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Calprotectin - A Promising Diagnostic Biomarker For Systemic And Periodontal Diseases – A Short Review

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ABSTRACT

Periodontal disease (PD) is a frequently occurring infectious and inflammatory disease characterized by tissue inflammation and destruction resulting in tooth loss. The primary etiology of the disease is the periodontopathic bacteria present in plaque biofilm and counteracting innate and adaptive immune responses in periodontal tissues. Calprotectin is a calcium-binding protein that in recent days is considered as an acute phase reactant, belonging to the S-100 protein family. The protein is found to be secreted in higher concentrations by activated granulocytes, monocytes, and epithelial cells. Calprotectin has been shown to play an important role in numerous chronic inflammatory systemic diseases. Accumulating evidences indicates that the protein is increasingly involved in the progression of periodontal disease, and its levels might be allied with disease progression, severity and outcome of periodontal treatments. This review emphasies on the history, evolution and role of calprotectin in various inflammatory systemic disorders and periodontal disease indicating it as an effective prognostic and diagnostic marker.

Key words: Calprotectin, Biomarker, Inflammation, Periodontitis.

INTRODUCTION

Inflammation is defined as the physiological response to aninjury or insult. The intial and first line of inflammation to occur is acute inflammation, where the reaction is rapid and of short duration. If the insult or injury persists, the response becomes chronic, which is pathologic in nature. In this stage, the adaptive immune response is activated with involvement of the cellular and non-cellular mechanisms of acquired immunity. Thus the immune mechanisms showcase further roles in the resolution of inflammation and in the wound healing process, including the repair and the regeneration of damaged tissues. Hence both innate immunity and acquired immunity must go hand- in- hand to bring back the state of homeostasis.[1]

Neutophils play an important role which are most abundant cell typesof white blood cells in the circulation play an essential role in regulating the inflammatory response and have been regarded as first line of defense in the innate immune system. After recognising pathogens, these cells catch and kill the invading microbes by phagocytosis and intracellular destruction, granule release, and by formation of neutrophil extracellular traps. Neutrophils also act as inflammatory

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mediators. However, recent evidences , have revealed that neutrophils exhibit a high degree of phenotypic variability and functional flexibility, making neutrophils key modulators of both inflammation and immunological responses.[2]

Calprotectin is an copious cytosolic protein complex (comprising of S100A8 and S100A9 subunits) that is indigenously expressed in neutrophils that consititutes 45% of total cytosolic protein content. [3] The expression of this protein is specifically triggered during the inflammation process.[3] This article aims in eliciting the role of calprotectin in various inflammatory disease processes thereby establishing its role as a biomarker for these chronic inflammatory diseases.

CALPROTECTIN – ITS HISTORY, EVOLUTION AND STRUCTURE

Calprotectin is a heterodimer protein consisting of two calcium-bindinglinkages, belonging to the S-100 family, mainly secreted in the cytoplasm of neutrophils accounting for 30 to 40% of its cytosolic protein content**.** The protein was discovered in the year 1980 by Fagerhol MK et al., with its name owing to its calcium-binding and anti-microbial actions. It weighs about 36kDa and has an established role to play in innate immune response due to its antimicrobial effects.This proteincan be physiologically detected in serum, amniotic fluid, saliva, GCF and other secretions. The protein is also referred to with various other names RP-8-MRP-14, Calgranulin A and B, Cystic fibrosis antigen, L1 and 60BB antigen. Calprotectin which is released following leukocyte activation exerts bacteriostatic and fungistatic effects by inhibiting zinc-dependent enzyme systems. Calprotectin also induced apoptosis in normal and cancer cells. Thus the protein has various roles to play in the protection and progression of various systemic disorders depending on the organ affected and the degree of inflammation present which makes it viable to consider it as a marker of inflammation.[4]

Structure of Calprotectin

Calprotectin is a dimer belonging to the S100 family, S100A8 (MRP8) and S100A9 (MRP14). The exact structure of calprotectin has been under debate where few authors quote it as a heterotrimer while few shreds of evidence state the protein is a heterodimer or heterotetramer due to the presence of calcium in the ring. The human analog of the protein is a dimer weighing about 24 to 36 kDa.[5]

Calprotectin has a high affinity for non-organic minerals which include calcium, iron, zinc and manganese. Each of the proteins consists of calcium-binding sites wherein each dimer molecule can bind with a total of four calcium ions. When a dimermolecule of calprotectin binds with the inorganic calcium, it induces a conformational change in the structural complexity of the dimer molecule which increases its affinity for transition metals and promotes the formation of the tetramer**.** A maximum of two transition molecules can bind to this complex.[5]

