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Salivary Calprotectin in Patient With Oral Candidiasis

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ABSTRACT
Calprotectin, a calcium and zinc-binding protein found in non-keratin squamous epithelial membranes, is a cytosolic protein produced by neutrophils and monocytes, which can be found in serum plasma, urine, saliva and other body fluids. Calprotectin has antifungal activity by inhibiting yeast development from becoming hyphal, in which the activity is associated with a zinc-binding capability. Calprotectin can inhibit the growth of Candida Albicans; the calprotectin level in saliva will affect oral candidiasis incidence. However, the mechanism action remains unclear. A literature review was carried out in this study to elucidate the mechanism action of salivary calprotectin toward the growth of Candida Albicans by searching for the top 20 articles on mechanism action of salivary calprotectin toward the growth of Candida Albicans. These articles were accessed from PUBMED/MEDLINE and Researchgate.com during December 2019. Calprotectin inhibits Candida Albicans growth by depriving the zinc, the findings support antimicrobial activity for this protein. Calprotectin activity has shown a synergistic increase in conjunction with lactoferrin (an iron-binding protein) produced by neutrophils in the infected area. Along with Lactoferrin, it controlled the fungal growth of Candida Albicans by retaining its essential metal nutrients. Therefore, this study aims to observe the activities of expression and release of calprotectin in immune and immunopathological reactions, especially in fungal infections. Calprotectin production, or release, or both, may increase within subjects with candidiasis.

Keywords: *Candidiasis, C.albicans, Calprotectin, S100A8, S100A9*

1. INTRODUCTION

Calprotectin, a cytosolic protein produced by neutrophils and monocytes, is a calcium and zinc-binding protein as part of non-keratin squamous epithelial membranes. It also can be found in serum plasma, urine, saliva and other body fluids. Moreover, Calprotectin, a non-covalent heterodimer of the S100A8 (A8) and S100A9 (A9) subunits, is the result of abundant main cytosolic neutrophil protein. It is a critical alarm that modulates inflammation and plays an essential role in hosting immunity by tightly binding the essential metals during the development of microorganisms consisting of two cysteine residues that act as redox switches. A study showed that most of the calprotectin was disulfide bonds between cysteine residues in the S100A8 and S100A9 subunits placed in healthy adults' saliva and lavage fluid from the lungs of patients with respiratory disease.[1]

The major and minor salivary glands always secrete saliva continuously. It makes the oral mucosa, a complex ecosystem composed of various epithelium types, always inundated by saliva. Extensive microbiota in the oral cavity includes a wide variety of microorganisms that live permanently and are not harmful or commensal. However, in certain circumstances, this condition can turn into potentially dangerous pathogens.[2] Candida albicans is a commensal fungus that generally occurs in humans in the skin and mucosa. A commensal relationship means that one organism benefits while the other neither benefits nor loses. This normal flora contributes to endogenous disease either by the presence of overgrowth at a “normal” location (overgrowth) or by translocation in a sterile site. Normal flora also contributes to disease with cross-infection, where organisms that colonize one individual can cause disease if transferred to other more susceptible individuals.[3] There are about 150 Candida species found in various environments; biotic and abiotic. However, only a few can cause disease in humans; Candida albicans, Candida glabrata, Candida parapsillosis, Candida tropicalis, Candida krusei, and Candida dubliniensis. These candida species are commonly associated with human diseases. While most candida species develop as a form of unicellular yeast, C. Albicans can also transform into hyphal or pseudohyphal morphologies, allowing it to form complex biofilms and contribute to its pathogenicity in humans.[2]

Calprotectin has antifungal activity by inhibiting yeast development from becoming hyphal; this activity is associated with a zinc-binding capability. Calprotectin can inhibit the growth of Candida Albicans in which the level of calprotectin in saliva will affect the incidence of oral candidiasis. However, mechanical action remains unclear. A literature review was performed in this study to elucidate the mechanism action of salivary calprotectin toward the growth of Candida Albicans.

2. CALPROTECTIN STRUCTURE, DISTRIBUTION AND FUNCTIONS

Being one of the low molecular weight sub-families of calcium S100 binding protein called calgranulin, Calprotectin consisted of S100A8 subunits (calgranulin A, MRP8) and S100A9 (calgranulin B, MRP14) as well as other various calprotectin literature found with several synonyms (protein complex S100A8 and S100A9, antigen 27E10, proteins related to macrophage inhibiting factors MRP8 / 14, proteins L1L and L1H, and protein calgranulin A / B). The S100A8 / A9 complex can also cause osteoclasts, keratinocytes, adult macrophages, fibroblasts, and microvascular endothelial cells produced by the myeloid cells lineage, including monocytes, neutrophils and in the initial differentiation state of macrophages. Calprotectin has extracellular function concerning the host's defense mechanism of anti-infection as well as involving the steps of kinase activity, cytoskeletal rearrangement, cell differentiation and migration.[4,5]

