Pentraxins as key disease markers for periodontal diagnosis

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Abstract. Periodontal diseases are characterized by a complex set of biologic interactions between a diverse and dynamic microbial ecosystem and the host's multifaceted and responsive immune and inflammatory machinery. Such interactions between microbial pathogens and various host response systems play a critical role in the development and progression of periodontal disease via the release of inflammatory and immune mediators. Advances in periodontal disease diagnostic are moving toward methods whereby periodontal risk can be identified and quantified by detecting such inflammatory mediators in its sequential pathophysiology. Pentraxins (PTXs) are classical mediators of inflammation and markers of acute-phase reaction. They are a super family of multifunctional molecules characterized by multimeric structure, divided into “short” PTXs and “long” PTXs. C-reactive protein (CRP) and pentraxin-3 (PTX3) are prototypic molecules of the short and long PTX family, respectively. Evidence suggests that PTXs acts as a non-redundant component of the humoral arm of innate immunity, downstream of, and complementary to, cellular recognition, as well as a tuner of inflammation. CRP is a cheaper biomarker and more readily available in everyday clinical practice compared with other inflammatory markers, on the other hand, PTX3 is believed to be the true independent indicator of disease activity and could have clinical implication in diagnosing the “at site” inflammatory status of the periodontal disease. These pentraxins are sensitive and specific in the diagnosis and prognosis of chronic diseases. Thus the pentraxins could be used as preferred biomarkers in periodontal disease diagnosis.

Keywords: Pentraxins, biomarkers, C-reactive protein, pentraxin-3, periodontal disease

1. Introduction

The incidence and rate of progression of periodontal diseases depends on the complex interactions between periodontopathic bacteria and cells of host immune system [1]. The interactions between the bacteria and the host are mediated by cytokines and chemokines, produced by both the resident and emigrant cells at the site of inflammation [2]. The bacterial lipopolysaccharides (LPS) and other virulence factors activates various pro-inflammatory signals and a variety of innate immune cells to produce numerous cytokines and acute phase proteins [3] (APPs), that are instrumental in generating a host mediated tissue destructive responses [4].

Periodontal clinical diagnosis is made by numerous traditional measures like visual examination, tactile appreciation, bleeding on probing, periodontal pocket depth, attachment level, and radiographic assessment of alveolar bone loss are still popular and universally used [5]. Albeit, easy to use, cost-effective and relatively noninvasive visual and tactile methods, requires the disease activity to be present for a while,
to be appreciated. Clinical attachment loss evaluation using the periodontal probe measures damage from past episodes of destruction and requires a 2–3 mm threshold change before a site with significant breakdown can be identified. The use of radiography offers a means to detect changes in alveolar bone calcium content but requires a whopping 30% bone loss before they are identified on the radiographs. The presence of bleeding on probing is still the best disease activity predictor available, but it is not specific enough and thus reveals too many false positives. The absence of bleeding on probing on the other hand is a very precise negative predictor of periodontal disease activity [6]. However, these traditional measures cannot reliably identify sites with ongoing periodontal destruction and does not provide any information of the cause of the condition or patient’s susceptibility to disease, whether the disease is progressing/remission or whether the response to therapy will be positive or negative [7]. Moreover these diagnostic methods are not precisely accurate and only allow retrospective diagnosis at one point of time [8].

Advances in periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers. In Periodontal diagnostics, it has been a great challenge to determine biomarkers for screening and predicting the early onset of disease (prognostic tests) or evaluating the disease activity and the efficacy of therapy (diagnostic tests). Hence the need of advanced markers to accurately identify the disease with high sensitivity (to identify the disease in its presence) and specificity (to exclude the disease in its absence) were sought. Acute phase proteins in general come close to fulfill these criteria.

