intervention.

KEYWORDS: C-Telopeptide, Oral Biomarkers, Proteome Diagnostics, Periodontal Disease, Peri-Implantitis, Collagen Degradation Products.

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INTRODUCTION

Periodontitis is a chronic, inflammatory disease of the supporting tissues of the teeth with an initial presentation of inflammation of the marginal and inter-dental gingiva. After its progression from gingivitis, the disease manifests as loss of connective tissue structures- specifically the loss of collagen fibers and its concomitant loss of attachment to the root surface, followed by apical migration of the pocket epithelium, formation of deep periodontal pockets and progressive resorption of alveolar bone leading to increased tooth mobility and finally tooth loss as an endpoint of the disease progression [1]. Traditionally diagnosis of periodontal disease is made using clinical parameters like measurement of probing depth, measurement of clinical attachment loss and the presence or absence of bleeding on probing to indicate periodontal disease sites. But the traditional clinical criteria used to identify periodontal disease are often insufficient for determining the presence of active disease sites, for monitoring quantitatively the response to periodontal therapy or for measuring the degree of susceptibility to future disease progression in healed or quiescent sites.

Hence the need for employing alternative diagnostic methods like microbiological, serological and radiographical to supplement clinical diagnosis becomes essential. Although serological biomarkers have been used with varying degree of success in diagnosing periodontal disease the justification of causality with remote origin of bone remodeling molecules does indicate a questionable pause in the widespread adaptation of serological markers.
for diagnosis of periodontitis.

Hence the ongoing process of developing biomarkers for diagnosis from areas closer to the periodontal disease sites has necessitated taking a closer look at oral biofluids which are at close proximity to the actual disease sites and can accurately reflect the changes taking place in the periodontal tissues.

One of the most exciting new developments in periodontal disease diagnosis is the use of biomarkers from oral fluids like saliva and GCF to obtain clinically relevant information and to supplement and reinforce clinical diagnosis.[2]. These biomarkers have become diagnostically important not only because they are specific for the unique pathological aspects of periodontal disease but also because the qualitative changes in the composition of these periodontitis specific bio-markers has a practical diagnostic value in identifying patients with enhanced disease susceptibility, in identifying sites with active continuing disease, in predicting sites that will have active disease in the near future and serving as surrogate end point for monitoring the efficacy of any therapeutic intervention undertaken to arrest the disease process and regenerate the lost tissues.[3].

Regardless of whether they are obtained from saliva or GCF these biochemical markers of bone remodulation processes are used to analyze changes in the organic bone matrix of the alveolar bone. The organic matrix of the periodontal tissues consists of predominantly collagen (approximately 95%) and non-collagenous proteins, the catabolic products of the organic bone components, enzymes of osteoblasts and osteoclasts like alkaline phosphatase, bone sialoprotein, osteopontin etc, as the calcium and phosphate levels in biologic fluids are the most reliable markers of active skeletal bone tissue remodulation. Hence the biochemical analyses of oral biofluids enable the determining of the rates of bone tissue reorganization and the current activity of osteosynthesis and osteoresorption processes with a fair degree of certainty.[4]

As collagen is the predominant connective tissue component of periodontal tissues a class of degradation molecules known as pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides which are released systemically during degradation of collagen matrix and bone resorption due to post-translational modification of collagen have emerged as valuable proteome markers for bone turnover and are very specific for periodontal disease. [5,6] Presence or absence of these markers accurately differentiates the active periodontal or peri-implant bone destruction from latent periodontal disease.[7]

Palys et al. related pyridinoline cross-linked carboxy terminal telopeptide of type I collagen (ICTP) levels to the subgingival microflora of various periodontal disease states in GCF and found ICTP levels differed significantly between health, gingivitis, and periodontitis subjects, and also related modestly to several clinical disease parameters in the progression of periodontal disease.[8] Furthermore the presence of depleted levels of ICTP subsequent to periodontal therapy imply that it is a good indicator of future alveolar bone and clinical attachment loss and can hence be used for risk assessment and prognosis of disease progression.[9] Hence along with the development of newer therapeutic modalities to achieve regeneration of hitherto lost periodontal tissues which is the gold standard of treatment endpoint, newer modalities for diagnosis too are being actively developed as there is an increasing need for research and development of original diagnostic tools that will expectantly demonstrate increased sensitivity and specificity enabling us to make correct therapeutic and prognostic decisions.[10]
Specific Diagnostic Tests

An oral diagnostic tool, in general, should provide pertinent information for differential diagnosis, localization of disease and severity of infection at a specific site level intraorally. The current paradigm of the pathogenesis of periodontal disease shows a wide variation in the magnitude of the inflammatory response around different teeth and in different sites suggesting periods of rapid progression interspersed with periods of quiescence. Factors such as smoking, diabetes, psychological stress, reduced serum antibodies, or biochemical mediators of inflammation are also linked to disease progression rates.

