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# DIAGNOSTIC ENZYMES IN PERIODONTAL DISEASE

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## ABSTRACT

Periodontal disease is a Chronic inflammatory disease that affect the connective tissue attachment and supporting bone around the teeth. It is widely accepted that the initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms and the host response of the individual to this pathologic infection.

Host responses to the periodontal disease includes the production of different enzymes released by connective tissue, epithelial or inflammatory cells. The enzymes like acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate or alanine aminotransferase (AST, ALT) and gama glutamyl transferase (GGT) are associated with cell injury and cell death. Alterations in enzymatic activity reflects metabolic changes in the inflammatory state of the gingiva and periodontium. Hence it is important to have a knowledge about this enzymes to have an insight in the disease activity.

Thus, the aim of this review is to provide a comprehensive details about various enzymes associated with periodontal disease activity .

**Key Words:** Chronic inflammatory disease, Gingival crevicular fluid, Periodontal disease

## INTRODUCTION

Prior to the 1980s, periodontitis was assumed to afflict all humans and to progress from gingivitis in a slow continuum until teeth were eventually lost .Studies since then have demonstrated these assumptions to be untrue for some individuals. For example disease activity may occur in episodes or bursts at a limited number of sites during a defined interval, with the clinical duration of activity occurring in short periods from days to months. On the other hand, there is evidence that indicates some sites in certain patients manifest periodontal breakdown that is slow but continuous in its progression. Importantly, there appears to be a small population of periodontal patients who do not respond to treatment and continue to lose attachment despite the best treatment provided. The ability to identify these various patients and diseases is poor. To avoid over treatment and to treat progressive disease appropriately, there is a need to distinguish between stable and progressive disease sites and to assess when these sites are adequately treated.

Traditional diagnostic instruments, such as the periodontal probe and radiographs are inadequate in diagnosing disease

activity as they indicate past tissue destruction and cannot distinguish prospectively between a progressive and a stable site. Thus ,to develop a diagnostic test, one must first define the parameters to be analyzed. For periodontics, the principal features to be established are the presence/absence of disease. Other features such as disease severity, current disease status and the possibility of future progression should also form part of the diagnostic process. A test that is easy to perform, particularly if it has chair side application and that is able to diagnose disease at a particular site will ensure periodontal treatment that is more effective and rational.

### Clinical importance of enzymes

1. Enzymes are the biocatalysts, which regulate the rates at which all physiologic processes take place and it has got the central role in health and disease.
2. In liver cirrhosis, the important key enzymes, which are responsible for urea formation, are impaired. As a result, there is ammonia intoxication and ultimately hepatic coma.
3. In the impairment of enzymes, digestion of foodstuffs is stopped causing mal absorption and metabolic dis-

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turbances. The net result is no energy production and no growth of the body.

4. Measurements of increased or decreased concentrations of certain enzymes in blood serum provide valuable diagnostic and prognostic information of the physicians.

Host response to periodontal infections includes the production of several families of enzymes that are released by stromal, epithelial or inflammatory cells (Table 1). Studies of these enzymes in Gingival crevicular fluid may lead to insights into pathogenesis and may provide a rational basis for the development of novel diagnostic tests.

### Potential Disease Markers in Gingival crevicular fluid (Table 1)

- I. Biochemical Mediators and products of inflammation
  1. Antibodies to periodontal bacteria. (PA Murray et al, 1989)
  2. Cytokines (HJ Lee et al, 1995)
  3. Complement (FJ courts et al, 1977)
  4. Prostaglandin (PA Heasman et al, 1998)
  5.  $\alpha$ 2-macroglobulins and  $\alpha$  1-antitrypsin (A Gustafsson et al, 1994)
  6. C-reactive protein. (E Megson et al, 2010)
  7. Myeloperoxidase. (QT Smita et al, 1986; CF Cao et al, 1989)
- II. Extra cellular matrix components
  1. Collagen (JT Talonpoika et al, 1994)
  2. Proteoglycans and Glycosaminoglycans (Smith AJ et al, 1997)
  3. Osteonectin (Bowers MR et al, 1989)
  4. Osteocalcin (Kunimatsu K et al, 1993)
  5. Fibronectin (J Talonpoika et al, 1989)
- III. Host derived enzymes
  1. Collagenase (T Nomura et al, 1998; R Romanelli et al, 1999)
  2. Elastase (KG Palkanis et al, 1992; A Gustafsson et al, 1992)
  3. Cathepsins (Kunimatsu K et al, 1990)
  4. Alkaline phosphatase (Yan F et al, 1995)
  5. Aryl sulfatase and  $\beta$ -glucuronidase. (Lamster IL et al, 1994)
  6. Aspartase aminotransferase. (Persson GR et al, 1992)