2. Acute phase proteins

Any of the plasma proteins whose concentration increases or decreases by at least 25% during inflammation. APPs include C-reactive proteins (CRP), several complement and coagulation factors, transport proteins, amyloid, and anti proteases enzymes. They help mediate both the positive and negative effect of acute and chronic inflammation including chemotaxis, phagocytosis, protection against oxygen free radicals and tissue repair. In clinical medicine erythrocyte sedimentation rate or serum CRP levels sometime is used as a marker of increased amount of APPs [9]. The serum and gingival crevicular fluid (GCF) concentrations of a number of these APPs, which increases rapidly during infection and concentration raise to several hundred folds, and remains elevated throughout infection [10]. Although there have been several pro-inflammatory APPs implicated in the immunopathology of periodontitis. The APP that receive maximum attention are CRP, plasminogen-activator inhibitor 1, and fibrinogen [11]. However, some of the most convincing evidence for destruction of the periodontium involves Interleukin (IL) -1/β and tumor necrosis factor-alpha (TNF-α) [12]. Recent novel research suggests IL-1/β and TNF α are the most potent inducers of a new PTX called Pentraxin-3 [13]. Short pentraxins are conserved during phylogensis and no deficiency state of either CRP or Serum Amyloid Protein (SAP) in humans has yet been reported. This possibly indicates that PTXs confer a survival advantage; however, the mechanisms by which they act are not yet defined. Thus the pentraxins promise to be key markers for periodontal disease diagnosis. This is the first review that compiles various PTXs that can be used for diagnosis of periodontal diseases and attempts to throw light on the numerous advantages of these molecules over other biomarkers.

PTXs are a superfamily of multifunctional conserved proteins, some of which are components of the humoral arm of innate immunity and behave as functional ancestors of antibodies (Abs). They are known as classical APP, known to the human race for over a century [13]. PTXs are characterized by a multimeric, usually pentameric structure by the presence in their carboxyl-terminal of a ∼ 200 aa-long conserved domain, called pentraxin domain. In addition, the members of this family share an 8 aa-long conserved sequence (HxCxS/TWxCxS, in which x is any amino acid) in the PTX domain, called pentraxin signature. Some PTXs, together with collectins and ficolins, constitute the humoral arm of innate immunity and behave as functional ancestors of Abs by mediating agglutination, complement activation, and opsonisation. The name “pentraxin” is derived from the Greek word ‘Penta’ meaning ‘five’ and ‘Ragos’ implying ‘berries’ relating to the radial symmetry of five monomers (pentameric) forming a ring approximately 95Å across and 35Å deep. They are considered to be markers of acute phase of inflammation [14] and are classified into “short” PTXs (CRP and SAP) and “long” PTXs (PTX3).

Long PTXs have an unrelated, long amino-terminal domain coupled to the carboxyl- terminal pentraxin domain and differ, with respect to short pentraxins,
in their gene organization, chromosomal localization, cellular source, and in inducing stimuli and ligand-recognition ability. The classical short pentraxins i.e. CRP and SAP are produced in the liver in response to inflammatory signals, most prominently by interleukin (IL)-6 [15]. On the contrary, PTX3 is produced on exposure to pro-inflammatory stimuli (Toll-like receptor (TLR) engagement and cytokines TNF α, IL-1β) and specific microbial constituents (LPS, lipoarabinomannans, outer membrane proteins) [16]. As these molecules are recognized as the most important mediators in the periodontal etiopathogenesis, one can expect PTX3, as a reliable biomarker in periodontal disease diagnosis.

CRP is the first described PTX, it binds to pneumococcal C-polysaccharides and is expressed during the acute phase response to bacterial infections [17]. In the 1930s, it was the first purified pattern recognition molecule, which was named after its ability to bind in a calcium-dependent fashion, C-polysaccharide of Streptococcus pneumonia [18]. Hence the name CRP. They are ligands for leukocyte Fcγ receptors. The CRP gene is located on first chromosome. CRP is a potent APPs that regulate innate resistance to microbes and scavenging of cellular debris. CRP is conserved from mammals to arthropods. In Limulus polyphemus, different forms of CRPs and SAP are normal and abundant, they are the constituents of the hemolymph and are involved in recognizing and destroying pathogens [13].