Hence, the severity and progression of periodontal disease has been linked to a combination of genetic, host response, microbial challenge and local environmental factors making it a multifactorial disease and consequently difficult to diagnose with single specific diagnostic methodologies. But the diagnosis of periodontal diseases and treatment outcome assessments are currently based predominantly on clinical signs such as tissue color and contour, the presence or absence of bleeding on probing, gingival recession, probing pocket depths, attachment levels, suppuratation, and tooth mobility as surrogate markers of the active disease progression taking place at site and tooth level. Even radiographs are only used as an additional tool to visualize the previous loss of periodontal tissue, by helping to determine the amount of bone loss around the affected teeth and hence cannot be characterized as a definitive diagnostic tool of active periodontal tissue destruction. As all these methods are only useful to assess the past disease activity they can be relied upon only to a certain level of significance.

A definitive and reliable diagnostic method essential to accurately assess the active disease status of specific sites and also for monitoring the site response to periodontal therapy is currently not available. Thus the development of more sensitive and specific tests whether clinical, biochemical or radiographical would help us to determine whether a patient has active periodontitis and what sort of attachment loss might be expected if the patient were not treated and the chronic inflammatory burden of the periodontal tissues continued unresolved.

Biomarker Tests

A biomarker, or biological marker, is in general a substance used as an indicator of a biological state in oral diagnostics. 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. Disease specific informative biomarkers can further serve as early sentinels of disease and thus have been considered as the most promising alternative to the currently available screening tests for periodontal diseases. As of now it has been a great challenge to determine specific biomarkers for screening, prognosis and evaluating the disease activity and also the efficacy of therapy (diagnostic tests versus therapy tests) in periodontal diseases as an oral diagnostic tool, in general, should provide pertinent information for differential diagnosis, localization of disease and severity of infection at a specific site level.

Biomarkers of Periodontal Disease Fall into Three Categories:

- Indicators of current disease activity;
- Predictors of future disease progression;
- Predictors of future disease initiation at currently healthy site
The Periodontal disease biomarkers can either be Salivary Biomarkers or GCF biomarkers or markers present in both oral fluids. Many different biomarkers associated with bone formation, resorption and turnover, such as alkaline phosphatase, osteocalcin, osteonectin and collagen telopeptidases, have been evaluated in gingival crevicular fluid and saliva. These disease specific informative biomarkers can further serve as early sentinels of disease and thus have been considered as the most promising alternative to the currently available screening tests for periodontal diseases. As of now it has been a great challenge to determine specific biomarkers for screening, prognosis and evaluating the disease activity and also the efficacy of therapy (diagnostic tests versus therapy tests) in periodontal diseases.

**Bone Metabolism in Destructive Periodontal Disease**

Bone, is characterized by two fundamental features, a high mechanical resistance due to its minerals and also considerable elasticity due to its organic components. The alveolar bone of the jaws is in a constant state of flux with continuous structural reshaping occurring through resorption and apposition simultaneously induced by the modification of the forces directed at both a macro and micro level of the alveolus, so that they withstand efficiently the mechanical stresses caused by both physiological and pathological biting forces. Hence the alveolar bone reshaping is controlled by a complex series of local and systemic factors that regulate both the structural and the metabolic functions of bone.

Collagen is not only the most abundant protein in the human body and at the same time, it is the most important one. Soft organs contain a relatively small amount of collagen, while the bone contains 23% from the dry mass. The main function of collagen is to provide resistance and to maintain the structural integrity of the tissues. Collagen undergoes some important metabolic synthesis and degradation processes in various conditions. The modifications in the metabolism of collagen explain a series of metabolic mechanisms that occur in conditions as diverse as diabetes mellitus and osteoporosis.

