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Aggregatibacter actinomycetemcomitans In Periodontal Disease

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ABSTRACT

It is well realized that periodontal diseases are primarily bacterial infections. The most common bacteria involved in periodontal pathologies of young individuals and in cases of refractory adult periodontitis is *Aggregatibacter actinomycetemcomitans*. This bacteria, was earlier names as *Actinobacillus actinomycetemcomitans*. A gram negative coccobacillus, these bacteria expresses numerous virulence factors that mediate tissue destruction. This review discusses the microbiology and pathogenesis of *A.actinomycetemcomitans* mediated periodontal diseases. The clinical relevance with regards to diagnosis and therapy have also been discussed.

Keywords: bacteria, periodontitis, aggressive periodontitis, leukotoxin, adhesins

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INTRODUCTION

Aggregatibacter actinomycetemcomitans is a major putative periodontopathic bacteria. It is closely associated with periodontitis in young individuals and in cases of refractory adult periodontitis [1]. *Aggregatibacter actinomycetemcomitans* can also seed to and produce severe infections in extraoral sites [2,3]. It belongs to the FAMILY Pasteurellaceae and GENUS *Aggregatibacter*. It is a gram negative, chemo organotrophic, facultatively anaerobic and fermentative bacteria. *Aggregatibacter* was first isolated in 1902 by Lignières and Spitz [4]. This genus contains 11 species with *Aggregatibacter actinomycetemcomitans* being the most important from the periodontal point of view. Its role in periodontal infections was first discovered by Jorgen Slots. *Aggregatibacter actinomycetemcomitans* was first described by Klinger. It was first named *Bacterium actinomycetemcomitans*.

The name *Actinobacillus actinomycetemcomitans* was given by Topley and Wilson [4]. *Aggregatibacter actinomycetemcomitans* is more closely related to *Haemophilus* than to the genus *Actinobacillus*. Hence *A.actinomycetemcomitans* is not a true *Actinobacillus*. That is the reason why it was renamed as *Aggregatibacter*. In 2006, Nørskov-Lauritsen and Kilian proposed that *Actinobacillus actinomycetemcomitans* be reclassified as *Aggregatibacter actinomycetemcomitans*. This bacterium is one of the most commonly implicated pathogen in periodontal pathoses. This review discussed the microbiological and pathological aspects of this bacteria.

Microbiology of *A. Actinomycetemcomitans*

Morphology

A.actinomycetemcomitans is a gram negative coccobacillus, approximately 0.4x1.0 µm in size [5]. It is predominantly bacillary with few coccal forms. It is a non sporulating, non-motile and non-branching bacteria. This name refers to the star-shaped inner structures that are sometimes seen in colonies on selective media and to the short rod or bacillary forms of the cells^{2,4}. This bacterium finds major ecological niches in the oral mucosa, dental plaque and periodontal pockets.

Biochemical properties [2,4]

It is a capnophilic (it needs an atmosphere with 5 – 10% carbon-dioxide for growth) microaerophilic bacteria which does not need the X (or) V factor for growth [5]. Following the reclassification by Nørskov-Lauritsen and Kilian, the species of the genus *Aggregatibacter* were stated to be independent of X factor and variably dependent on V factor for growth in vitro. It is a facultative anaerobe which is oxidase and catalase positive. It is non-haemolytic. It decomposes hydrogen peroxide. It is known to ferment sucrose, glucose and mannose. It produces strong alkaline and acid phosphatases. It reduces nitrate to nitrite.

Chemo taxonomic tests based on the following are used to differentiate *A.actinomycetemcomitans* from closely related taxa: cellular and vesicular fatty acids,

cellular proteins, cellular sugars, vesicular and whole cell enzymes, bacteriolysis, metabolic enzymes, respiratory quinones – menaquinones, DNA – DNA hybridization, DNA – r RNA hybridization, genetic transformation, ribotyping and analysis of r RNA.

