C-Reactive Protein and its Association with Periodontitis and Atherosclerosis: A Review

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ABSTRACT

Periodontal disease is a chronic inflammatory process resulting from interaction of specific gram negative bacterial species with the host defense in disease susceptible individuals. The host responds to the microbial challenge, with increased levels of pro and anti-inflammatory cytokines like IL-1, IL-10, IL-8, IL-6, TNF-α etc. These mediators endorse activation of the acute phase reactants resulting in elevated serum levels of CRP, α1-acid glycoprotein, ceruloplasmin, serum amyloid A. CRP thus produced due to local periodontal infection has negative after effects on systemic health. High serum CRP concentrations can be of high risk for future cardiovascular disease and events. Pre-assessment of serum C reactive protein in chronic periodontitis can predict the risk of developing cardiovascular disease in future and thereby can help in diagnosis at earlier stages and can aid in providing screening services and advice to seek immediate dental care. This review highlights the bidirectional associations between the cardiovascular disease marker CRP and periodontitis.

Keywords: Periodontitis, Periodontal Therapy, atherosclerosis, C-reactive protein.

INTRODUCTION

CRP is a pentameric plasma protein which is involved in the systemic response to inflammation. It is an extremely sensitive and non-specific acute-phase marker for inflammation. CRP is produced in response to many forms of injury such as bacterial, viral or parasitic infection, mechanical or thermal trauma, ischaemic necrosis or malignant growth. CRP binds to specific molecular configuration, or found on the surfaces of pathogens. C-reactive protein when bound to bacteria promotes the binding of complement, which facilitates their uptake by phagocytes. CRP increases 100 to 1000 increase within hours of tissue injury. C-reactive protein may be considered as a primitive form of antibody specifically interacting with cell membrane components of microorganisms such as bacteria and fungi as well as for damaged mammalian cell membranes. When complexed, C-reactive protein can activate complement to enhance opsonization and clearance of the bacteria prior to the production of specific IgM or IgG. C-reactive protein bound to bacteria or cells can interact with natural killer cells and with monocytes and may increase the tumoricidal activity of these cells. Serum CRP concentration closely follows the course of the acute-phase response to inflammation and tissue necrosis. Acute phase proteins may provide essential pathways for modulating macrophage activity, since macrophages contain CRP receptors and CRP may effectively upregulate the formation of proinflammatory cytokines. Periodontitis is a polymicrobial disease and these bacteria tend to disrupt host mechanisms involved in their clearance by activating a number of host immune-inflammatory processes.
These processes are subject to environmental, genetic and acquired potential risks. Although periodontitis is initiated by microbes, host modifying factors play an important role in determining severity and extent of disease. Periodontal disease occurs due to a complex interplay of bacterial infection and host response. Bacteria interacts with host through there virulence factors and induce an innate and humoral immune response. Immune responses to bacterial challenge shows interindividual variations and leads to release of proinflammatory factors, PGE2, IL-1β. Inflammatory processes at periodontium results in increase level of CRP and other mediators such as fibrinogen leading to systemic response. Annually CVD accounts for 40% of all deaths worldwide, with atherosclerosis as underlying etiology in majority of cases. Atherosclerosis is a disease process in which fatty deposits, inflammation, cells and scar tissue buildup within the walls of arteries. Inflammation plays a central role in the pathogenesis of atherosclerosis, from its initial stage to development of clinical signs and symptoms. Several factors are defined as risk factors for cardiovascular diseases; however, incidence of atherosclerosis cannot be explained by traditional factors alone. American Heart Association (AHA) working group concluded that periodontal disease is associated with atherosclerotic vascular disease (ASVD) independent of known confounders.

C-reactive protein (CRP): CRP is plasma protein which was first discovered by Tillet and Francis in 1930, as a protein which could precipitate “C” Polysaccharide derived from Pneumococcal cell wall. CRP is not only a marker of inflammation, but also may directly modify the inflammatory process. CRP can interact with various ligands, can activate classical complement pathway, can stimulate phagocytosis and bind to FcγR immunoglobulin receptors. CRP act as a pattern recognition molecule which binds to specific molecules configuration formed on the surface of pathogen. Interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) regulates CRP. As it was elevated in people with a variety of illnesses including cancer It was initially thought that CRP might be a pathogenic secretion however, discovery of hepatic synthesis demonstrated that it is a native protein. It is normally present in ng/ml quantities but may increase dramatically to hundreds of μg/ml within 72hrs following tissue injury. Periodontal pathogens affect the immune system and promote local and systemic inflammatory responses. Persistent localized infection may influence the systemic levels of inflammatory mediators.