### Alkaline Phosphatase

It is active on a wide range of phosphate esters and is widely distributed in various mammalian tissues, but its exact role in metabolism is not known. It probably plays a role in calcification and its determination in serum is of practical importance in bone disease (Moss DW, 1982)<sup>1</sup>. Analysis of Alkaline Phosphatase activity measured at pH 10 with P-nitrophenyl phosphatase as substrate, have shown that, in human gingival fluid collected from a series of patients, the

concentration of Alkaline Phosphatase was found to be significantly correlated with pocket depth (Ishikawa and Cima-soni 1970)<sup>2</sup>.

ALP is known to be present in PMNS where the enzymes is found exclusively in specific or secondary granules. These cells are probably the main source of enzymes in the gingival sulcus, although it has been shown that oral bacteria, including some gram (-) ve micro organisms typical of sub gingival plaque also produce ALP (Frank RM et al 1978)<sup>3</sup>The concentration of ALP in GCF was the best indicator of active disease. (Lamster I.B et al, 1992)<sup>4</sup>. Chapple IL et al, 1999<sup>5</sup> developed a chair side chemiluminescent assay for the enzyme in sub microtitre volume of GCF and serum and reported that the mean ALP concentration was 2135-IU/L for GCF and 183 IU/L for serum ( 12 fold difference).

### COLLAGENASE

The presence of collagenase in GCF has been of particular interest as collagen constitutes one of major extra cellular matrix protein in the periodontium, and because significant destruction of collagen occurs during periodontal destruction. These are specialized enzymes that have evolved specifically to hydrolyze collagens because the triple helical collagen structure is resistant to more common proteinases. They belong to a family of enzymes called Matrix metallo Proteinases (MMPs) that consist of at least 13 members with closely related remain structures and discrete function. Based on their substrate specificity, MMPs are classified as Collagenases, Geletinases, Stromelysins, Matrilysin. These interstitial collagenases capable of degrading native matrix collagen fibrils have be identified so far. Collagenases –I (MMP-1 or fibroblast type collagnase) is produced by a variety of human epithelial and mesenchymal cells types including keratinocytes, fibroblasts and macrophages. This enzyme can hydrolyze type I, II, III, VI, VIII and X collagens and gelatin. It hydrolyzes type III molecules faster than it does type – I.

Collagenase-2 (PMN) type collagenase or MMP-8) also hydrolyzes type I and III collagen but their enzymes degrades type I faster than it does type III. Collagenase-2 is found only in the granules of PMNs.

Gelatinase Group of MMPs has two prominent members, the 72 kd. (gletinase-A, MMP-2) and 92 kd. (geletinase B O<sub>2</sub> MMP – 90 geletinases. Both these enzymes have a high affinity for gelatin, but they also degrade type IV, VII, X and XI collagens and elastin. MMP-9 is produced by eosinophils, macrophages and keratinocytes and it is stored in PMN granules. Its synthesis is regulated by several inflammatory mediators. MMP-2 is not regulated by most mediators other than TGF-/3 and is produced by most cell types.

Stromelysins have broad specificity with the ability to degrade proteoglycans, basement membranes, laminin and fibronectin, in addition to collagens. Three stromelysins-1 (MMP-3), stromelysins-2 (MMP-10) and stromelysins-3 (MMP-11) have been described.

Matrilysin (MMP-7) and metalloelastase (MMP-12) are other MMPs.

JM. Korostoff 2000<sup>6</sup> substantiate the proposed role of host-derived proteases in the pathogenesis of chronic adult periodontitis. Specifically, they indicate that activated MMP-2 and a 40 kd serine protease are involved in tissue destruction associated with this form of periodontal disease.