Type I collagen comprises 90% of the organ matrix of the bone and it is the most abundant collagen in the bone tissue. The degradation products of collagen are considered to be important markers of the bone turnover in several osteolytic or bone metabolic diseases. Several investigators have implicated bone specific pyridinoline cross-links as markers of bone resorption in periodontitis and peri-implantitis.

Hence analysis of antigens related to collagen formation and degradation in bone can provide good and specific estimates of both bone resorption and bone formation rates. A study by Erikesen et al measured serum levels of the pyridinoline cross-linked telopeptide domain of type I collagen (ICTP) as a marker of bone resorption and serum carboxy-terminal propeptide of type I pro-collagen (PICP) as a marker of bone formation. Serum levels of the two antigens were correlated to histomorphometric indices of bone resorption and bone formation with high confidence.

Thus, assays employing antigens that reflect collagen formation and degradation are useful instruments for the evaluation of rates of bone remodeling. A reliable increase in the serum levels of bone tissue resorption markers (deoxypiridinolin and C-terminal telopeptide of type I collagen) and a decrease of markers reflecting bone formation (osteocalcin, bone-specific alkaline phosphatase) was noted in patients with exacerbated generalized periodontitis as compared with the control group by Mazur et al.

Biochemical markers of bone turnover which provide an insight into the dynamic changes of the skeletal bones can also be used as research tools to study the pathogenesis and treatment of bone diseases. Research using bone biomarkers has suggested their clinical use to monitor the effect of antiresorptive therapy. Collagen turnover predict
bone loss and fracture in osteoporosis [24], predict complications of metastatic bone disease [23], and to identify the progression of joint damage in rheumatoid arthritis [25] and the extent of bone involvement in metastatic cancer and multiple myeloma [26, 27]

C-Telopeptide

C-TeloPeptide is a member of a family of pyridinoline cross-links which are specific for osseous and cartilaginous tissues [9]. The pyridinoline cross-links include free pyridinoline, deoxypyridinoline, the C-terminal and N-terminal telopeptide molecules. Hence, CTP represents one member of an important group of molecules which are highly associated with bone resorptive diseases.

The Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (CTP) is derived from the carboxyterminal telopeptide regions of type I collagen cross-linked via pyridinoline or deoxypyridinoline [28]. Following procollagen synthesis and its release into the maturing extracellular matrix, collagen fibrils undergo post-translational modification resulting in cross-links between the telopeptide regions of type I collagen chains by lysyl oxidase. The ICTP cross-link contains two carboxyterminal telopeptides of the α1 chain and one helical region from the α2 chain. These cross-links are essential for providing mechanical stability to the maturing matrix and are specific to bone and cartilage and are not found in soft tissues such as skin where the cross-link is initiated by histidine residues [22]. Elevated serum levels of Type1 collagen have been shown to correlate with bone resorptive metabolic diseases such as primary hyperparathyroidism and post-menopausal osteoporosis [6, 29].

The release of pyridinoline cross-links into urine or blood can be reversed by estrogen replacement or bisphosphonate therapy which inhibit osteoclastic bone resorption [24, 25]. When released from the extracellular matrix of mineralized tissues such as alveolar bone, pyridinoline cross-links can be detected in the GCF by immunological methods. Therefore, CTP may be a first generation pyridinoline cross-link detectable in GCF. Newer methods may involve the use of deoxypyridinoline and other cross-links as immunodetection methods are optimized. Various studies have shown a correlation between the level of the PICP and the bone resorption rate as propeptides share these properties with most of the parameters of the metabolism of collagen [30].

Serum CTP values have been used as biochemical markers of bone formation and resorption. Variables that affect CTP measurement include age, alcohol consumption, smoking, ovulation, gender, drugs (e.g., corticosteroids), disease (e.g., diabetes), exercise, and circadian rhythms [31, 32]. Overnight fasting is one of the most commonly used techniques to minimize the variability of bone turnover markers as variation during fasting is 8.8%. [7].

Talonpoika, Hämäläinen et al in 1994 demonstrated a relationship between ICTP and inflammatory periodontal disease [18]. The initiation of osteoclastic bone destruction and the corresponding elevation in deoxypyridinoline could be detected within 3 days of disease induction by immunohistochemical staining of TRAP+ osteoclast-like cells. Therefore, pyridinoline cross-links may be useful for predicting future bone loss in the periodontium [9, 18].