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A calprotectin dimer molecule can bind to only one manganese or iron ion only in the presence of calcium. Zinc can bind to two sites within the calprotectin structure even in the absence of calcium.[6]

This signaling protein due to its high affinity to zinc ion binding property encodes for its anti-microbial and apoptotic inducing properties where the heterotetramer binds and sequesters zinc thereby inhibiting zinc-dependent enzymes and microbial growth. Due to its high calcium-binding capacity, it has been implicated in the wound healing process and is a clinically relevant marker of inflammatory diseases like cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease, and periodontitis.[6]

GENES THAT CONTROL CALPROTECTIN EXPRESSION

The genes that encode for the production of this heterodimeric molecular complex are located on chromosomal locus 1q21.3 of the epidermal differentiation complex. The extracellular calprotectin secretion exhibiting proinflammatory and antimicrobial properties is by signaling through RAGE and TLR4 receptors located in the neutrophil cellular complex. The protein subunit S100A8/9 of the calprotectin molecule is essential for myeloid cell differentiation. Within the epithelial cells, calprotectin activates NADPH oxidase generating reactive oxygen species. This activation of NADPH oxidase is dependent on the binding capacity of arachidonic acid to the C- terminal region of the S100A9 protein subtype. It also activates the nuclear factor – kappa B signaling pathway. The genes that encode calprotectin production include the S100A8 gene, HGNC 10498 gene,Entrez gene, and P05109 gene.[7]

This protein functions as a casein kinase inhibitor and as a cytokine. Thus during the acute and chronic inflammatory process, the calprotectin that is released functions as an effector molecule in modulating the innate immune response. Calprotectin represents a key antimicrobial protein found in neutrophil extracellular traps in the extracellular environment. Thus it is considered as a biomarker for inflammatory diseases like periodontitis and inflammatory bowel disorders. [8]

FUNCTIONS OF CALPROTECTIN

The functions of calprotectin could be classified as intracellular and extracellular activities. The intracellular activity includes regulatory and protective functions in the cytosol of the cell. S100A8 and S100A9 subunits of the protein helps in a rapid rearrangement of the cytoskeleton which is a prerequisite for cell migration, phagocytosis. It results in effective leukocyte migration through calcium and MAPK signaling pathways during inflammation-causing reversing of microtubule formation. Calcium-infused heterodimer of calprotectin plays a crucial role in stabilizing the microtubule network that facilitates phagocytic induction. Activation of NAPH oxidase is implicated in respiratory burst in the presence of calcium. The activation of NADPH oxidase generated reactive oxygen species in phagocytes contributing to the initiation and regulation of inflammatory responses. It also enhances epithelial cell resistance to enteric pathogenic bacteria like *Porphyromonas gingivalis, Salmonella, and Listeria monocytogenes*.[9]

The extracellular functions include leukocyte recruitment, the release of proinflammatory cytokines, modulation of cellular proliferation, differentiation and apoptosis, antimicrobial and anti-inflammatory action.[10]

CALPROTECTIN AND INFLAMMATION

Inflammation is the basic protective response generated by the human body in response to a noxious stimulus. Various cells and molecules are involved in regulating a massive host immune response during the inflammation process, which helps in the elimination of exogenous and endogenous pathogenic substances to protect the body. However, an imbalance in this cell population and prolonged excessive inflammatory status results in tissue damage.[11]

Calprotectin (S100A8/A9) is a protein mainly derived from immune cells such as neutrophils and macrophages that have been confirmed to play a pivotal role in the development of inflammation.[12] The protein plays a dual role in regulating both intracellular and extracellular microenvironments. This protein plays an important role in infection-induced inflammation.[12]Once there is bacterial invasion, the recruitment of neutrophils, monocytes and macrophages eventually increase, which secrete calprotectin protein to modulate the inflammatory process with the induction of inflammatory cytokines, reactive oxygen species (ROS) and nitric oxide (NO).[13]The early expression of S100 proteins during infection-induced inflammation that calprotectin plays a vital role in mediating innate inflammatory response owing to the antimicrobial actions of zinc ion binding to the heterodimer molecule.[13] The expression of calprotectin protein during infection-induced inflammation is restricted by a negative feedback mechanism.Excessive expression of the protein magnifies the inflammatory response thereby causing increased release of pro inflammatory cytokines by neutrophils and monocytes.[14] This is more concurrent with chronic inflammatory diseases like periodontitis, chronic obstructive pulmonary disorder (COPD) where the levels of the protein expressed in saliva, GCF and serum are exponentially increased with the progression of the disease marking it as a pro inflammatory marker.[15]

