



## Acute phase proteins

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### ABSTRACT

An assembly of systemic and metabolic changes that occur within 90 minutes after the onset of a systemic inflammatory reaction is termed as acute phase reaction/response. This is caused due to the change in the concentrations (increase/decrease) of a class of proteins called as acute phase proteins. In periodontitis, subgingival gram negative bacteria release endotoxins that interact with Toll-like receptors, expressed on polymorphonuclear leucocytes and monocytes resulting in the formation of a complex that activates the immune system. This results in a cascade of inflammatory changes resulting in the production of acute phase proteins by the pro-inflammatory cytokines and glucocorticoid hormones. Recent studies have shown increased levels of acute-phase proteins with gingival inflammation, including experimental gingivitis and periodontitis reflecting the locally stressed environment. New acute-phase phenomena continue to be recognized, and the mechanisms mediating them are becoming better understood. This article therefore highlights about the vital acute phase proteins and their association with periodontal diseases.

**Keywords:** acute phase response/proteins, inflammation, cytokines

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### INTRODUCTION

Periodontitis (PD) is one of most predominant worldwide chronic inflammatory disease that is characterized by the host-mediated destruction of soft and hard tissues of the periodontium. Even though the etiological role of bacteria has been decisively established in vitro and in vivo, it is only recently that researchers have begun to identify the role of both local and systemic inflammatory processes in encouraging a pathological response to the oral micro flora. [1]

As periodontitis supervenes, the local host inflammatory mediators undergo alterations resulting in the initiation of a localized specific host response, followed by a serum antibody reaction to the bacteria. [2] This stimulus for a strong antibody response is exhibited by the increased production of plasma proteins which results in the initiation of the inflammatory acute phase response.[3]

Acute phase reaction/response is a general term attributed to a group of systemic and metabolic changes that occur within hours of an inflammatory stimulus. It represents an organism's early and/or highly complex reaction to a variety of injuries such as bacterial, viral or parasitic infection, mechanical or thermal trauma, ischemic necrosis, or malignant growth.[4] A range of changes in the organism occurs that act together to neutralize the inflammatory agent.

Characteristic features of the systemic acute-phase response include the following:

- (i) Fever,
- (ii) Neutrophilia,
- (iii) Changes in lipid metabolism,
- (iv) Hypoferremia,
- (v) Increased gluconeogenesis,
- (vi) Increased (muscle) protein catabolism,
- (vii) Activation of the complement and coagulation pathways,
- (viii) Hormonal changes, and
- (ix) Induction of acute-phase proteins.[5]

The results include activation of macrophages, platelet aggregation, increased blood vessel permeability, transudation of biological fluids into the tissues and migration of circulating leukocytes. This leads to many reflective changes in the biosynthetic profile of various acute phase proteins (APPs).

#### **ACUTE PHASE PROTEIN**

An acute phase protein has been defined as one whose plasma concentration increases (positive APPs) or decreases (negative APPs) by at least 25 percent during inflammatory disorders.[6]

#### **REGULATION OF ACUTE-PHASE CHANGES**

- Studies have shown that cytokines and to a lesser extent the glucocorticoid hormones regulate the synthesis of these acute-phase proteins.
- Cytokines are intercellular signaling polypeptides produced by activated cells that are produced during inflammatory processes.
- They are the chief stimulators of the production of acute phase proteins.[6]

Subjectively, cytokines related to the acute phase response can be divided into three groups:

(i) Pro-inflammatory cytokines initiating or enhancing the cascade of events (tumor necrosis factor  $\alpha$ , interleukin 1 (IL-1), interferon- $\gamma$  and IL-8);

(ii) Cytokines that are responsible for the main systemic features of acute-phase response in a variety of tissues (Interleukin-6- type cytokines, leukemia inhibitory factor, IL- 11, oncostatin M, ciliary neurotrophic factor and cardiotrophin-1); and

(iii) Anti-inflammatory cytokines down regulating the acute-phase response (IL- 10, IL-4, IL-13 and transforming growth factor  $\beta$ ).[4]

- The most potent recognized inducers of acute-phase proteins include the  $\alpha$ -helical cytokines, IL-6 and oncostatin M.[7]
- The IL-6-like cytokines synergize with IL-1-like cytokines to induce type I acute-phase proteins. This occurrence is thought to be chiefly controlled by IL-6 that acts on the hepatocyte and induces transcriptional activation of the acute-phase protein genes.[8]
- The reciprocal interaction between IL- 6 and functionally related other inflammatory cytokines, as well as the hypothalamo-pituitary-adrenal axis, represents a separate facet of the complex web of regulatory neuroendocrino-immunological interactions.
- Gluco-corticosteroids decrease the level of IL- 1, tumor necrosis factor, and IL-6 in the peripheral blood via transcriptional and post-transcriptional routes. By doing this, they prolong their impact on the target cells through the elevation of the expression of their receptors.[9] Lastly, there exist apparent feedback mechanisms involving

both liver synthesized acute-phase proteins and neuroendocrine factors from the central nervous system, which contribute to regulation of the acute-phase response to inflammation.

