WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Candida Albicans - A Partner in Crime in Early Childhood Caries

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Acknowledgment – NIL
Financial support and sponsorship – Nil
Conflict of Interest – NIL
DOI: https://doi.org/10.55248/gengpi.234.4.36868

ABSTRACT –

Early childhood caries (ECC) is associated with early colonization and high levels of cariogenic microorganisms. The association of Streptococcus mutans and *Candida albicans* with the onset of ECC is well known. Thus, the present review aims to affirm the established relationship between ECC and *C.albicans* and to analyse and understand the association between *S.mutans* and *C.albicans* in the dual species pathogenesis.

INTRODUCTION

Dental caries is the amongst the most common and preventable oral disease known to mankind.[1] When comparing it with other common diseases, dental caries is five times as frequent as asthma and seven times as common as hay fever.[2]

The earliest comprehensive description of childhood caries, then known as nursing bottle mouth, was published by Dr. Elias Fass (1962). The compelling first sentence of the paper begin as:

"Nothing is so shocking to a dentist as the examination of a child patient suffering from rampant caries." [3]

Concepts and beliefs about the cause of dental caries have evolved over many centuries, with the involvement of microorganisms being recognized since the late 1800s. Antoine van Leeuwenhoek in the 17th century recorded how he was fascinated by the seething activity he could observe when he examined scraping from his teeth, and took great delight in the variety of shapes, sizes and movement of the 'little animalcules' that he saw. He appeared not to have made any connection between what he observed and dental disease. Almost 200 years later this observation was the basis of 'germ theory' of disease.[4]

Early childhood caries is an infectious, multifactorial disease defined by American academy of pediatric dentistry as the presence of one or more decayed (noncavitated or cavitated lesions), missing (because of caries), or filled tooth surfaces in any primary tooth in a child aged 71 months or younger. ECC process begins as soon as the first teeth erupt in the oral cavity and develops on smooth surfaces. [5]

ECC is a "family malady" in that the disease is infectious, transmissible, and is often associated with poor (sugar-laden) dietary habits. is still one of the most prevalent diseases in children worldwide. ECC leads not only to temporary pain, but more importantly has major effects on the quality of life of the family/caregivers including financial and health implications. Various microorganisms appear to be involved in the formation of cariogenic biofilms.[6]

*Streptococcus mutans*has often been regarded as one of the key etiologic agents of ECC although other organisms may also contribute to its pathogenesis.[6] *Candida* is a normal commensal in the oral cavity of healthy individuals. The percentage of *Candidal* infection colonization ranges from 20% to 40% in healthy individuals, whereas it is about 60% in immuno-compromised people. There are many species of *Candida*, of which *C.albicans* is the most prevalent one recovered from the oral cavity.[5] It is a dimorphic species able to switch morphology between yeast and hyphal forms, a property that is central to its pathogenesis and ability to form biofilms.[6]

C.albicans ferment glucose and maltose, producing both acid and gas showing a high acidogenic potential and biofilm formation.[7] Putative virulence factors of *Candida* include ability to adhere to host surfaces, produce filamentous growth form and release hydrolytic enzymes capable of inducing damage to host cells.[8]

Interestingly, this opportunistic fungal pathogen has been frequently detected with *S.mutans* in the plaque biofilms formed on the tooth surface of children with ECC. It is also noteworthy that it enhances co-colonization and proliferation in the oral cavity of ECC patients. Co-aggregation reactions of *C.albicans* with *S.mutans* play a large role in the colonization of oral mucosal and hard tissues. Carious lesions offer the highest colonization site for *C.albicans* as it provides an ecologic niche.Hodson and Craig have reported that, *C.albicans* in the biofilm of ECC is twice more prevalent than the caries free children.[7]

Thus, the present literature review aims to affirm the established association between *C.albicans* and Early childhood caries.

EARLY CHILDHOOD CARIES

ECC in pre-school children has been discussed extensively in the scientific literature over the past 50 years. Caries in infants and young children has long been recognized as a clinical syndrome, described by Belterami in 1930s as “Les dents noire de tout-petits” which means “black teeth of the very young.” Fass is perhaps the most preeminent in this perspective for defining the term “Nursing bottle mouth.” Subsequently, other terms such as “baby bottle tooth decay”, “nursing bottle syndrome”, “bottle mouth caries”, “nursing caries”, “rampant caries”, “nursing bottle mouth”, “milk bottle syndrome”, “breast milk tooth decay” and “facio-lingual pattern of decay” have also been used to describe this condition.[9]

During the last 20 years, different research groups have attempted to develop classification systems for ECC.