Serotypes [2]

A. actinomycetemcomitans was initially divided into 24 groups by Pulverer and Ko based on tube agglutination assays. These 24 groups were further divided into 6 agglutinating antigens. Non oral *A. actinomycetemcomitans* was divided into 3 serogroups by King and Tatum based on a heat stable component. Taichman et al. divided *A. actinomycetemcomitans* into 4 serogroups based on surface antigens and proteinaceous leukotoxin. Zambon divided *A. actinomycetemcomitans* into three serotypes – a, b and c. Serotypes a and b are common in the oral cavity and serotype c is found in 10% of human isolates and is of importance in extra oral infections.

Serotype b is commonly implicated in Localized Aggressive Periodontitis while serotype c is related to periodontal health in adults. The number of serotypes has now been extended to 6 – a,b,c,d, e and f based on the differences in the carbohydrate moiety of the cell surface lipopolysaccharide [6-9].

A. actinomycetemcomitans is differentiated into 8 biotypes based on fermentation reactions with galactose, mannitol and xylose. It is divided into 10 biotypes based on fermentation of dextrin, maltose, mannitol and xylose. Serotype a does not ferment xylose while serotype b ferments xylose. Serotype c has both xylose positive and xylose negative strains [9].

A. actinomycetemcomitans population is genetically heterogenous. Spontaneous (or) treatment induced change in oral strains is extremely rare and the same strain and biotype seem to be remarkably persistent. Individuals within a family with Localized Aggressive Periodontitis harbor the same biotype and serotype of *A. actinomycetemcomitans* [10]. In individuals with periodontal disease, elevated antibodies to multiple serotypes were found. However, serotype b was found to be the most consistent feature [10]. Antibody reactive to *A. actinomycetemcomitans* serotype b lipopolysaccharide was found to be protective in generalized early onset periodontitis. Intra familial transmission is also found in *A. actinomycetemcomitans* [2]. Prevalence of *A. actinomycetemcomitans* serotypes in different populations shows three predominant serotypes – a,b,c and a lesser frequency of the other two serotypes – d,e. Proportion of *A. actinomycetemcomitans* serotype b is significantly greater in culture positive patients with aggressive periodontitis than those with chronic periodontitis [11]. Serotype antigens of *A. actinomycetemcomitans* have high molecular weight and are heat stable. They have primary carbohydrate moieties. The serotype specific antigens are the most immunodominant antigens of *A. actinomycetemcomitans*.

Pathogenesis of *A. Actinomycetemcomitans* Mediated Periodontal Infection

The distinction between exogenous and indigenous periodontal pathogens is important because the nature of periodontal infection significantly influences the clinical

approach to disease management. *A. actinomycetemcomitans* may constitute exogenous species because of the rare occurrence in periodontally healthy individuals. Transmission studies and vigorous host response to periodontal infection by this organism and the ability of appropriate therapies to eradicate the organism completely from the oral cavity (which is not the case for indigenous pathogens) also appear to confirm this fact.

Host response

Periodontopathogenic potential of some bacteria may be due to their ability to manipulate the immune response of the host¹². T lymphocytes are believed to be the regulators of immune response. The best characterized immunomodulatory bacterial products are the lipopolysaccharides of gram negative bacteria, which can activate B cells, monocytes, macrophages and polymorphonuclear neutrophils [2,13,14].

A. actinomycetemcomitans contains proteinaceous products that have the ability to selectively stimulate T suppressor cells and also potentially suppress immunoglobulin production. Super antigens are T cell modulating components of bacteria and viruses. These super antigens, though potent T cell stimulators are ultimately immunosuppressive [14]. This feature is also thought to be present in *A. actinomycetemcomitans* [12].