3. Functions of CRP

A major function of CRP is its ability to bind phosphocholine and recognize some foreign pathogens as well as phospholipids of damaged cells. It can activate complement system when bound to one of its ligands and can bind to phagocytic cells. An observation suggests that it can initiate the elimination of targeted cells by its interaction with both humoral and cellular immunity. Other proinflammatory effects of CRP include induction of inflammatory cytokines and tissue factor in monocytes. It is said to assist in complement binding to foreign and damaged cells and affect the humoral response to disease [19]. CRP has the ability to prevent the adhesion of neutrophils to endothelial cells by decreasing the surface expression of L-selectin, inhibit the generation of superoxide by neutrophils, and stimulate the synthesis of interleukin-1 (IL-1) receptor antagonist by mononuclear cells [19]. CRP has also been reported to stimulate tissue factor production by human peripheral blood monocytes and has a procoagulant effect. In addition, CRP recruits monocytes by receptor-mediated chemotaxis into the arterial wall. It colocalizes with foam cells in atherosclerotic lesions [20,21].

It is a type 1 APP that is produced on stimulus like heat, trauma, infection and hypoxia. It is found in traces in healthy individuals, that is, < 0.3 mg/L serum. But in case of severe systemic infection its level could exceed 100 mg/L. This marked increase in its level is used extensively as a marker for tracking the course of infection [22]. Thus CRP has been the focus of attention as a key marker of most systemic diseases discovered till date. Its elevated levels predict an increased risk of cardiovascular disease (CVD) by a factor of approximately 2.1 (1.4–2.78) [23]. According to the recommendations of Centre for Disease Control and Prevention (CDC), serum CRP levels of ≤ 1 mg/l are considered as low risk, 1 to 3 mg/l as average risk, and ≥ 3 mg/l are considered as high risk for the development of CVD [24]. Baseline hs-CRP concentrations in healthy patients have been shown to be one of the most powerful predictors of both long- and short-term cardiovascular risk in both men and women [25–27]. Also, CRP appear to add to the predictive value in lipid screening [26,28].

CRP are considered to be one of the stronger AP proteins that respond rapidly to inflammatory stimuli and their serum levels can increase significantly in all kinds of inflammations [10–13]. Boulman et al. in 2004 reported its correlation with polycystic ovary syndrome (PCOS). High level of CRP were observed in patients suffering from PCOS than the controls [29].

In periodontal inflammation, CRP are considered to be one of the stronger APPs that respond rapidly to inflammatory stimuli and their serum levels can increase several hundred folds. Novel research has shown that measurements of CRP in serum or GCF might help identify a subset of patients who are at higher risk of destructive periodontal diseases or those who are undergoing a process of periodontal breakdown [30]. Although CRP is produced by hepatocytes, there is evidence of it being present in cells associated with acute connective tissue inflammation [32]. The rapid rise of CRP in serum following exposure to interleukin-1, which is a potent bone resorber (found in GCF), made the search of CRP in periodontitis reasonable. Numerous studies have shown a positive association between chronic periodontal infections and elevated CRP levels. These include large-scale, cross-sectional studies [31] and studies analyzing data from the Third
National Health and Nutrition Examination Survey, which have included over 10,000 patients [32]. Since many years poor periodontal health is been linked to higher systemic CRP levels [33] with a higher sensitivity, specificity and a greater predictive value. Thus is in not surprising that till today, there have been more than 333 studies reported in Medline pertaining to as periodontal disease and CRP [34]. Although CRP is produced by hepatocytes, there is evidence of it being present in cells associated with acute connective tissue inflammation. The rapid rise of CRP in serum following exposure to interleukin-1, which is a potent bone resorber also found in GCF substantiates this fact [35]. It was reported that elevation of CRP in GCF increases the odds of periodontitis [36]. Also, in our previous study we reported the simultaneous elevation of GCF and serum CRP levels in a progressive periodontal disease [37]. Moreover, its levels in GCF of periodontitis patients correlate significantly with systemic disease [38] and periodontal treatment decreased CRP levels significantly as it decreases the inflammatory burden, of the systemic disease. Recently we evaluated the periodontal inflammatory burden index (PIBI) associated with chronic periodontitis. PIBI reduction post non-surgical periodontal therapy correlated significantly with the post therapy CRP levels. (Unpublished data, Kathariya R et al.) [39]. Thus, CRP levels associated with periodontal disease might play a significant role in predicting high-risk patients for the most common diseases of the present era. Periodontal therapy might also lower the risk of severity of these systemic diseases, as it reduces the inflammatory burden, not only in systemically-compromised individuals, but also in otherwise healthy individuals [40].