In a first approach by Wyne in 1999, the severeness of ECC can be defined in three types and associated with different aetiology.

1. In Type I (mild to moderate) ECC was defined as the existence of ‘isolated carious lesion(s)’ involving incisors and/or molars. The most common causes are usually a combination of semi-solid or solid food and lack of oral hygiene.

2. In Type II (moderate to severe) ECC was described as ‘labiolingual lesions’ affecting maxillary incisors, with or without molar caries, depending on the age of the child and stage of the disease. Typically are the unaffected mandibular incisors. The cause is usually inappropriate use of a feeding bottle or at-will breast-feeding or a combination of both, with or without poor oral hygiene.

3. Finally, a Type III (severe) ECC was described as carious lesions affecting almost all teeth including the mandibular incisors. A combination of cariogenic food substances and poor oral hygiene is the cause of this type of ECC.[10]

The primary risk factors in Early childhood caries are the susceptible tooth/host, substrate present in the oral cavity and/or presence of cariogenic microorganisms in dental plaque. The associated risk factors are night bottle feeding, compromised oral health, parents/guardians socioeconomic status etc.[9]

ORAL MICROBIOTA IN EARLY CHILDHOOD CARIES

The oral cavity is a highly diverse ecosystem with up to 600 different microbial species colonizing it. The colonization begins as early as birth during the passage of the new born through the birthing canal. Studies have reported that the oral cavity of children is sterile until 10 hours and 24 hours after birth and other findings point to an increase in the number of viable microorganisms between 6 and 10 hours after birth.[11] The growth of these microbes in an infant's mouth follows a pattern of microbial ecological succession, analogous to the succession that occurs in forests, grasslands and other ecosystems.[12]

The oral microbiota of children with primary dentition in relation to other groups has a higher prevalence of bacteria belonging to the class *Gammaproteobacteria*, particularly the families of *Pseudomonaceae* (genus *Pseudomonas*), *Moraxellaceae* (genera Acinetobacter, Moraxella, and Enhydrobacter), Enterobacteriaceae and Pasteurellaceae (genus Aggregatibacter). As the dentition evolves from deciduous to permanent, the population of the bacteria belonging to the Veillonellaceae family (genus Veillonella and Selenomonas) and the genus Prevotella increases, while the bacteria of the Carnobacteriaceae family (genus Granulicatella) decreases.[13]

Directly or indirectly the resident microflora in the oral cavity helps in the normal development of the host and functions as a part of the innate host defence. These microflora acts as a barrier to prevent the permanent colonization of the transient bacteria, some of which are potentially pathogenic. If this barrier is broken down by external causes such as antibiotic therapy, then the oral cavity becomes susceptible to various pathogenic bacteria.[2]

ECC AND THE BIOFILM MATRIX

A biofilm is described as a mutual bacterial association which adheres to a solid (such as denture prosthesis or an intravenous catheter) or with one another protected in an extracellular polysaccharide matrix. Nearly 65% of human infections are considered to be linked for microbial biofilms. Dental plaque formed on tooth surfaces is a typical illustration of a biofilm.[14]

The exopolysaccharides (EPS) are the prime biofilm building blocks, which form the core of the matrix. EPS are chiefly produced by bacterial exoenzymes (*e.g*., glucosyltransferases) at the biofilm-tooth interface utilizing dietary sucrose and starch. *S.mutans*, appear to be the main organisms associated with the production of the insoluble EPS matrix. *S.mutans*-released glucosyltransferases (Gtfs) become constituents of the pellicle and remain active despite major conformational changes, producing large amounts of glucans *in situ* upon sucrose exposure. The glucans formed on pellicle provide new microbial binding sites that promote local colonization of *S.mutan* and other microorganisms.[15]

CANDIDA ALBICANS: AN OPPORTUNISTIC FUNGUS

*Candida*spp are asexual yeasts of the genus ascomycetes and genetically diploid with the presence of eight chromosomes. Out of more than 200 species, the most commonly encountered in medical practices are *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*.