#### CLASSIFICATION OF ACUTE-PHASE PROTEINS

GROUPS	PROTEINS	NORMAL PLASMA CONCENTRATION (mg/ml)
I (concentration may increase by 50%)	Ceruloplasmin	150-600
	Complement C3	800-1700
	Complement C4	150-650
II (concentration may increase two to fivefold)	AGP (Alpha1-Acid Glycoprotein)	550-1400
	PI	2000-4000
	ACT (Alpha1-Antichymotrypsin)	300-1600
	Hp (Haptoglobin)	400-1800
III (concentration may increase up to 1000 fold)	Fibrinogen	2000-4500
	C-reactive protein	<5.0
	Serum Amyloid A	<10.0

#### Classification according to the changes in concentration of APPs[10]

Proteins whose plasma concentrations increase (Positive APPs)

- Complement system
  - C3
  - C4
  - C9
  - Factor B
  - C1 inhibitor
  - C4b-binding protein
  - Mannose-binding lectin
- Coagulation and fibrinolytic system
  - Fibrinogen
  - Plasminogen
  - Tissue plasminogen activator
  - Urokinase
  - Protein S
  - Vitronectin
  - Plasminogen-activator inhibitor 1
- Antiproteases
  - $\alpha$  1-Protease inhibitor
  - $\alpha$  1-Antichymotrypsin
  - Pancreatic secretory trypsin inhibitor
  - Inter  $\alpha$ -trypsin inhibitors
- Transport proteins
  - Ceruloplasmin
  - Haptoglobin
  - Hemopexin
- Participants in inflammatory responses
  - Secreted phospholipase A2
  - Lipopolysaccharide-binding protein
  - Interleukin-1-receptor antagonist
  - Granulocyte colony-stimulating factor 17
- Others
  - C-reactive protein
  - Serum amyloid A

- $\alpha$  1-Acid glycoprotein
- Fibronectin
- Ferritin
- Angiotensinogen

Proteins whose plasma concentrations decrease (Negative APPs)

- Albumin
- Transferrin
- Transthyretin
- A 2-HS glycoprotein
- Alpha-fetoprotein
- Thyroxine-binding globulin
- Insulin-like growth factor I
- Factor XII

## SIGNIFICANT ACUTE-PHASE REACTANTS AND THEIR ROLE IN PERIODONTITIS

### 1. C- REACTIVE PROTEIN (CRP)

CRP has derived its name because of its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae*. It was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage.[11] It is a member of the pentraxin family of proteins, which are serum opsonins. Its functions include the ability to recognize pathogens and to mediate their elimination by recruiting the complement system and phagocytic cells.

The median concentration of CRP is 0.8 mg/l in healthy young adults but, following an acute-phase stimulus, values may increase from 50  $\mu$ g/l to more than 500 mg/l, that is, 10,000 fold.[12] Plasma CRP is produced only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6.

Periodontal diseases are associated with an increase in CRP levels. The systemically disseminated bacteria, lipopolysaccharide (LPS) as well as the cytokines in periodontitis may stimulate hepatocytes and circulating leukocytes to produce CRP and IL6, respectively.[13] Dentate people with extensive periodontal disease had an increase of approximately one third in mean CRP and a doubling in the prevalence of elevated CRP, compared with periodontally healthy people.[14] Periodontal pathogens like *Porphyromonas gingivalis*, *Prevotella intermedia*, *Campylobacter rectus*, and *Bacteroides forsythus* were found in the subgingival samples obtained from patients with elevated CRP levels.[15]

### 2. SERUM AMYLOID A

Serum amyloid A (SAA) is a multi-gene family that consists of highly conserved protein sequences that cluster on chromosome 11 in humans.[16] They are secreted predominantly by hepatocytes but the extra-hepatic production of acute-phase SAA occurs in other cells like the vascular smooth muscle cells and endothelial cells.[17]

Aoki Nonaka *et al.* have reported that periodontal infections induced elevated levels of SAA and *Porphyromonas gingivalis* specific IgG in the serum of Wild type mice.[18] In a very recent study conducted by Carlos *et al.*, SAA levels were significantly higher in patients with chronic periodontitis than in individuals without periodontitis.[19]

### 3. ALPHA 1- ACID GLYCOPROTEIN

AAG is an acidic glycoprotein of about 41 kDa in molecular weight with a tertiary structure. The normal concentration of AAG is between 0.6-1.2 mg/mL (1-3% of plasma proteins). It is mainly synthesized by liver and its production increases in response to stimuli like trauma or infection.[20]

Ingrid *et al.* has reported a trend towards higher levels of AAG in subjects with both periodontitis and cardiovascular disease in his study.[21]

### 4. ALBUMIN

HSA is a single-chain, nonglycosylated polypeptide with a molecular weight of 66,500 Da containing 585 amino acids.[22]