Some periodontitis patients show high systemic and topical antibody levels against *A. actinomycetemcomitans* [2]. Despite the high antibody levels, *A. actinomycetemcomitans* persists in the periodontal pockets. The anaerobic environment of the periodontal pocket can impair the bactericidal effect of polymorphonuclear leukocytes. *A. actinomycetemcomitans* demonstrates resistance to complement mediated killing [2]. It may escape the antibacterial activity of the immune system by surviving within the epithelial cells.

Virulence factors [2]

Virulence is the ability of an organism to cause infection. Virulence in microbes includes the ability to enter the host, find a unique ecological niche, subvert the host's normal defenses, replicate in the new environment and express specialized pathogenic traits. In order to produce periodontal disease, *A. actinomycetemcomitans* must be able to infect periodontal sites by attaching to epithelial cells, existing microbes (or) the tooth surface, by competing with the resident flora in an effective manner and also by overcoming the cellular and humoral host defense mechanisms.

Surface ultrastructure of *A. actinomycetemcomitans*

- Fimbriae - These are small cell surface appendages that are associated with bacterial colonization of host tissue. The fimbriae are peritrichous, more than 2 μm in length and 5 nm diameter [4].
- Vesicles – Blebs are a prominent feature of *A. actinomycetemcomitans*. These are lipopolysaccharide units. It is continuous with the outer membrane. Leukotoxic strains have abundant extracellular membranous vesicles. These vesicles per se exhibit leukotoxic activity. These membranous vesicles also have endotoxins,

adhesions, bacteriocins and bone resorption activity. Bacteria in contact with cell surface have been found to exhibit vesicles. The role of vesicles as a virulence factor is yet to be conclusively determined.

- Extracellular amorphous material – This frequently embeds adjacent cells in a matrix. The production of this material is based on culture conditions. The adhesion of the microbe is increased by this material. Conveyed adhesion is the result of a direct transfer of the material onto the surface of the bacterium.

Tissue destruction potential

The tissue destruction potential of *A.actinomycetemcomitans* may be by toxin production, enzyme production or induction of immunopathological reactions [2,12] (Table 1)

Table 1: Factors expressed by *A.actinomycetemcomitans* involved in tissue destruction

Factors involved in colonization and persistence in oral cavity	<ul style="list-style-type: none"> • Adhesins • Invasions • Bacteriocins • Antibiotic resistance
Factors influencing host defence mechanisms	<ul style="list-style-type: none"> • Leukotoxin • Chemotactic inhibitors • Fc binding proteins • Immunosuppressive proteins
Factors causing host tissue destruction	<ul style="list-style-type: none"> • Cytotoxins • Collagenase • Bone resorption factors • Stimulation of inflammatory mediators
Factors inhibiting host tissue repair	<ul style="list-style-type: none"> • Inhibitors of fibroblast proliferation • Inhibitors of bone formation

Leukotoxin

The Repeats-in-Toxin (RTX) exoprotein is produced by several gram negative bacteria [15]. RTX exoprotein exhibits clear protein sequence homology and similarities in operon arrangement and secretion pathways to cytolytic toxins, metallodependent proteases, lipases and exoproteins of unknown function. These toxins have a varying number of glycine rich Ca²⁺⁺ binding tandem repeats in the N-terminal end of the structural toxin molecule.

Leukotoxin is one of the most important virulence factors of *A.actinomycetemcomitans* [16]. It is an RTX exoprotein that plays an important role in periodontal disease pathogenesis [1]. This leukotoxin is lethal to neutrophils from humans and some non-human primates, but cells from other mammalian species are unaffected in vitro. It is a member of pore forming toxins. It was thought that the toxin remains associated with the bacterial cell rather than being released into the culture medium. However latest

studies show that some strains secrete the toxin into the environment during the early growth phase.