Gul Atilla et al ,2001<sup>7</sup> evaluated MMP 8 & 9 and neutrophil elastase in GCF of cyclosporin treated patients and Suggested that low GCF PMN-elastase levels can be an important factor in the pathogenesis of Cyclosporin induced gingival over growth.

Sabrina Mancini et al 1999<sup>8</sup> developed and validated a novel specific, simple, rapid and reproducible assay (soluble biotinylated collagen assay; SBA) based on chemiluminescent detection of biotinylated collagen digestion products and its results indicate that active MMP-8 is detected in GCF by this assay that facilitate diagnostic discrimination between stable and progressive lesions.

Sylvie Segulier, 2001<sup>9</sup> undertook a study to quantify the amount of MMPs and 71TIMPs (tissue inhibitors of MMPs) in order to investigate the possible correlations between such molecules, collagen loss and inflammatory cell subsets. The study showed an imbalance between MMPs and TIMPs associated with the pathologic breakdown of the extracellular matrix during periodontitis. This active form of MMP-9 could be marker for the clinical severity of periodontal disease.

### Cathepsin D

It is a carboxypeptidase and one of the chief acid enzymes in lysosomes, present at high concentration in inflamed tissues, particularly abundant in human mononuclear leukocytes, while PMNs contain less enzymes. Its concentration was found to be 10 times higher in crevicular fluid than in serum and this concentration was positively correlated with periodontal destruction<sup>10</sup>. In gingival washings collected from human volunteers, the activities of both free and total cathepsin D were found to increase during a period of experimental gingivitis<sup>11</sup>. In spite of its unphysiological pH, the enzyme may be capable of attacking various components of the connective tissues. Sections of gingivitis cut in a cryostat and exposed to the granular fraction of human leukocytes at a pH of 3-5, which is optimum for cathepsin D activity, showed extensive destruction of both epithelium and connective tissue<sup>12</sup>.

### Cathepsin G

It is a serine endopeptidase contained in the azurophilic granules of human PMNs and also called chymotrypsin like, because it attacks a number of synthetic substrates typical for chymotrypsin and is inhibited by the same inhibitors. It has an optimum pH of 7.5 and a molecular weight of about 20,000. This enzyme has been shown to hydrolyze hemoglobin and fibrinogen, casein and collagen<sup>13</sup>. Determination of cathepsin G was done using N-acetyl-DL-phenylalanine-naphthylester, as substrate and fast garnet salt as coupling agent, and its activity could be shown in the concentrated supernatant of gingival washings.

### Cathepsin B

GCF cathepsin B was investigated in a two years longitudinal study in which the levels of cathepsin B were found to be significantly higher in sites which went on to experience attachment loss.<sup>14</sup>

### Elastase

Neutrophil elastase is a serine endopeptidase that can degrade both collagenous and non collagenous extracellular matrix proteins. It is released at sites of inflammation. The levels of this enzyme in GCF have been noted to increase with development of gingivitis as well as at sites of established periodontitis. In addition, the levels of neutrophil elastase have been found to decrease following treatment of affected periodontal sites<sup>15</sup>. Longitudinal studies have indicated that GCF levels of neutrophil elastase have some of further breakdown<sup>16</sup>.

PERIOCHECK® is a rapid chair side kit which detects neutral proteases in GCF<sup>17</sup>. The GCF is collected on filter paper and placed in a collagen dye – labelled gel matrix, where dye – labelled fragments are produced by the neutral collagenase activity. These soluble products diffuse into the sample strip, creating a blue color which is in proportion to the neutral activity of the GCF. However the authors confirmed that this test can detect neutral proteases in GCF and reported that the highest neutral protease activity values were found in patients clinically diagnosed as having periodontal disease. However, Jepsen S et al (1996)<sup>18</sup> tested the Periocheck system in a longitudinal study of peri-implantitis lesions. They found that the neutral protease test and bleeding on probing had high negative predictive values for future progressive peri-implantitis.