Structure:
C-reactive protein is a plasma protein which along with serum amyloid P component (SAP), forms the pentraxin family of proteins, which is a part of lectin fold superfamily. Human CRP (Mr 115,135) consists of five identical noncovalently associated 23KDa protomers arranged symmetrically around a central pole. Each protomer contains "lectin fold," that is composed of a double-layered β sheet with flattened jellyroll topology. The ligand binding site, contains loops with two calcium ions bound 4 Å apart by protein side-chains, is located on the concave face. The other face carries a single α helix . CRP has two different conformational forms: the native pentameric isoform (pCRP) and the monomeric isoform (mCRP), which possess distinct antigenic, electrophoretic, and biological features.

C-Reactive Protein: From Pentameric to Monomeric:
pCRP is a stable molecule and is the main form detected in serum. Many studies have confirmed that conformational subunits from pCRP can be dissociated, both in vitro and in vivo, into individual mCRP units. Although independent mCRP synthesis also be an important source of this
There are two main mechanisms for \textit{in vivo} generation of mCRP.

\textbf{a. Local expression:} Presence of mCRP mRNA in various extrahepatic tissues has been reported by various studies, which includes adipocytes, smooth muscle cells, and inflammatory cells within atheromatous plaques. Mechanisms for synthesis of subunits and their assembly into pCRP has not been understood clearly. Some \textit{in vitro} studies support local expression, with the detection of mCRP mRNA in U937 macrophages of atherosclerotic lesions.\textsuperscript{17,18}

\textbf{b. Local dissociation:} dissociation of pCRP into mCRP has been observed in membranes of apoptotic cells and activated platelets in atherosclerotic plaques.

Phosphatidylcholine molecules in the cell membrane of activated platelets appear to play an important role. Initially a hybrid intermediate molecule termed mCRP\textsubscript{mn} is formed, exhibiting CRP subunit antigenicity, yet retaining native pentameric conformation. This molecule rapidly detaches from the cell membrane and finally dissolves in solution into mCRPs.

\textbf{Ligands and Receptors:}

Volanakis and Kaplan (1971) identified the specific ligand for CRP in the pneumococcal C-(capsular) polysaccharide as phosphocholine, part of the techio acid of the pneumococcal cell wall. Human CRP also binds to a variety of other autologous and extrinsic ligands. Autologous ligands include damaged cell membranes, apoptotic cells, native and modified plasma lipoproteins, small nuclear ribonucleoprotein particles, a number of different phospholipids and other related components. Extrinsic ligands include many phospholipids, glycans and other constituents of microorganisms, such as capsular and somatic components of bacteria, parasites, fungi, and other plant products. The CRP-binding sites for phosphatidylcholine are located on the lateral surfaces of each subunit and require binding of two calcium ions at specific hydrophobic pockets centred on Phe66, in an event known as calcium-dependent ligand binding. Then, CRP is able to interact with C1q, activating the classical pathway of the complement system.\textsuperscript{19} CRP can be recognized by immunoglobulin G (IgG) and Fc\textgamma R receptors, expressed in numerous cells, including macrophages, mast cells, platelets, and leukocytes. Fc\textgamma R receptors are classified either as high-affinity Fc\textgamma RI(CD64) or as low-affinity receptors, such Fc\textgamma RII (CD32) and Fc\textgamma RIII (CD16). Several studies points towards CRP-binding capacity of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1).

\textbf{Properties and functions:}

1. C-reactive protein when bound to bacteria, can activate the complement to enhance opsonisation and clearance of the bacteria prior to the production of specific IgM or IgG. This process of protein coating to enhance phagocytosis is similar to opsonization by antibodies.

2. CRP induces activation of the classical complement pathway and act as scavenger for chromatin fragments.

3. C-reactive protein bound to bacteria or cells can interact with the natural killer cells and with the monocytes and may increase the tumoricidal activity of these cells.\textsuperscript{20}

4. C-reactive protein can induce the synthesis of IL-1\alpha, IL-1\beta, TNF\alpha and IL-6 in human peripheral blood mononuclear cells and alveolar macrophages.

5. CRP modulates macrophage function.

6. Bound CRP may produce secondary binding sites for factor H and thereby regulate alternative-pathway amplification and C5 convertases.\textsuperscript{14}

\textbf{Methods of measuring CRP levels}

CRP can be measured using immuno-turbidimetric or immuno-electrophoretic assays or latex slide
agglutination method. In an enzyme-linked immunosorbent assay (ELISA) or an immune-fluorescent assay high-sensitivity CRP (hs-CRP) assay uses labeled monoclonal or polyclonal anti-CRP antibodies. To detect hs-CRP levels Laser nephelometry can also be used. As CRP is a non-specific marker its value needs to be correlated with clinical and laboratory findings. Clinically, CRP can be measured in serum in 2 ways i) routine CRP which measures level above 3mg/L in adults and ii) High sensitivity (hs-CRP) measuring range up to 3mg/L. Routine CRP level measurement can be done for a) Screening for organic disease, b) Monitoring of extent and activity of disease: infection, inflammation, malignancy and necrosis. c) Detection and management of recurrent infection.