It is a heat labile toxin. It is protease sensitive. It is secreted outside the periplasmic space, but remains adherent to the nucleic acids that coat the outer surface of *A.actinomycetemcomitans*. The toxin is associated with the outer cell membrane in contrast to other RTX secreting bacteria. It can kill both lymphoid and myeloid leukocytes [2]. Target cells include human polymorphonuclear leukocytes, monocytes and macrophages. Human platelets, fibroblasts, endothelial and epithelial cells are resistant to the effects of Itx A. Target cell susceptibility is due to the cell surface expression of $\beta 2$ integrin molecule, lymphocyte function – associated antigen 1 (LFA 1) suggesting that killing is a receptor mediated process.

The expression of leukotoxin varies amongst the various strains of *A.actinomycetemcomitans*. It is dependent in part, on the structure of the Itx promoter region. Leukotoxic strains are characterized by a 530 base pair deletion within the Itx promoter in aggressive periodontitis patients. Insertion of the transposable DNA element correlates with the high level of leukotoxin expression [2]. RTX leukotoxin is encoded by an operon of 4 genes – Itx A, Itx B, Itx C, Itx D. The A gene encodes the leukotoxin itself and is produced as an inactive protoxin.

Mechanism of action of Leukotoxin

Two Itx A mediated mechanisms of cell death are known to exist: Necrosis and apoptosis. The Itx A forms pores in the target cell membrane leading to water influx and osmotic lysis. This is the case when the Itx A is present in high concentrations. At low concentrations, Itx A mediates cell death via apoptosis. This leukotoxin is thought to be the most important virulence factor in the pathogenesis of localized aggressive periodontitis and other forms of early onset periodontitis. Some highly leukotoxic strains of *A.actinomycetemcomitans* produce about 10 – 20 times more leukotoxin than the other minimally leukotoxic strains. Examination of the distribution and clonality, intrafamilial transmission of the highly leukotoxic strains of *A.actinomycetemcomitans* reveals that localized aggressive periodontitis and other forms of early onset periodontitis are primarily associated with the highly leukotoxic clones of *A.actinomycetemcomitans*.

Bacteriocins [2]

These are proteins produced by bacteria, that are lethal for other strains and species of bacteria. Actinobacillin is a bacteriocin which is active against *S.sanguis*, *S.uberis* and *Actinomyces viscosus*. It is associated with both the bacterial cell surface and the extracellular vesicles. It acts by increasing the permeability of the cell membrane to the target bacillus. It may be responsible for the reciprocal relationship between *A.actinomycetemcomitans* and *S.sanguis* / *Actinomyces viscosus* in plaque and in patients with localized aggressive periodontitis.

Collagenase [2]

Collagenase activity is commonly seen in *A.actinomycescomitans* and *Porphyromonas gingivalis*. This may cause reduction in collagen density, which is a common feature of periodontal disease.

Cytotoxins

A.actinomycescomitans produces a heat labile cytotoxin which exhibits virulence by its impact on fibroblast activity. Most strains of *A.actinomycescomitans* produce a 115 KDa heat labile protein that specifically lyses human polymorphonuclear leukocytes and macrophages [2,17].

Immunosuppressive factors

A.actinomycescomitans produces an immunosuppressive factor that affects both B lymphocytes and T regulatory cells. The factor is a protein capable of inhibiting DNA, RNA and protein T cells activated by mitogens (or) antigens.

Surface associated material [2,18]

The surface associated material of *A.actinomycescomitans* has several putative virulence factors. This material has potent osteolytic activity. It contains a protein which blocks cell cycle progression. It also has potent proinflammatory cytokine stimulating activity with extremely potent induction of IL – 6 and IL – 8 synthesis by monocytes and fibroblasts.

Prostaglandin E2 is also involved in the mechanism of formation of osteoclast – like cells, mediated by the *A.actinomycescomitans* Y4 capsular polysaccharide antigen. This may play an important role in inflammatory bone resorption by promoting osteoclast formation in periodontal disease.