C-Telopeptide In Saliva

Easily collected and containing local and systemic derived biomarkers of periodontal disease, oral fluids may offer the basis for patient-specific diagnostic tests for periodontal disease. Secretions from the major salivary glands (parotid, submandibular and sublingual), which have a large number of proteins and peptides, are responsible for maintaining the integrity of the oral cavity. Also, because of its importance in oral biofilm formation and host defense,
secreted saliva may have a significant role in the establishment and progression of periodontal disease. Saliva (oral fluid) is a mirror of the body. It could be used to monitor the general health and the onset of specific diseases. Informative biomarkers from saliva can further serve as early sentinels of disease, and this has been considered as the most promising alternative to classic environmental epidemiology.

Saliva is simple, non-invasive, readily available and easily collected without specialized equipment or personnel. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting breast cancer, oral cancer, caries risk, salivary gland diseases, periodontitis and systemic disorders such as hepatitis C and the presence of human immunodeficiency virus (HIV).

Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases. Also, for some diagnostic purposes, salivary biomarkers proved more useful than serum analysis. C-reactive protein is a systemic marker released during the acute phase of an inflammatory response. C-reactive protein is produced by the liver and is stimulated by circulating cytokines, such as tumor necrosis factor-a and interleukin-1, from local and/or systemic inflammation such as periodontal inflammation. Circulating C-reactive protein may reach saliva via gingival crevicular fluid or the salivary glands. High levels of C-reactive protein have been associated with chronic and aggressive periodontal diseases and with other inflammatory biomarkers. Studies have demonstrated that periodontal patients display elevated concentrations of serum C-reactive protein when compared with healthy individuals. C-reactive protein has recently been shown to be measurable in saliva from periodontal patients using a lab-on-a-chip method.

Matrix metalloproteinases are host proteinases responsible for both tissue degradation and remodeling. During progressive periodontal breakdown, gingival and periodontal ligament collagens are cleaved by host cell-derived interstitial collagenases. MMP-8 is the most prevalent MMP found in diseased periodontal tissue and gingival crevicular fluid. Elevated MMP-8 levels in active disease progression were observed in a longitudinal study of patients with gingivitis and with nonprogressive and progressive periodontitis. Recently, the level of MMP-8 was demonstrated to be highly elevated in saliva from patients with periodontal disease using a rapid point-of-care microfluidic device.

Many different biomarkers associated with bone formation, resorption and turnover, such as alkaline phosphatase, osteocalcin, osteonectin and collagen telopeptidases, have been evaluated in gingival crevicular fluid and saliva. These mediators are associated with local bone metabolism (in the case of periodontitis) as well as with systemic conditions such as osteoporosis or metastatic bone cancers. Khashu H, Baiju CS, Bansal SR et al, in 2012 have reported that pyridinoline cross-linked carboxyterminal telopeptide of type I collagen is a valuable diagnostic aid for periodontal disease due to the ability of pyridinoline cross-links to detect bone resorption. Furthermore, the levels of carboxyterminal telopeptide of type I collagen were strongly correlated with clinical parameters and putative periodontal pathogens, and demonstrated significant reductions after periodontal therapy. Controlled human longitudinal trials are needed to establish fully the role of salivary carboxyterminal telopeptide of type I collagen as a predictor of periodontal tissue destruction, disease activity and response to therapy in periodontal patients.

**C-Telopeptide in GCF**

In recent years Gingival crevicular fluid (GCF), has gained great interest on possible diagnostic value in periodontal disease. It contains a large number of proteins and peptides derived from inflamed host tissues and hence the analysis of the GCF components are used to isolate the disease status of individual sites and thus, identify potential
biomarkers of periodontitis. [38] Gingival crevicular fluid is both a physiological fluid as well as an inflammatory exudate, originating from the gingival plexus of blood vessels in the gingival corium, subjacent to the epithelium lining of the entogingival space.

As gingival crevicular fluid traverses through inflamed periodontal tissues en route to the sulcus, biological molecular markers are gathered from the surrounding areas and are subsequently eluted into whole saliva. Gingival crevicular fluid (GCF) is a complex mixture of substances derived from serum, host inflammatory cells, structural cells of the periodontium, and oral bacteria. [30] GCF originates from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. GCF can be isolated from healthy sulcus, although only in small amounts. In the healthy periodontium, GCF represents the transudate of gingival tissue interstitial fluid produced by an osmotic gradient.