C-reactive protein and Periodontitis:

Periodontal disease is a chronic inflammatory processes resulting from interaction of selected gram negative bacterial species with the host defense in disease susceptible individuals, leading to progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both. This condition occurs in response to a predominantly gram-negative bacterial infection originating from dental plaque. The host responds to the microbial challenge, with a high inflammatory response with increased levels of cytokines like IL-1, IL-6, TNF-α. These mediators promote activation of the acute phase reactants resulting in elevated serum levels of CRP, α1-acid glycoprotein, ceruloplasmin, serum amyloid A. In healthy young adult volunteer blood donors, the median concentration of CRP is 0.8 mg/l, the 90th centile is 3.0 mg/l, and the 99th centile is 10 mg/l, but, following an acute-phase stimulus values may increase from less than 50 mg/l to more than 500 mg/l, that is, 10,000-fold. This effect is mainly seen as periodontal pathogens do not induce only local inflammation and tissue destruction but they are also involved in systemic increase in inflammatory and immune response. Trang N. Salzberg et al demonstrated that patients with AgP demonstrate elevated serum concentrations of CRP. It was reported that periodontitis had an impact on systemic markers of inflammation in a relatively young subject group. Boucher et al. reported that CRP appears in the serum of patients with some forms of inflammatory oral disease. The highest incidence of positive CRP tests and the strongest CRP test reactions were observed in patients with acute alveolar abscesses. Recent study have shown that patients with severe periodontitis have increased serum levels of CRP, when compared with unaffected control population. Various studies have proved a positive association between the presence of chronic periodontitis and high serum CRP levels. Role of C - reactive protein in the Pathophysiology of Atherosclerosis: Atherosclerosis is a chronic inflammatory disease; inflammatory processes are the vital part of pathophysiology of atherosclerosis and are supposed to be involved from initiation to final stages of infarction. Three different risk categories based on serum CRP levels have been identified in a consensus conference of the American Heart Association (AHA) and the Center for Diseases Control (CDC). CRP concentrations less than 1 mg/l are considered to be at low risk, concentrations in the 1–3 mg/l range are assigned a medium risk level and those with more than 3 mg/l in serum CRP are considered to be at high risk for future cardiovascular disease and events. High-sensitivity CRP (hsCRP) has been identified to be a key marker of atherosclerosis, and elevated levels constitute a risk factor for ACVD. Expression of adhesion molecules, chemokines, and cytokines by endothelial cells can directly be activated by CRP. CRP can directly influence atherogenesis by
activation of the complement system, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation, and thrombosis. pCRP can generate inflammatory responses, binding to the phosphatidylcholine on the exterior of LDLox and the surface of apoptotic cells, while mCRP is able to modulate platelet function inducing aggregation and contributes to atherothrombotic complications by promoting thrombosis.

Effect of periodontal therapy on serum CRP level:
Periodontal therapy includes mechanical disruption of the dental biofilm of the diseased dentition. After periodontal therapy reduction of serum levels of CRP and an improvement of measures of endothelial function has been observed. Treatment of periodontal infections, whether by intensive mechanical therapy, drug therapy or extraction, can significantly lower serum levels of CRP. Median decrease in serum CRP level after completion of periodontal therapy has been reported, and found that control of periodontal disease can be observed with non-surgical periodontal therapy, along with decrease in the serum mediators and markers of acute phase response.

A recent meta-analysis of 10 cross-sectional studies showed that CRP in periodontitis patients is elevated in comparison to controls without periodontitis. Results from a meta-analysis indicate that the periodontal treatment could lower the levels of CRP after therapy. Furthermore, a recent multi-centred randomized control study (the Periodontitis and Vascular Events; PAVE) showed that periodontal treatment can reduce the CRP levels from high to moderate levels in non-obese periodontitis patients. Several short term intervention studies reported that treatment of periodontitis reduces the serum concentrations of inflammatory markers which are thought to be initiating factors for cardiovascular disease.

CONCLUSION
C-reactive protein is a nonspecific marker of inflammation. Elevated levels of serum C-reactive protein are found in subjects with cardiovascular diseases. Chronic periodontitis results in higher systemic levels of CRP. The elevated inflammatory factors may increase inflammatory activity in atherosclerotic lesions, thereby increasing the risk for cardiovascular events. CRP plays an important role in activation of complement system, metalloproteinases and recruitment and activation of inflammatory cells. Many studies have shown that periodontal therapy can lower the levels of CRP in serum of patients with periodontitis. Further research is needed to find out whether the changes in cellular and molecular markers in peripheral blood in periodontitis are indeed causally related to cardiovascular events and whether periodontal therapy and maintenance of periodontal health will reduce the risk for such events.

REFERENCES
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