As a commensal, *Candida* resides in yeast form and multiplies by budding into blastospores, but during weakened immunity of the host it transforms into the hyphal form as the start of pathogenesis. It is the most common opportunistic pathogen, utilizing several kinds of virulence factors. Some of the commonly studied virulence factors in *C.albicans* are briefly described here –

1. Adhesion –

Adherence of candidal cells to host tissues is a complex multifactorial phenomenon utilizing several types of adhesins expressed on morphogenetically changing cell surfaces. But the striking feature of *Candida* cells is the formation of biofilms in host tissue, resulting in enhanced adherence

2. Morphogenesis –

Morphogenesis in *C.albicans* is defined as transition from unicellular yeast form to filamentous form (pseudohyphae or hyphae). Of all the species only *C.albicans* and *C.dubliniensis* are able to undergo morphogenesis. This transition is strongly required for pathogenesis.

3. Phenotyping Switching –

Colonies of *C.albicans* show morphological variation, including smooth, rough, star, stippled, hat, wrinkle, and fuzzy at high frequency. This switching is reversible, occurs spontaneously in stress, and results in changes in cell surface behavior, colony appearance, and metabolic, biochemical and molecular attributes to become more virulent and effective during infection.

4. Phospholipases –

Phospholipases are enzymes that hydrolyse ester linkages of glycophospholipids and hence impart tissue invasiveness to *Candida* cells. In *C.albicans*, four types of phospholipases are classified by researchers on the basis of the ester bond they cleaved, viz., phospholipase A, B, C, and D. All types possess hydrolase activity.

5. Proteinases –

Secretion of proteinases by pathogen is mandatory in order to degrade the tissue barriers and obtain nutrition at the infection site. Secreted aspartyl proteinases (SAPs) from Candida have been reported that hydrolyse many proteins such as albumin, haemoglobin, keratin, collagen, laminin, fibronectin, mucin, salivary lactoferrin, interleukin1b, cystatin A, and Immunoglobulin A.

6. Biofilm formation –

Biofilms are the organized structures involving microbial communities that are attached to some inanimate surfaces or tissues and circumvented in a matrix of exopolymeric materials. Biofilm formation is initiated by irreversible adherence of microbial cells to tissues or devices and followed by growth and maturation to form a mesh of cells with altered phenotype, growth rate, and gene expression compared to planktonic cells.

Dental plaque is a well-known example of biofilm formation from *Candida* cells. Biofilm formation on oral tissues is favored by a high concentration of glucose, serum, and other proteins.[16]

ASSOCIATION OF *C.ALBICANS* AND EARLY CHILDHOOD CARIES

Streptococcus mutans and *Candida albicans*, are the most common bacterium and fungus in the oral cavity respectively, and are considered microbiological risk markers of early childhood caries.[17]

S.mutans are major cariogenic organisms—the result of their ability to produce large quantities of glucans as well as acid, exceeding the salivary buffering capacities, which gives the bacteria an advantage to outcompete noncariogenic commensal species at low pH environments. This ability to survive in an acid environment by modulating sugar metabolic pathways coupled with irreversible binding to teeth is a key component to *S.mutans* pathogenesis.[18]

C.albicans may be a "keystone commensal" of plaque biofilms and can work synergistically with classic cariogenic bacteria. The cross-kingdom interactions of *S.mutans* and *C.albicans* are considered to be associated with severe dental caries, which has become of increasing interest.[17]

Bacterium-fungus interactions occur commonly in humans and may influence the transition from a healthy to a diseased state within a specific host niche. Although *S.mutans*-derived EPS appears to be an articulation point between the two species, it is conceivable that *C.albicans* may contribute its own extracellular substances that help mediate this interaction. *C.albicans* alone produces matrix materials (β-glucans, chitin, β-*N*-acetylglucosamine) during biofilm formation on other surfaces, and they confer protection from antifungal agents. β-Glucan is a component of the cell wall and can also be actively secreted by *C.albicans* during biofilm formation on silicone or polystyrene surfaces. The antibody-labelled β-glucan is closely associated with the bacterial microcolonies, and it does not appear visually to be in a 1:1 ratio with the *C.albicans* cells present. It has also been found that GtfB binds in an active form to β-1,3-glucan, which could explain why β-glucan is closely associated with both *C.albicans* cells and the Gtf-derived α-glucan produced by *S.mutans*. The observations in studies reveal that β-glucan contributes to the structural organization of the extracellular matrix in cospecies biofilms and may play a functional role yet to be elucidated.