CRP is a cheaper alternative and more readily available in everyday clinical practice compared with other inflammatory markers, it has a better sensitivity and specificity, its measurement could have clinical implication in diagnosing the “at site” inflammatory status of the patient [41].

SAP is different from serum amyloid A. It is a second and less known member of short PTXs in the PTX super family. SAP is a 25 kDa pentameric protein which shares 51% sequence homology with CRP. It was first identified as the pentagonal constituent of in vivo pathological deposits called “amyloid” [42]. SAP makes up 14% of the dry mass of amyloid deposit and is thought to be an important contributor to the pathogenesis of amyloidoses [43]. These conditions are characterized by the ordered aggregation of normal globular proteins and peptides into insoluble fibers which disrupt tissue architecture and are associated with cell death. SAP is thought to decorate and stabilize aggregates by preventing proteolytic cleavage and hence inhibiting fibril removal via the normal protein scavenging mechanisms [44]. SAP is present in human serum at approximately about 65 pg/ml and does not vary significantly with inflammation [45]. It is relatively stable, even during the early acute-phase response. It is conserved protein in mice and is of little importance in human. However recent studies have shown that significantly higher levels of amyloid protein (AP) in periodontitis and gingivitis tissue [46]. It is correlated with the intensity of plasma cell accumulation and the extent of connective tissue matrix degradation. AP was concentrated in the deep connective tissue and perivascular areas; also, the deposition was associated with nerve bundles and, occasionally seen in the extracellular matrix of the lining epithelium. These findings have potential significance in relation to the pathology of chronic periodontitis as AP has been shown to interact in a calcium-dependent manner with a number of ligands including fibronectin, elastic fibres, C-4 binding protein and amyloid fibrils. Also, amyloid-like fibrils (AP) composed of immunoglobulin light chains were extracted from the lesions of chronic periodontitis [47]. However, SAP sustained a reduction at 4 weeks after periodontal therapy [48]. Cho et al. in 2004, observed elevated levels of SAP in cases of nasopharyngeal cancer. The level increased drastically with the disease, correlated with relapse and decreased with chemotherapy [49]. Literature links radiation therapy carried out for the treatment of the carcinoma causes periodontitis, but, lacks proper evidence on linkage between the two [50]. Further research are warranted to correlate nasopharyngeal carcinoma and periodontitis, and its mechanism of cause. In 2008, Bozinovski et al. concluded that CRP is a novel biomarker of acute exacerbation of congestive obstructive pulmonary disease (COPD) [51]. Later in 2009 Deo et al. confirmed the relationship of COPD and periodontitis. The authors claimed periodontitis as a potential risk factor for COPD [52]. The association of SAP with periodontal diagnosis is sparse and need to be explored further.

PTX3 is a recently discovered first identified prototypic long pentraxin in the PTX super family. It is structurally related to (although distinct from) classical short PTXs- CRP and SAP. It has a high degree of conservation from mouse to man. Both resident and innate immunity cells produce PTX3 in peripheral tissues in response to inflammatory cells, bacterial cyto-
toxins and Toll-like receptors (TLR) activation. PTX3 is a first cloned long PTX as an IL-1β-inducible gene in endothelial cells and TNF-α stimulated gene in fibroblasts [16].