Although much attention is grabbed to the pro inflammatory effect of the protein, it also exerts an anti inflammatory effect under specific conditions to prevent tissue damage. The protein under certain circumstances suppresses mase cell degranulation, eosinophil infiltration, leukocyte adhesion and migration. In addition, the protein as well exerts a regulatory activity in inflammation by its apoptosis inducing potential. The phagocytic activities of neutrophils have been reinstated when a medium is depleted with calprotectin protein thus indicating that the protein is an important player in causing suppression of pro inflammatory activation of macrophages. Thus calprotectin protein is considered as a double-ended sword where it could aid and induce inflammation in various systemic disorders under specific conditions.[15]

CALPROTECTIN AS A DIAGNOSTIC BIOMARKER

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Due to its extensive role in potentiating inflammation, calprotectin could be used as a diagnostic biomarker in various chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, diabetic nephropathy, cardiovascular diseases, periodontitis and autoimmune diseases such as juvenile dermatomyositis.

ROLE OF CALPROTECTIN IN VARIOUS SYSTEMIC DISORDERS

I. Calprotectin and Rheumatoid arthritis

Rheumatoid arthritis is an inflammatory autoimmune disorder characterized by higher circulating inflammatory infiltrates, erosion of joints, bone, and cartilage.[16] Calprotectin is potentially considered as a sensitive biomarker of disease activity in rheumatoid arthritis compared to other conventional acute phase reactants such as erythrocyte sedimentation rate (ESR) and C- reactive protein as it directly reflects the inflammation in synovial membrane and cavity rather than systemic inflammatory activity. It was observed that increased levels of calprotectin were found in serum and synovial fluid of rheumatoid arthritis patients reflecting the progressive disease activity. However, the mechanism linking calprotectin in the disease progression of rheumatoid arthritis remains unclear. Some studies have demonstrated pro-inflammatory functions of calprotectin by activation of the TLR receptor pathway. TLRs are detected on the membranes of fibroblast-like synoviocytes. The hyperinflammatory response in rheumatoid arthritis may aggravate inflammation causing tissue damage. Few studies also quoted calprotectin interaction with TLR4 activates osteoclast differentiation and bone resorption worsening the disease progression.[17]

II. Calprotectin and Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic fatal gastrointestinal disorder caused by recurrent episodes of intestinal inflammation.[18] It clinically contains Crohn's disease, lcerative colitis and other inflammatory conditions. The disease is characterized by bouts of stomach discomfort, diarrhea, bloody stools, weight loss, and the invasion of neutrophils and macrophages that generate cytokines, proteolytic enzymes, and free radicals that cause inflammation and ulceration of the intestinal mucosa. [18]Faecal calprotectin is a diagnostic marker of intestinal inflammation that is chiefly used in the diagnosis of Crohn's disease and ulcerative colitis (IBD). Fecal calprotectin is biochemical analysis of the levels of calprotectin in the stool. Increased levels of calprotectin indicated the migration of neutrophils to the site of inflammation that is intestinal mucosa. Calprotectin is a stable protein not digested by digestive enzymes present in the gut hence it can be employed as a sensitive marker in detecting disease progression.[19]

III. Calprotectin and Cardiovascular diseases

Cardiovascular disease describes a group of disorders affecting the blood vessels and heart. It is a broad term that includes a variety of diseases like coronary heart disease, cerebrovascular

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disease, rheumatic heart disease, congenital heart diseases, and thromboembolic disorders. Researches have suggested higher levels of plasma calprotectin expression associated with cardiovascular diseases. Calprotectin, predictably considered as an acute-phase protein, its concentration in blood may upturn by 40–130 times during the process of inflammation.[20]

The substantiation that calprotectin production may be brought about in mature myeloid and non-myeloid cells cardiovascular cell types has unveiled a novel and intriguing scenario, wherein this protein may play a noteworthy role in the pathobiology of cardiovascular disease (CVD). The expression of calprotectin is virtually absent under normal conditions in cardiomyocytes, and endothelial and vascular smooth muscle cells (VSMC). Therefore, some pro-inflammatory cytokines such as LPS, IL-1β, and TNF-α may competently promote the synthesis of the protein in these cells], and its release into circulation upon injury of the plasma membrane, as may frequently occur in atherosclerotic lesions. Furthermore, it has been shown that calprotectin produced by endothelial cells may promote atherogenesis by increasing adhesion molecule expression, chemokine production, and endothelial layer permeability. Therefore, calprotectin is not just considered as another acute phase reactant, but it may play an active role in the pathogenesis of cardiovascular diseases.[21]