Calprotectin as the binding protein between calcium and zinc are located in monocytes/macrophages, but not in platelets and lymphocytes. The concentration of calprotectin in neutrophils is almost half (30-60%) of the total Calprotectin cytosol initially found in neutrophils and mononuclear phagocyte subpopulations. Monoclonal antibody reactivity 27E10 shows the limited distribution in myeloid cell lineages with weak variable expressions in endothelial and epidermal cells. Calprotectin is released from outside the cell to stimulate neutrophils and monocytes or be released due to cell disorders or death. After cell death, calprotectin is released into the pus or fluid abscess along with a microbicide nucleohiston.[5,6]

Calprotectin has antimicrobial activity linked to at least some of its abilities to bind zinc, which blocks the organism's needed nutrition. It has been proposed that calprotectin can protect the mucosa intracellularly against microorganisms due to poor opsonization to avoid phagosomes. Moreover, it successfully gains access to the phagocytic cell of cytosols. Calprotectin and its derivations involved in the inflammatory process can be recognized as the markers of various inflammatory diseases.[7] Like many other pathogens, Calprotectin potentially acts as antifungal activity against *C. Albicans* as well as Neutrophil lysates and NETs with highly enriched Calprotectin, competent in inhibiting *C. Albicans'* growth *in vitro*. Meanwhile, purified calprotectin and mice with lack calprotectin showed a significant number of susceptibility-increases toward *C. Albicans* infections in the lung. [8]

3. CALPROTECTIN ACTIVITY IN ORAL CANDIDIASIS

C. Albicans is regarded as the essential agent causing oral candidiasis for at least 95% of cases. Despite being a pathogen, *C. Albicans* commonly colonizes the oral mucosa by isolating healthy people's oral cavities. In fact, around 80% of people act as asymptomatic carriers, while a simple carriage does not predictably lead to infection. C. Albicans adapts well to human hosts due to their versatility. In addition, whatever changes, the host's microenvironment support proliferation provides these pathogens with an opportunity to attack almost all sites. It can demonstrate the fragmentary mucosal invasive infections disseminated by the diseases involving various organs.[9,10]

The body's response to pathogenic fungi is primarily carried out by cell-mediated immunity (CMI) in which T cells work. There are two T cell classes involved in the CMI's response to the pathogenic invasion, namely CD4 T cells and CD8 T cells. Antigen presented by DC will activate T cells.[11] Additionally, the T cell activation is also influenced by cytokines secreted from macrophages and DCs. It secretes cytokines that support the immune system. Meanwhile, Epithelial cells (EC) produce the IL-17 receptor (IL-17RA / RC). Furthermore, these cells are involved in fungal clearance in the production of various pro-inflammatory cytokines and antimicrobial peptides during infection.[12] Interleukin-17 (IL-17) differentiated into three main subsets, namely Th1, Th2, and Th17 cells, depending on the transcription factors, is a pro-inflammatory cytokine produced by CD4 + T helper cells. The Th17 will produce IL-17 to recruit neutrophils, increase the inflammatory response and anti-fungal; IL-17F and IL-22. [13] Oral Candidiasis in the early phase are strongly influenced by the neutrophil's response; Th17 cells recognize pathogen-related molecular patterns (PAMPs) via several type C lectin receptors (CLRs) and inflammation. PAMPs release IL-23, IL-1β, IL-6, and TGF-β, which leads Th17 cells to the mucosal region during the response. The cytokines releases (IL-17A, IL-17F, IL-22) at the infection site recruit neutrophils and amplify secretion of proinflammatory cytokines and chemokines. Indeed, IL-17 and IL-22 produced by Th17 cells also cooperatively increase the numbers of AMPs, such as β-defensins, calprotectin, and histatins. [11]

Epithelial cell damage caused by candida Albicans will activate the production of "alarmins". Alarmin's function is to remind the immune system of tissue damage caused by microorganisms' trauma or infection. It promotes recruitment and activates the innate immune cells. Alarmin consists of structural different endogenous mediators, including the S100 protein, heat shock proteins, and nucleosomes. The 14,15-concentration in Calprotectin was correlated with candida infection intensity; the high calprotectin levels were merely found in subjects with candidiasis.

When epithelial cells are damaged, alarmin will be released as IL-1, S100A8 / S100A9 (calprotectin). The S100 alarmin is produced abundantly by neutrophils, both oral and vaginal epithelial cells. Calprotectin, which belongs to the S-100 protein family, binds Ca2 + - and Zn2 +. It is produced by mucosal keratinocytes and hosts immune cell varieties, such as PMNs, monocytes, and macrophages.[16] In vivo and vitro studies, it was found that calprotectin was part of a neutrophil extracellular trap contributing to the antifungal function.[17] Calprotectin activity also showed a synergistic increase of lactoferrin, a ubiquitous iron-binding protein in human exocrine secretions. Neutrophils also produce it in the infection area. Combining these two proteins reduces the fungal growth of C. Albicans by retaining the essential metal nutrients. Saliva also contains proteolytic lysozyme enzymes

generated by oral neutrophil leukocytes and anti-leukoproteases. These are secretory protease leukocyte inhibitors with a potential candidacidal activity which inhibits the growth of C.albicans.[2]