A.actinomycescomitans surface associated material at very low concentrations inhibits fibroblast proliferation [18]. The active component of this surface associated material is termed gapstein. Capsular polysaccharide¹⁹ from *A.actinomycescomitans* Y4 completely inhibits IL – 6 and IL – 8 production from human gingival fibroblast. This suggests that *A.actinomycescomitans* Y4 modulates the inflammatory response in periodontitis. This inhibitory effect has been found to be reversed by specific anti *A.actinomycescomitans* Y4 capsular polysaccharide, suggesting an important relationship between the organism and humoral immune response.

Lipopolysaccharide [2,13,14]

The lipopolysaccharide of *A.actinomycescomitans* contains 30% carbohydrate, 30% lipid A, 10 – 12% hexosamine, 03 – 10% phosphate, heptose. It is known to inhibit collagen and DNA synthesis as well as stimulation of bone resorption in a dose dependent

fashion. It stimulates interleukin 1 inhibitor (interleukin -1ra) released by macrophages. This plays an important meditative role in the development of periodontal disease.

The lipopolysaccharide of *A.actinomycetemcomitans* stimulates macrophages to produce interleukin 1 α , interleukin 1 β and tumour necrosis factor, mRNA and proteins involved in tissue inflammation and bone resorption [17]. This lipopolysaccharide is cytotoxic to fibroblasts. It is a potent inhibitor of fibroblast proliferation [19].

Fc Bonding proteins

The Fc region of antibody is important in the binding of antibody to specific receptors on polymorphonuclear leukocytes. Any competing proteins for this region inhibits antibody binding, and hence phagocytosis is inhibited. A heat modifiable membrane protein of *A.actinomycetemcomitans* has been identified to serve this purpose. Biotinylated Fc molecules can inhibit binding of Fc molecules to *A.actinomycetemcomitans*. Complement activation is also inhibited.

Bone resorption [2]

A.actinomycetemcomitans stimulates bone resorption by lipopolysaccharide mediated mechanisms, proteolysis sensitive factor in microvesicles, surface associated material (molecular chaperone GroEL). Bone resorption exhibits a “burn out phenomenon” whereby bone resorption reduces due to the host immune response.

Inhibition of neutrophil function

A.actinomycetemcomitans secretes a low molecular weight compound that inhibits polymorphonuclear leukocytes’ chemotaxis, which is abrogated by treatment with proteinase K, suggesting that the compound is proteinaceous in nature. *A.actinomycetemcomitans* is capable of inhibiting neutrophils from producing antibacterial agents [20]. It produces a heat stable protein that inhibits hydrogen peroxide production by leukocytes. *A.actinomycetemcomitans* is also resistant to defensins (cationic peptides found in neutrophils)

Penetration of epithelial cells

A.actinomycetemcomitans can penetrate the gingival epithelium [21]. It is seen on the epithelial wall, enlarged intracellular spaces of the pocket epithelial surface, epithelial side of the basal lamina, connective tissue and the alveolar bone. The primary receptor of *A.actinomycetemcomitans* invasion is transferrin. The phospholipase C present in this microbe is implicated in vacuole lysis. *A.actinomycetemcomitans* shows rapid intracellular replication and this is attributed to the *A.actinomycetemcomitans* – microtubule interaction.

It can invade the oral epithelial cells and spread from cell to cell by endocytosis. *A.actinomycetemcomitans* colonizes the sub epithelial gingival tissue. The adherence ability of *A.actinomycetemcomitans* to titanium implant surfaces is dependent on the strain [3].

Serotype a has the highest affinity while serotype e has the least adherence capability. Significant associations between the periodontal status and several health conditions were found in the adult population, including gender, smoking habit, diastolic blood pressure, white blood cell counts, C-reactive protein and serum IgG antibodies to IgG of *A.actinomycetemcomitans* whole cell titres [3].