IV. Calprotectin and Diabetes

Researches have found that circulating calprotectin is an impending biomarker for endovascular inflammation in type 2 diabetes mellitus (T2DM) patients. The possible associated mechanism linking calprotectin and type 2 diabetes is the association of possible risk factors of the disease like smoking, sedentary lifestyle, obesity that could trigger oxidative stress, and proinflammatory cytokine production resulting in the accumulation of neutrophils to the site of inflammation in blood vessels. The second possible linking mechanism is that calprotectin can bind to receptors of advanced glycation end products (AGE receptors) and potentiate the inflammation process.[22]

CALPROTECTIN AND PERIODONTAL DISEASE

Periodontitis is a chronic inflammatory disease affecting the soft and hard tissues of the oral cavity which on progression might result in periodontal pocket formation, clinical attachment loss, alveolar bone loss and finally leading to tooth loss caused by pathogenic microflora which colonize on the hard surfaces of the teeth. Although it is initially thought that the pathogenic plaque biofilms are the primary etiological agents of the disease, the progression and clinical outcome of the periodontal disease is primarily characterized by the host immune responses which are modified by behavioral and environmental factors. There are various molecules like cytokines, chemokines, enzymes like matrix metallo proteinases which are released during the pathogenesis of the disease that determines the severity and progression.[23]

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Calprotectin is a calcium-binding protein that is mainly secreted by neutrophils in a major quantity while monocytes and macrophages secrete it in minor amounts. Researchers have found that the production of the protein has been attenuated to certain conditions by several epithelial cells, microvascular endothelial cells, fibroblasts, keratinocytes, and osteoclasts after activation. However, the protein is nonexistent in resting tissue macrophages and lymphocytes. Calprotectin accumulates on the inflammation sites and interacts with the cell surface proteins where it triggers special kinetic pathways. Calprotectin binds to Toll like receptor (TLR – 4) the receptor for AGE and extracellular matrix metalloproteinases belonging to the family of pattern recognition receptors. TLR has been known to identify pathogen associated molecular patterns (PAMP) which immediately triggers signal transduction events to activate an active inflammatory response.` Calprotectin has been identified as an important damage associated molecular pattern (DAMP) recognized by TLR that exerts double effects on phagocyte homeostasis. This further results in activation of nuclear factor kappa B signaling pathway that triggers the production of various proinflammatory cytokines like IL-1β, IL-6, TNF –α which leads to activation of innate immune response and tissue destruction. [24]

Calprotectin as a potential biomarker in periodontal disease

Calprotectin recently considered as an acute-phase protein has the uniqueness of not synthesized in liver and is comparatively stable when compared with similar linear groups. Moreover, the protein is expressed in the saliva, GCF, serum in the affected patient's samples which makes it a more evident and available diagnostic marker to predict the staging and grading of the periodontal disease progression.[25] A study by Que et al., quantified the protein during the early phase of experimental gingivitis and periodontitisfound that the levels of the protein were significantly elevated in patients with aggressive periodontitis and chronic periodontitis when compared with gingivitis subjects. [26]Zheng et al., found that calprotectin is highly expressed in GCF of aggressive periodontitis than compared with controls.[27] In an interventional longitudinal study conducted by Kaner et al., he found that the levels of calprotectin eventually decreased after initial non-surgical therapy in aggressive periodontitis patients. [28]Kajiura et al., compared the levels of calprotectin in diabetic periodontitis and periodontitis subjects wherein the author concluded that patients with diabetic periodontitis have a two-fold increase of the protein level when compared with chronic periodontitis.[29] Kido et al demonstrated that lipopolysaccharide of Porphyromonas gingivalis bacteria increased the release of calprotectin from neutrophils.Calprotectin exhibits extensive antimicrobial activity through zinc chelation. [30]In periodontally healthy individuals, calprotectin was found to be expressed in spinous layers of the gingival epithelium. Nishikawa et al., studied the effects of calprotectin in human gingival fibroblasts wherein the author demonstrated a lower inductive effect of monocyte chemotactic protein (MCP -1), IL-6, TNF – α productions in human gingival fibroblasts.[31]

The above-mentioned studies and researches promise the role of calprotectin as a promising diagnostic biomarker for periodontal disease.

CONCLUSION

Calprotectin, in particular, can be considered as an effective biomarker for monitoring the disease activity and predicting the outcome of treatment modalities in various systemic conditions especially in periodontitis owing to its extraordinary properties of being a stable protein, not synthesized by the liver and its collateral link with various systemic disorders. The protein also exerts a protective and destructive effect on the progression of the pathogenesis of the periodontal disease. However, the exact mechanism of calprotectin in periodontal diseases is still under interrogation whether the overall effect of calprotectin is to combat the infection or to contribute to tissue destruction by evoking inflammatory response. Hence future studies unwinding these mechanisms linking calprotectin and periodontal disease would promise its place as a chair side diagnostic biomarker for predicting the disease progression.

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