Recent research suggests that, PTX3 expression is induced in response to inflammatory stimuli, including TNF α, IL-1β and microbial moieties, such as LPS, lipoarabinomannans, and outer membrane proteins by a variety of cell types abundant in periodontal tissue like neutrophils, fibroblasts, monocytes/macrophages, dendritic cells, epithelial cells, endothelial cells, and vascular smooth muscle cells, adiposities, dendritic cells etc. [15] in response to inflammatory cytokines and TLR engagement. Hence hypothesizing that, it is elevated in almost all kinds of inflammation and infections in the body and periodontitis is no exception. However, the plasma levels of PTX3 are very low in normal subjects and are raised in inflammatory conditions resulting from a wide range of disease states from infections to autoimmune and/or degenerative disorders [53]. Moreover, PTX3 is produced from vascular endothelial cells and macrophages, its levels may directly reflect the inflammatory status. Because of its extracellular synthesis, on contrary to CRP; PTX3 levels are believed to be a true independent indicator of disease activity [54]. Thus, measurements of PTX3 in GCF or plasma may help identify a subset of patients who are at a higher risk for destructive disease, or those patients who are undergoing a process of periodontal breakdown [55]. Ours was the first study to report the use of PTX3 in periodontal disease diagnosis [56], and periodontal disease associated with chronic kidney diseases (CKD) [57]. We found that, greater the amount of periodontal tissue destruction, greater was the mean PTX3 concentration in both GCF and plasma, as its levels correlated positively with all clinical parameters. We also concluded that GCF PTX3 levels could be considered as ‘marker of inflammatory activity in periodontal disease’ [56]. In the later study, we found strong correlation between PTX3 levels periodontal parameters in patients with periodontitis associated with CKD and concluded that periodontal disease could serve as a risk factor for developing CKD [57].

Recently PTX3 has been detected in various chronic inflammatory conditions, like atherosclerosis [58], and is recognized as an early marker for unstable angina [59] and myocardial infarction [60]. It was found that it is released by the endothelium and by activated myofibroblasts in systemic sclerosis [61]. It was suggested that PTX3 may contribute to the pathogenesis of atherosclerosis [59], and hence thrombo-embolic events leading to CVDs and CKDs. It is believed to be a true independent indicator of disease activity at sites of inflammation and vasculitis [54]. Also, PTX3 can act as a natural inhibitor of influenza virus [62]. Moreover it was also found to be elevated to its prognostic value in CVD [63], CKD [64], rheumatoid arthritis [65], preeclampsia [66], inflammatory bowel diseases [67], and severe dengue virus infections [68] etc. Recently, it is also recognized as a diagnostic biomarker for lung pathologies. Its diagnostic sensitivity and specificity was found to be similar to other potential biomarkers for lung carcinoma, acute lung injury and acute respiratory distress syndrome [69,70]. Also, circulating levels of PTX3 significantly correlated with the severity of infection in critically ill patients [71]. The evidence that PTX3 could serve as a potential marker of periodontal diagnosis comes from that fact that neutrophils represent a reservoir of pre-stored PTX3 in its ready for rapid release-specific granules and release it in response to inflammatory signals. Neutrophils arrive early at sites of periodontal destruction and it is believed that tissue loses its cohesiveness only after neutrophil infiltration increases to more than 60% [72].

PTX4 is a new unique member of the pentraxin superfamily which consists of 470 amino acids. It has been found that human, murine, and rat PTX4 show 30% identity at the pentraxin domain level with other long pentraxins. Like other members of this family, the gene encoding PTX4 is well conserved from mammals to lower vertebrates. However, PTX4 has a unique pattern of mRNA expression, which is distinct from that of other long pentraxins [13,20]. In a study authors reported that the expression of PTX4 in endothelial cells, leukocytes, monocytes, lymphocytes (B and T), neutrophils, and NK cells. In endothelial cells, monocytes, neutrophils, and lymphocytes, PTX4 expression was very low, and IL-1 or LPS did not induce it [13]. Presently there is only one reported study in Medline on PTX4. So, further studies are required to define its role as a biomarker and its possible relation to periodontitis.