Factors influencing growth of *A.actinomycetemcomitans*

- Appropriate culture media – microbes need a suitable culture medium to support their nutritional needs. Selective media for *A. actinomycetemcomitans* include
 - MGB – utilizes trypticase soy broth with malachite green and bacitracin (inhibitory to other indigenous flora).
 - TSBV – trypticase soy agar with serum, bacitracin and vancomycin.
 - “A” medium – TSBV with spiramycin, fusidic acid and carbenicillin.
 - Defined media :
 - RPMI – 1640
 - Dulbecco’s modified eagle medium
- Supplements
 - Yeast extract added to trypticase soy broth
 - Cysteine
 - Thiamine
 - Steroid hormones
 - Iron compounds
- pH – the optimum pH for growth of *A.actinomycetemcomitans* is 7.0 – 8.0 ²². Environmental pH is a critical physiological parameter that determines the growth and metabolism of microbes.
- Salt concentration – optimal growth of *A.actinomycetemcomitans* is in the salt concentration between 85.1 mEq/l – 170.0 mEq/l [22].

Implication of *A.actinomycetemcomitans* in disease

A.actinomycetemcomitans is an important pathogen in severe and recurrent forms of periodontitis. It is frequently associated with rapidly progressive periodontitis. Serotype b of this microbe is most commonly implicated in localized aggressive periodontitis.

Role of *A.actinomycetemcomitans* in localized aggressive periodontitis

Localized aggressive periodontitis is a periodontal condition in adolescents that exhibits rapid destruction of periodontal tissue, which slows with time. It shows molar – incisor localization and burn out phenomenon. It was previously termed localized juvenile periodontitis. Periodontal bone loss resembles a “mirror image” pattern. *A.actinomycetemcomitans* has been implicated as the organism causing localized juvenile periodontitis.

Large numbers of *A.actinomycetemcomitans* are routinely isolated from lesions of localized aggressive periodontitis, whereas isolation of the bacterium from healthy sites is low. Isolation is positive in 97 % of aggressive periodontitis cases. Eradication of the

organism from diseased sites is usually correlated with a significant humoral immune response. Presence of large numbers of the bacterium in the periodontal pocket is correlated with a significant humoral immune response. *A.actinomyetemcomitans* produces a wide variety of potent, cell bound and secreted virulence factors that are implicated in the pathogenesis of aggressive periodontitis.

Rapidly progressing periodontitis is characterized by severe and rapid bone loss. The organism commonly implicated is *A.actinomyetemcomitans*, either alone or in association with *Porphyromonas gingivalis*, *Campylobacter*, *Prevotella intermedia*, *Eikenella corrodens*. *A.actinomyetemcomitans* is also associated with refractory periodontitis. This is thought to be so because it is more difficult to eradicate this bacteria from the subgingival area than other bacteria because of its invasive capability. It can also cause re-infection from other sites in the mouth.

The evidence supporting the role of this microbe as a pathogen in periodontal disease based on Socransky’s criteria has been presented in Table 2. However, conflicting views are seen in this regard. Some reports find no apparent association between *A.actinomyetemcomitans* and progressive adult periodontitis while some reports do [3].

Table 2: Socransky’s criteria applied to explain role of *A.actinomyetemcomitans* in periodontal diseases

ASSOCIATION	<ul style="list-style-type: none"> • Increased in localized aggressive periodontitis • Increased in some lesions of chronic periodontitis • Detected in the tissues of localized aggressive periodontitis lesions
ELIMINATION	<ul style="list-style-type: none"> • Suppressed (or) eliminated in successful therapy • Found in recurrent lesions
HOST RESPONSE	<ul style="list-style-type: none"> • Increased serum and local antibody levels in localized aggressive periodontitis
ANIMAL STUDIES	<ul style="list-style-type: none"> • Capable of inducing disease in gnotobiotic rats
VIRULENCE FACTORS	<ul style="list-style-type: none"> • host tissue cell invasion • leukotoxin • collagenase • lipopolysaccharide – endotoxin • epitheliotoxin • fibroblast inhibiting factor • bone resorption inducing factor

Extra oral infections

Periodontal disease is known to influence the systemic condition in various ways and the bacteria and their products such as lipopolysaccharides may spread from the periodontal lesion via the systemic circulation to affect distant organs.