The products of the inflammatory response which occur during the disease process can be found in the GCF [10]. Monitoring of the presence of such components can be of potential value in evaluating periodontal disease status or outcomes of periodontal therapy. Gingival crevicular fluid sampling methods have been shown to capture inflammatory and connective tissue breakdown mediators accurately. To date, more than 90 different components in gingival crevicular fluid have been evaluated for periodontal diagnosis. [38] Of the numerous constituents in gingival crevicular fluid, however, the vast majority constitute soft-tissue inflammatory events, while only a few are regarded as specific biomarkers of alveolar bone destruction. [3] The most promising gingival crevicular fluid markers of disease progression/connective tissue destruction are probably host breakdown products (as opposed to the enzymes that break down host tissues). This is especially true of markers that represent bone destruction and include chondroitin-4-sulfate, pyridinoline cross-links of the carboxyterminal telopeptide of type I collagen, and RankL (receptor activator for NF-κB ligand). [39]

Given the specificity and sensitivity for bone resorption, pyridinoline cross-links, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen represent a potentially valuable diagnostic aid for periodontal disease. Several investigations have explored the ability of pyridinoline cross-links to detect bone resorption in periodontitis and peri-implantitis, as well as in response to periodontal therapy. [18, 19] As the cross-linked telopeptides resulting from posttranslational modification of collagen molecules cannot be reused during collagen synthesis they are considered specific biomarkers for bone resorption. [6] Pyridinoline cross linked carboxyterminal telopeptide of typeIcollagen (ICTP) is 12 to 20 Kd fragment of bone typeIcollagen released by digesting with trypsin or bacterial collagenase. According to Palys et al in 1998, GCF ICTP levels were related to subgingival microflora of periodontal diseases and hence GCF ICTP levels are a good predictor of future alveolar bone and attachment loss and are strongly correlated with clinical parameters and putative periodontal pathogen. [8]

Talonpoika JT, Hämäläinen MM studied a total of 126 gingival crevicular fluid (GCF) samples from 20 adults using paper strips and concluded that GCF ICTP reflects the local type I collagen degradation in periodontal tissues and also gives information about the tissue destruction process beyond the reach of the clinical parameters. [18] These investigations have shown that ICTP correlated strongly with radiographic bone level and pocket depth and was significantly higher at periodontitis sites compared to non-periodontitis sites [18]. Moreover, ICTP was highly sensitive and specific for predicting future alveolar bone loss as measured by computer-assisted digitizing radiography. [34].
CONCLUSIONS

Periodontal diagnosis and treatment plan are based on the assessment of probing depth, clinical attachment level, plaque index, gingival index, bleeding on probing, suppuration, furcation involvement, mobility, and radiographic findings. However, these clinical parameters are not sufficiently sensitive and specific to identify disease activity in individual sites or to predict future attachment loss. Hence, attention is focused on the development of diagnostic tools that could screen and differentiate the active inflamed sites and predict future tissue destruction.

It is noteworthy that in developing newer treatment strategies and diagnostic tests, it has been essential to become more knowledgeable about the patho-physiological mechanisms underlying periodontitis. And that with this information the physiological and patho-physiological continuum that connects tissues in the oral cavity to other remote organ systems in both health and disease can also been defined and recognized with increasing clarity. This concept also presages a more biological approach being taken by dentists regarding diagnosis and management of oral diseases. The periodontists of the 21st century will approach oral diseases using a more biological model of health, disease and disease management; an approach more befitting physicians of the oral cavity.

While the future of periodontal disease diagnosis using oral fluid biomarkers looks promising, obstacles to these approaches may appear in the clinical setting. Validation of novel periodontal diagnostics will need to be benchmarked with existing gold standards of disease, such as alveolar bone levels and clinical attachment levels, in large patient populations. Acceptance by clinicians is also necessary and may prove difficult if technical feasibility is complicated. However if a demonstrably more efficient periodontal therapy can be delivered, clinicians will be more likely to utilize the new diagnostic approaches. Although challenges remain ahead, the use of biomarkers based oral fluid diagnostics appear promising for future application to diagnose periodontal diseases and to prognosticate periodontal treatment outcomes.

REFERENCES