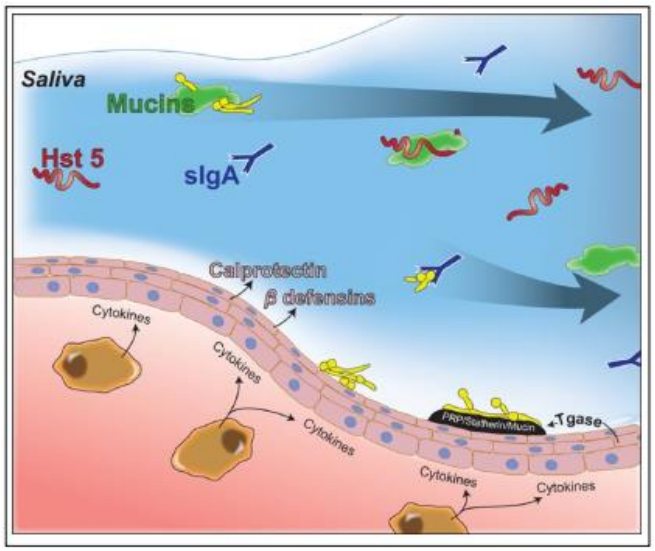

Figure 1. Role of salivary proteins in candidiasis. Salivary proteins (proline-rich proteins [PRPs], statherins, mucins) and oral epithelial transglutaminase (Tgase) can aid *Candida albicans* growth by promoting adherence to oral tissues or inhibiting growth through immune exclusion by binding and aggregating fungal cells (mucins and secretory IgA [sIgA]) to facilitate their clearance by swallowing. Antifungal proteins, including salivary histatin 5 (Hst 5) or calprotectin and β-defensins (secreted by oral tissues), prevent *C. Albicans* growth. [2]

Micronutrients such as iron and zinc are essentials trace metals for cells' life. At least one-third of proteins interact with metal cofactors. Zinc contributes to eukaryotes as about 9% of the proteome requires this metal to function. However, these pathogenic microorganisms face a complex relationship during the process of nutritional immunity. The micronutrients in mammalian-hosts use high antimicrobial concentrations of metals and metal absorption to kill microbes or inhibit their growth. "The battle for iron" is a well-established paradigm in host-pathogen interactions. Lately, the importance of manganese, copper and zinc have existed in the method of nutritional immunity.[18,19] In particular, Zinc represents a double-edged sword for its potential-invasive species; it is very limited due to the systemic availability of zincaemia. However, it locally produces zinc chelating agents such as calprotectin, whose absorption is crucial for pathogenicity. In accordance, recent studies demonstrated the magnitude of importing zinc with high-affinity znuABC for the spreading of dangerous microorganisms.[20]

A zinc absorption depends highly on the acidity surrounding the environment, which, interestingly, this pH dependence seems to be concentrated in the mushroom kingdom. Therefore, a potential experiment is proposed for the evolution of zinc absorption in existing fungal species. Following cellular assimilation, C. Albicans transports this potential poisonous transition metal to a subcellular compartment called zincosome. It is shown that zinc uptake and compartmentalization are essential for the growth of C. Albicans, both in laboratory conditions and as an experimental model of invasive candidiasis. [21]

Previous studies related to experimental models of invasive candidiasis showed that zinc absorption and compartmentalization were essential for the growth of Candida albicans as well as manganese. Although manganese is not susceptible to *Fenton* reactions than most transition metals, manganese functions as the antioxidant of SOD protein in Candida albicans, mainly when copper is not available. Reciprocally, calprotectin isolates manganese as a mechanism of antimicrobial activity in the oral environment.[22]

In accordance, other studies showed that calprotectin also absorbed Cu (Cuprum) by attaching Cu (II) with sub-picomolar affinity. CP blocks the acquisition of Cu fungus from serum and induces starvation of Cu stress response involving SOD1 and SOD3 superoxide dismutations. This transcription change is reflected when C. Albicans attacks the kidneys of an experimental mouse disseminated by candidiasis. However, there is a difference between the response to Cu's and Zn's restrictions; the Cu's response lasts for 72 hours, while the Zn's responses are relatively short-lived. Indeed, this stress response is attenuated in null CP rats; however, it is only in the early stages of infection. Thus, the Zn and Cu pools become dynamic host to the pathogenic interface, while CP acts from the start of the infection to minimize the metal nutrients from C.albicans.[23]

4. CONCLUSION

In conclusion, calprotectin inhibited Candida Albicans growth by depriving the zinc of antimicrobial activity's support mechanism. It also isolated manganese as a mechanism of antimicrobial activity and absorbed Cu limiting the metal nutrients. Calprotectin's activity showed a synergistic increase in conjunction with lactoferrin (an iron-binding protein) produced by neutrophils in the infected area. Calprotectin and Lactoferrin, together, reduced the fungal growth of candida Albicans by retaining essential metal nutrients. Expression and calprotectin release were essential activities in immune and immunopathological reactions, especially in fungal infections. Calprotectin production, or release, or both, might increase within subjects with candidiasis.

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