A.actinomyetemcomitans is indigenous only to the oral cavity (this is a conflicting view as some authors consider it an exogenous species). The occurrence of this organism in extra oral sites, therefore, suggests translocation of the organism from oral to non-oral sites. The dental health of a patient (dental diseases en masse) has a bearing on the general health. Periodontal diseases have been linked to coronary heart disease, including

myocardial infarction, ischaemia, coronary atherosclerosis [3]. The leukotoxin of *A. actinomycetemcomitans* is a proven virulence factor in periodontal disease. Systemic diseases from this toxin are being investigated. It is most commonly associated with prosthetic valve endocarditis as well as native valve endocarditis. Individuals with a locus minoris resistentiae are at elevated risk.

The ability of *A. actinomycetemcomitans* to reduce the amount of oxygen, is important in the synergism between this organism and *Actinomyces* in causing infection. The most common extra oral infections mediated by *A. actinomycetemcomitans* are endocarditis, embolism, pericarditis, meningitis, osteomyelitis and subcutaneous abscesses.

It may also be present in atheromatous plaques that expresses molecules that cross react with the antibodies to HSP 60 (Heat Shock Protein). The level of lipopolysaccharide in plasma from periodontally diseased patients is found to be very low and this low lipopolysaccharide level is suspected to have a priming (or) desensitizing effect. Pretreatment with 5 pg/ml *A. actinomycetemcomitans* lipopolysaccharide significantly enhances IL - 1 β and IL - 6 production. A low dose of blood stream lipopolysaccharide found in periodontitis patients appears to prime monocytes and may be capable of affecting the systemic response of the immune and inflammatory cells

Routes of infection

Saliva is considered to be the most important transport vehicle for *A. actinomycetemcomitans*, as it can be cultured from salivary samples²³. It can survive in saliva during transportation to a new host. Mucosal contact or toothbrush sharing may allow implantation of bacteria to potential growth locales. The salivary and subgingival serotypes of *A. actinomycetemcomitans* are the same in a patient.

Subgingival prevalence

Subgingival prevalence of *A. actinomycetemcomitans* is found to be as high as 80 %. Destructive periodontal disease in children is frequently associated with this bacterium. In prepubertal periodontitis and other types of early onset periodontitis, the prevalence is about 40 – 100 %. *A. actinomycetemcomitans* is also associated with periodontal lesions of Papillon – Lefèvre syndrome.

The dynamics of subgingival *A. actinomycetemcomitans* is the result of a complex bacterium – host interaction. *A. actinomycetemcomitans* is also isolated rarely from healthy mucosal sites around integrated dental implants. It has also been detected in failing root formed dental implants. It is a major pathogen in infectious implant failure. *A. actinomycetemcomitans* is also known to get attached to barrier membranes used in periodontal regeneration and result in failure of regeneration [24].

Diagnostic Tests [2]

- Culture: TSBV agar is the medium of choice for culturing *A. actinomycetemcomitans*. It is unable to grow on McConkey agar, in contrast to other members of the genus

Actinobacillus. On primary isolation, it forms small colonies measuring about 0.5 – 1.0 nm in diameter. The colonies are translucent/transparent, with irregular edges, smooth, circular, convex in shape. Fresh isolates have a “star shaped” (or) “crossed cigar” morphology form, embedding in the agar .

- Immunodiagnostic methods include indirect immunofluorescence, flow cytometry, EvaluSite™ test – an antibody based sandwich enzyme linked immunosorbent assay, bacterial concentration fluorescence immunoassay
- Nucleic acid probes: Digoxigenin labeled whole genomic DNA, Radiolabelled cloned DNA, Digoxigenin labeled genomic DNA, DMDx™ detection method, Radiolabelled oligonucleotide. These DNA probe methods are rapid and are efficient tools for clinical detection of periodontopathic bacteria, mainly A.actinomycetemcomitans and Porphyromonas gingivalis. For A.actinomycetemcomitans , the DNA from ATCC 43718, JP2, ATCC 29524, 310a, 146 HE are used for the probes.
- Polymerase chain reaction²⁵.