Gtf-derived glucans formed on the *C.albicans* surface enhance the ability of the fungal cells to colonize and form cospecies biofilms. Results from previous studies have shown that *S.mutans*-derived Gtfs (particularly GtfC) present on sHA surface rapidly form an amorphous glucan layer, which masks host-derived microbial binding sites in the salivary pellicle. These observations are relevant because *C.albicans* itself adheres poorly to the preformed EPS layer on sHA surfaces, or binds poorly to *S. mutans*, unless the fungal cells are first coated with Gtf-derived glucans. Study reveals that fungal cells are detected only after the initial polymeric matrix and *S.mutans* microcolonies are formed on the sHA. Furthermore, the lack of *GtfB* and/or *GtfC* expression by *S.mutans* severely disrupts the ability of *C.albicans* to colonize, accumulate, and form cospecies biofilms. These findings are supported by the observation that *C.albicans* is detected at low numbers or not at all in the plaque of ECC-free children. The potential of *C.albicans* to contribute to the pathogenesis of caries disease has often been associated with its ability to produce and tolerate acids. It has been found that the pH values of the culture medium surrounding cospecies biofilms were highly acidic, though not significantly different from those of single-species *S.mutans* biofilms. Although an acidic pH is undeniably the immediate cause of tooth enamel dissolution, the environment within which the acid is produced plays a crucial role in cariogenesis.

The results of the previous studies have shown that the synthesis of Gtf-derived glucans leads to the formation of an insoluble EPS-rich matrix scaffold that acts as a diffusion-limiting barrier. In parallel, the metabolic activity of *S.mutans* clustered within the microcolony can produce copious amounts of acids that accumulate locally. It is conceivable that the alterations in the extracellular matrix containing a dense population of bacterial cells help to prevent acid within the biofilm from diffusing outward, thus prolonging and intensifying the acid attack.

The presence of *C.albicans* dramatically modifies the physical environment and the 3D architecture of the biofilm. It alters the volume and the structure of the extracellular matrix by –

I. Increasing the amount of insoluble Gtf-derived EPS, which has been shown to have diffusion-limiting properties, and

II. Independently contributing to the matrix through the production of extracellular β-glucans. It is also possible that the presence of insoluble β-1,3-glucan embedded in the extracellular matrices of cospecies biofilms may help limit diffusion while contributing to stability of the 3D matrix scaffold.

Furthermore, *S.mutans* microcolonies form more rapidly, and their size more than doubles, when the biofilms are grown in the presence of *C.albicans*. It has been shown previously that the pH inside the microcolony becomes more acidic as the structure increases in size, due to a high density of acidogenic organisms and limited diffusion into and out of the structure. Thus, the elevated and localized concentration of *S.mutans* cells sheltered by an abundant extracellular matrix would maximize the ability of acids to demineralize teeth by retaining the acids in close proximity to the tooth surface.

In this scenario, EPS may be both the point of articulation for the coexistence of *S.mutans* and *C.albicans* and a diffusion barrier that helps to maintain an acidic environment, which could explain why the transcription of *S. mutans* acid tolerance genes (*fabM* and *atpD*) is induced in cospecies biofilms relative to single-species biofilms. Such changes in expression suggest that *S.mutans* may be able to sense *C.albicans* within the surrounding biofilm milieu, in turn increasing the production of proteins involved in virulence and/or stress defence. Overall, the data from studies indicate that the presence of *C.albicans* might accentuate the fitness *S.mutans* of which may help to account for the enhanced virulence.[19]

Clearly, unusual combinations of organisms may generate biofilms with unique virulence properties and demonstrate the need to explore the interactions of mixed flora so frequently observed in medically relevant biofilms formed *in vivo*. The presence of abundant EPS matrix (surrounding a dense population of acidogenic microbial cells) could effectively block access by saliva to the interior of the biofilm and/or prevent acid within biofilm from diffusing outward.

This phenomenon would ensure that the acid formed within the biofilm would remain unneutralized and lead to a protracted acid dissolution of the enamel.[20]

CONCLUSION AND FUTURE PROSPECTS –

The oral cavity is a unique site where host and microbes, including *Candida*, have a delicate balance. They live in harmony with each other until the balance is disrupted. The factors that affect this relationship are host immunity, physiology and habits. Oral diseases continue to increase despite the best efforts of the medical and scientific communities. The most complicated pathologies derive from microbial biofilms (plaque), formed by a consortium of microorganisms, which are protected by a net of polymers (e.g., EPS, DNA). The biofilm matrix delays or blocks the antimicrobials'

diffusion, making treatment much more difficult or even unsuccessful. The oral *S.mutans–Candida* spp. mixed biofilm has been subject to various studies involving alternative therapeutics, new chemical structures (natural and synthetic) with antimicrobial and antibiofilm activities, and nanotechnology, revealing different but promising antimicrobial properties. Nonetheless, more and deeper studies involving in vivo and clinical approaches are still needed.

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