Neuronal PTXs which includes NP1, NP2 and NPR define a specific family of pentraxins. All three have approximately 50% identity to each other, and each has 20–25% identity to the smaller acute phase pentraxins (CRP and SAP). It is suggested that NP1, NP2, and NPR mediate a similar function for neurons [73]. NPTXs have been shown to be involved in the excitatory synaptic remodeling. NPTX2 has been implicated
Many dental and periodontal conditions lead to xeros-NP1 protein levels in cerebellar granule neurons [76]. that potassium depletion produces a marked increase in expression is restricted to the nervous system. The amino-terminal half of NP1, also NP2, encodes a series of amino acids that are often associated with the inner side of the plasma membrane, suggesting that neuronal pentraxins also have physiological functions as intracellular proteins [75]. NP1 is a glycoprotein, involved in both synaptogenesis and synaptic remodeling whose expression is restricted to the nervous system. The amino-terminal half of NP1, also NP2, encodes a series of coiled-coil domains that seem to be essential for homomultimerization. Recent studies have shown that potassium depletion produces a marked increase in NP1 protein levels in cerebellar granule neurons [76]. Many dental and periodontal conditions lead to xerostomia, which in turn leads to hypokalemia that might trigger NP1 secretion. Moreover, JG Meechan et al. reported local anesthetic agents used for dental or periodontal surgeries had a significant influence on serum potassium levels [77].

In a study, it was interesting to discover that hypokalemia due to CKD increases NP1 levels [77], and it can be hypothesized that NP1 levels might influence periodontitis. Exclusive expression of NP1 in the central nervous system, its characteristic feature as a secretary protein, and its large molecular size relative to the classical pentraxins suggest that NP1 may have novel unknown functions [78].

NP2 also known as neuronal activity-regulated pentraxin (Narp); gene symbol NPTX2 (11 kb in length) on 7q21.3-q22.1 chromosome is a recently discovered PTX.

NPTX2 promotes neuritic outgrowth and is suggested to mediate uptake of degraded synaptic material during synapse formation and remodeling and cause aggregation of neurotransmitter receptors at synapses. Recent studies have shown the potential role of NPTX2 as a biomarker [79]. Further studies are warranted to evaluate the role of NPTX2 as a biomarker in periodontal disease diagnosis.

NPR is a third member of the NP family, primarily expressed in the central nervous system, and physically associated with NP2 and NP1. The sequence of NPR has significant homology to the classical pentraxins and to neuronal pentraxin 1 and 2 in particular [73]. In a study by Yin et al. in 2009 [80] showed that NPR is a candidate biomarkers in cerebrospinal fluid for neurodegenerative diseases, and that the changes in the cerebrospinal fluid level of NPR may be specific for Alzheimer’s disease [81]. Several reports confirm a tie-up between periodontal infection and Alzheimers disease. Thus the role of neuronal pentraxins as periodontal biomarker needs to be evaluated [81].

4. Conclusion

Pentraxins (PTXs) are classical mediators of inflammation and markers of acute-phase reaction. They are a super family of multifunctional molecules characterized by multimeric structure, divided into “short” PTXs and “long” PTXs, CRP and PTX3 are respective prototypic molecules. Evidence suggests that PTXs acts as a non-redundant component of the humoral arm of innate immunity, downstream of, and complementary to, cellular recognition, as well as a tuner of inflammation. CRP is a cheaper biomarker and more readily available in everyday clinical practice compared with other inflammatory markers, on the other hand, PTX3 is believed to be the true independent indicator of disease activity and could have clinical implication in diagnosing the “at site” inflammatory status of the periodontal disease. These pentraxins are sensitive and specific in the diagnosis and prognosis of chronic diseases like periodontitis. The newly discovered PTXs were hypothesized to be related in periodontal pathophysiology in some or the other ways. Thus the pentraxins could be used as preferred biomarkers in periodontal disease diagnosis.

However, further research is required to assess the levels of different and newer PTXs in periodontal disease and its influence on systemic disorders and its interrelationship. This study contributes to helping health care providers identify and develop ideal biomarkers for periodontal disease diagnosis. Indentifying them at an early stage might prove vital in intervention strategies to reduce patient risks and prevent systemic disease outcomes.
Conflict of interest

The authors report no conflict of interests. It is a self-funded research.

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