Treatment Modalities for Periodontal Diseases Mediated By A.Actinomycetemcomitans

Convincing data exist that A.actinomycetemcomitans is an etiologic agent of periodontal disease. Systemically administered antibiotics are recommended for elimination of this bacterium from the subgingival and adjacent intra oral areas⁴. Longitudinal studies conducted to compare the clinical and microbial data in patients with severe periodontal disease, representing either subgingival suppression or recurrence of A.actinomycetemcomitans found that in destructive adult periodontitis, the periodontal treatment response is negatively affected by the persistence of subgingival A.actinomycetemcomitans over a 3 year maintenance period [4].

Study of the microbiological effects of initial periodontal therapy using DNA probes and PCR, indicates that the initial conventional therapy can eliminate Porphyromonas gingivalis and Bacteroides forsythus , but not A.actinomycetemcomitans [26]. This indicates that monitoring the levels of these periodontopathic bacteria and the elimination of all these three microorganisms is a prerequisite for successful treatment.

Antibiotic resistance has been described amongst bacterial species colonizing the periodontal pockets. Some strains of A.actinomycetemcomitans have been found to exhibit resistance to metronidazole, but a combination of amoxicillin and metronidazole has been found to be effective against subgingival aerobic and capnophilic mixed flora²⁷. Pristinamycin and ciprofloxacin are effective alternate monotherapies against A.actinomycetemcomitans²⁸. Threat of β lactam resistance by production of β lactamase production is currently not a problem seen with A.actinomycetemcomitans [4].

Host modulation therapy with low dose doxycycline – 20 mg doxycycline hyclate is also followed contemporarily. 250 mg tetracycline over a period of 2 – 7 years is a regimen that has been used over the past several years. For tetracycline resistant strains, combination of amoxicillin and metronidazole is the drug of choice. Mechanical debridement (scaling and root planning) in combination with amoxicillin – metronidazole therapy is effective in subgingival suppression of A.actinomycetemcomitans in patients with severe periodontitis [27].

Overview of treatment modalities and effects of treatment

- Scaling and root planing alone cannot remove *A.actinomycetemcomitans* from lesions of localized aggressive periodontitis
- Non surgical therapy has the least effect on *A.actinomycetemcomitans* counts in heavily infected periodontal lesions. This is because of the ability of the organism to invade gingival tissue and evade the effect of mechanical debridement and periodontal healing. The cells of the bacterium in gingival may constitute a reservoir for repopulating periodontal pockets.
- Periodontal therapy often fails to effectively control subgingival *A.actinomycetemcomitans*. Modified Widman flap surgery is shown to have about 50 % effect.
- An apically displaced flap with osseous recontouring is more effective than an apically displaced without osseous recontouring in reducing subgingival levels of *A.actinomycetemcomitans*.
- Superior performance of resective periodontal surgery may be due to the excision of *A.actinomycetemcomitans* – infected gingival tissue and reduction of pocket depth.
- Systemic metronidazole has good anti *A.actinomycetemcomitans* activity in localized aggressive periodontitis patients, but not in cases of adult periodontitis.
- Systemic amoxicillin-metronidazole combination shows striking clinical results in treatment of localized aggressive periodontitis, adult periodontitis and refractory periodontitis, even in the absence of other periodontal therapy. The prescribed regimen is 250 mg amoxicillin and 250 mg metronidazole- thrice daily for 8 days.

In summary, for complete treatment of *A.actinomycetemcomitans* mediated periodontal infections, the treatment plan should include scaling and root planning with a surgical procedure with/ without osseous recontouring along with systemic and local antibiotic therapy.

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