Protein depletion results in hypoproteinemia with many pathologic changes including degeneration of the connective tissue of the gingival and periodontal ligament, osteoporosis of the alveolar bone, impaired deposition of the cementum, delayed wound healing, and atrophy of the tongue epithelium. Mojon *et al.* has demonstrated in his study that older adults with vertical tooth mobility and periodontal pockets greater than 6mm had a significantly lower albumin concentration.[23] Studies by Ogawa *et al.* and Ramesh *et al.* have also described the negative association between albumin concentration and periodontal diseases in their respective studies.[24, 25]

### 5. FIBRINOGEN

The fibrinogen molecule is a soluble, large, and complex glycoprotein. It is a 340 kDa plasma glycoprotein, which is converted by thrombin into fibrin during the blood clot formation. It has a rod-like shape with dimensions of  $9 \times 47.5 \times 6$  nm and it shows a negative net charge at physiological pH (IP at pH 5.2).[26] It is one of the acute phase proteins that originate from the liver. The concentration of fibrinogen in the blood plasma is 200– 400 mg/dL.

Fibrinogen plays two important roles in the body.

- Firstly, it is an important component of the common pathway of coagulation.
- Secondly, it takes part in the acute phase response after tissue inflammation and damage.

Page *et al.* showed that fibrinogen levels are elevated in any form of inflammation, as it is an acute phase protein;[27] like especially in human gingival tissues during the initial phase of periodontal disease. Ritam *et al.* has demonstrated in his study that statistically significant reduction was observed in the level of the inflammatory marker *i.e.* plasma fibrinogen after SRP.[28]

#### OTHER ACUTE PHASE PROTEINS AND THEIR BIOLOGICAL FUNCTIONS

Acute-phase protein	Main biological function
<b>Proteins whose plasma concentration increase</b>	
Fibronectin	Wound healing
Ferritin	Iron binding
Angiotensinogen	Renin substrate
Complement factors: C3, C4, C9, factor B, C1 inhibitor, C4b binding protein, mannose-binding lectin	Enhancing phagocytosis of antigens, attracting macrophages and neutrophils, lysis membranes of foreign cells, clumping of antigen-bearing agents, altering the molecular structure of viruses
Coagulation and fibrinolysis factors: plasminogen, tissue plasminogen activator, Urokinase, Protein S, Vitronectin, Plasminogen-activator inhibitor 1	Coagulation, degradation of blood clots, trapping the invading microbes, chemotaxis
Ceruloplasmin	Contains copper, has histaminase-and ferroxidase-activity; scavenges $Fe^{2+}$ and free radicals
Haptoglobin (Hp)	Binds haemoglobin; binds to CD11b/CD18 integrins
Interleukin1-receptor antagonist	modulates a variety of interleukin-1 related immune and inflammatory responses
Alpha1-Antitrypsin (AAT)	Inhibits proteolytic enzymes, immune-modulatory activity
Alpha1-Antichymotrypsin (ACT)	Inhibits proteolytic enzymes
Alpha2-macroglobulin	Inhibits proteolytic enzymes
<b>Proteins whose concentration decrease</b>	
Transferrin	Carrier protein, immunoregulation
Transthyretin	Binds to aromatic compounds, carrier of retinol
Alpha-fetoprotein	Binds various cations, fatty acids and bilirubin
Alpha2-HS glycoprotein	Carrier protein, forms soluble complexes with Calcium and Phosphate
Thyroxine-binding globulin	Binds thyroid hormone

#### CLINICAL ASSESSMENT OF ACUTE-PHASE PROTEINS

The measurement of APPs has become popular because:-

- (i) Inflammatory diseases represent a common circumstance in clinical practice,
- (ii) The increase of APP constitutes one of the major characteristic changes which can objectively document the occurrence of an inflammatory process for the clinician,
- (iii) The relative merits of the different APP are now well documented,
- (iv) Rapid and precise methods of measurement are available.

- Some 30 proteins have been reported to increase in serum during the acute phase response.
- The most commonly measured proteins are CRP, Haptoglobin,  $\alpha_1$ - Acid-glycoprotein or orosomucoid,  $\alpha_1$ - Protease inhibitor (al - PI) previously termed  $\alpha_1$ - Antitrypsin and  $\alpha_1$ - Antichymotrypsin.[29]

### CONCLUSION

A number of the participating AAPs are multifunctional and contribute to both the improvement and the inhibition of inflammation in all stages. The aftermath of the acute inflammatory response is thus likely to be determined by the coordinated generation of a group of APPs, their concentrations and molecular forms in the microenvironment.

Recent data have resulted in the emergence of periodontitis as a disease that can have a significant effect on the systemic health of both humans and animals. Therefore, an understanding of the relationship between the progression of periodontitis and risk factors associated with systemic diseases like cardiovascular disease (such as diet, serum lipids, acute-phase responses, etc.) or others (such as low-birth weight infants, diabetes and systemic inflammatory diseases) would have a profound effect on the approaches for treatment of the periodontal diseases.

Acute phase protein is a non-specific marker of inflammation which can act as a useful testing tool for the assessment of health in general, the pathogenesis of various diseases and also to determine the spread of infection or the efficacy of treatment. There is a broad spectrum of possible applications of acute phase protein-based diagnostics in periodontics and hence it is necessary to develop and augment rapid field tests that allow their measurement in a short period of time.

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