### ARYL SULPHATASE AND β-GLUCURONIDASE

These lysosomal enzymes are involved in the degradation of glycosaminoglycans (GAG) and are released by activated PMNs. Following hyaluronidase action on GAGs, β-Glucuronidase may act on oligosaccharides and aryl sulphatase to catalyze the hydrolysis of sulphatase to cata-

lyze the hydrolysis of sulphate esters. Lamster IL and Colleagues(1994, 2003) demonstrates that enzymes activity is related to the severity of inflammation and to pocket depth, but there appears to be a stronger relationship between absolute amounts of  $\beta$ -Glucuronidase and pocket depth than with the conc. of the enzymes and also In longitudinal study designs, the activity of  $\beta$ -glucuronidase was significantly elevated during monitoring periods that coincided with attachment loss of 2mm or more . The persistent and Long-lasting elevation of enzymes levels and the strong temporal association with clinical detection of attachment loss indicates that  $\beta$ -glucuronidase may exhibit some of the hall marks of a useful diagnostic test. Measurement of this enzymes may provide an assessment of PMN activity, critical element in the current concept of disease progression.

### LYSOZYME

Lysozyme has bactericidal property, linked to its ability to hydrolyse  $\beta$ ,1-4, glycosidic bonds of peptidoglycans within the bacterial cell wall. Found in a soluble and free form in fluids like saliva, nasal, gastric and intestinal secretions. Surna A et al ,2009<sup>19</sup> observed a significantly higher lysozymal activity in crevicular fluid and saliva than in serum. Also reported higher lysozymal activity in patients with severe periodontal destruction. The free enzyme may contribute to the formation of pocket by its detrimental effect upon epithelial cell stickiness and lytic activity upon the ground substance of connective tissue and can also accelerate the local release of intra cellular bacterial enzymes.

### Aspartate aminotransferase (AST)

Is an enzyme released from dead cells, and many studies have demonstrated a marked elevation of AST in GCF from sites with severe gingival inflammation and in sites with a history of progressive attachment Loss<sup>20</sup>.

A chair side kit has been developed for detecting GCF AST levels and is called Periogard™.

This commercially available test kit detects AST in GCF. The GCF is collected using a paper strip which is placed in the crevice for 30S and is then placed in tromethamine hydrochloride buffer, to which a reaction mixture (L-aspartic and L-ketoglutaric acid) is added. After 10 minutes, oxalacetate and glutamate are produced if AST is present, and these react with an indicator such as fast red to produce an easily detectable colour change which is proportional to the GCF AST activity.

The problem with using AST is that it has a strong relationship with gingival inflammation, which may or may not be present in site which are actively losing attachment.

Persson et al (1995)<sup>20</sup> examined the ability of Periogard™ to distinguish between diseased and healthy sites in a before

and after treatment designed longitudinal study.

### Pocket watch™.

The principle of this method is that, in the presence of pyridoxal phosphate, AST catalyzes the transfer of an amino group from cysteine sulfinic acid , by L-ketoglutaric with to yield  $\beta$ -sulfinyl pyruvate and glutamate. AST catalyze the formation of  $\beta$ -sulfinyl pyruvets.  $\beta$ -sulfinyl pyruvate spontaneously and rapidly decomposes and releases inorganic sulfite. The sulfite ion instantaneously reacts with malachite green (MG), simultaneously causing MG to convert from a green dye to its colourless form, thereby allowing the pink-colored rhodamine B dye to show through. The rate of conversion of MG is directly proportional to the AST concentration.(Shimada K, et al,2000)<sup>21</sup>

### Lactate dehydrogenase

It is a cytoplasmic enzyme in the anaerobic glycolytic pathway. It catalyses the reversible reduction of pyruvate to lactate. Its occurrence extra cellularly indicates cell death. Bang et al (1972)<sup>23</sup> found GCF to contain 10-20 times more total LDH than blood. Several studies by Lamster IL et al (1985,1988,1989) have indicated a positive correlation between enzyme levels and inflammation Measurement of LDH is by a spectrophotometric assay, and like AST, it is not possible to know which dying cells are measured O<sub>2</sub> where they are located.

## CONCLUSION

From available data, it appears that PMN enzymes are not only linked casually to disease progression, but can be readily measured in GCF and will likely provide a good reflection of the global host response in the periodontium. Of the enzymes discussed above, currently available evidence supports the efficacy of the MMPs, AST and elastase as the most promising enzyme markers for progressive disease.

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