Monografia de investigação – Artigo de Revisão Bibliográfica

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

CANDIDA SPP. AND ITS ROLE IN DENTAL CARIES

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1. Abstract

Introduction: Dental caries is an infectious disease and is a major concern for Dentists. M*utans streptococci* and lactobacilli were considered for a long time the major aetiology agents responsible for caries. More recently, other microorganisms presenting acidogenic and aciduric characteristics were also involved in the onset and development of cariogenic lesions. *Candida*, in particular *Candida albicans* is highly acidogenic and aciduric. Although is considered commensal, *Candida* may lead to serious opportunistic infections, due to its virulence and pathogenicity.

Aim: The aim of the present work is to review the bibliography relating *Candida* and dental caries.

Material and methods: An extensive bibliography search was performed in "PubMed®" including papers written in English, Portuguese or Spanish. The final set of references used for this review included 44 papers.

Development: Dental caries results from the demineralization of the hard tissues leading to the destruction of the organic matter of the tooth, frequently due to the production of acid by oral microorganisms. The acidic environment may result from metabolic end products of dental plaque, usually organic acids produced through the fermentation of carbohydrate. Dental plaque is the result of a large number of interactions between microorganisms that are present in the oral biofilm on the tooth surface.

A newly proposed cariogenic microbiota also includes the yeast *Candida* as an etiologic agent for dental carie. Though, it is not known if this yeast is a real cariogenic pathogen or simply an indicator of oral conditions favorable for the infectious disease – dental caries – or a structural component of the biofilm.

Conclusion: The results support the previous suggestion that *C. Albicans* plays an important role in dental caries. However, there are some mechanisms that still unclear because of the lack of literature.

Key-words: *Candida, Caries, Dental caries*

2. Resumo

Introdução: A cárie dentária é uma doença infeciosa que afeta a cavidade oral e representa uma das preocupações dos Médicos Dentistas. *Streptococcus* do grupo *Mutans* e *Lactobacillus* foram considerados, durante muito tempo, os principais agentes etiológicos responsáveis pela cárie. Mais recentemente, outros microrganismos com características acidogénicas e acidúricas foram também envolvidos no aparecimento e desenvolvimento de lesões cariogénicas. A *Candida*, particularmente a *Candida* albicans, é bastante acidogénica e acidúrica. Embora seja considerada um fungo comensal, a *Candida* pode levar à ocorrência de infeções oportunistas sérias, devido à sua virulência e patogenicidade.

Objetivo: O objetivo deste trabalho é fazer uma revisão bibliográfica, relacionando a *Candida e* a cárie dentária.

Materiais e métodos: Foi realizada uma extensa pesquisa bibliográfica no motor de busca "PubMed®", incluindo artigos escritos em Inglês, Português ou Espanhol. O conjunto final de referências usadas nesta revisão incluiu 44 artigos.

Desenvolvimento: A cárie dentária resulta da desmineralização dos tecidos duros, levando à destruição da matéria orgânica do dente, frequentemente devido à produção de ácido pelos microrganismos orais. O ambiente ácido pode resultar do metabolismo dos produtos finais da placa bacteriana, normalmente ácidos orgânicos produzidos através da fermentação dos hidratos de carbono. A placa bacteriana é o resultado de um grande número de interações entre microrganismos que estão presentes no biofilme na superfície do dente.
A microbiota cariogénica recentemente proposta também inclui o fungo *Candida* como um agente etiológico para a cárie dentária. Contudo, não é conhecido se este fungo é um verdadeiro patogéneo cariogénico, ou simplesmente um indicador de condições orais favoráveis para a doença infeciosa – cárie dentária – ou um componente estrutural do biofilme.

Conclusão: Os resultados apoiam a ideia anterior de que a *C.albicans* desempenha um papel importante na cárie dentária. Contudo, alguns mecanismos continuam pouco claros devido à falta de literatura sobre o assunto.
Palavras-chave: *Candida, Cáries, Cárie Dentária*

3. Introduction

Dental caries is a multifactorial disease associated with many factors such as: cariogenic microorganisms, high level of carbohydrate consumption, poor oral hygiene, malnutrition and low socio-economic status (1, 2). Dental caries results from the demineralization of the hard tissues (enamel, dentin and cementum) leading to the destruction of the organic matter of the tooth, frequently due to the production of acid by oral microorganisms. The acidic environment may result from metabolic end products of dental plaque, usually organic acids produced through the fermentation of carbohydrates (3, 4). Dental plaque is the result of a large number of interactions between microorganisms that are present in the oral biofilm on the tooth surface (5, 6).

Streptococci and *Lactobacilli* were considered the main aetiological agents of human dental caries (7-11). More recently, other oral microbiota has also been implicated in dental decay by numerous studies, mainly acidogenic and aciduric microorganisms (7, 10, 12, 13). These newly proposed cariogenic microbiota also includes the yeast *Candida* (8, 14-20). Though, it is not known if this yeast is a real cariogenic pathogen or simply an indicator of oral conditions favorable for the infectious disease – dental carie – or a structural component of the biofilm (21).

A strong association has been found between the prevalence of *C. albicans* and dental caries – particularly in children, adults and young adults (15, 22, 23). However, the host age is a determining factor in yeast carriage (20, 24-26) as well as the geographical area (27).

Candida albicans, an opportunist microorganism found in the oral cavity (13, 28), may cause serious candidosis and is the major cause of denture stomatitis (29). Candidosis is an infection that is particularly important in immunocompromised individuals (28, 30), for example, children and elderly or patients of AIDS, cancer (31) and diabetes (28, 32-34).

In the population of elderly people studied, a worsening of the overall state of health (like lowering of cell-mediated immunity) (35), poor oral status and the side effects of the main treatments used contributed, directly or indirectly, to the risk factors for *Candida spp.* (36). Another problem in this population are exposed root surfaces (37, 38) because of gingival recession (10, 39). These exposed surfaces become vulnerable against the acidic attack by oral

microbiota (7, 40, 41). These carious foci may serve as reservoirs of yeasts and cause debilitating opportunistic infections in the oral cavity and systemically (42). Also, it was shown an increased oral carriage rate of *Candida* with the use of acrylic prothesis (42-45), although some authors (39) refer that there are no significant differences in root caries microbiology between denture wearers and non-denture wearers.

The presence of *C. albicans* in the oral cavity of young subjects should be taken into account as a risk factor for dental caries (46) because a study revealed that the oral cavity of children with healthy teeth is almost devoid of *Candida albicans* (27). In Signoretto et al. review (8), it was suggested that low level of fluoride in drinking water (11), high sugar consumption, together with inefficient oral hygiene, open caries lesions and, as in the case of babies, the extensive intake of sweet substances via the feeding-bottle are the factors mainly responsible for the high prevalence of this yeast in the mouths of children (47). The oral cavity of children therefore becomes one of the main sources of transmission of this fungus and its eradication should start with a reduction in the concentration of this microorganism in their mouths (48). Several approaches are recommended for this reduction, including tooth brushing, fissure sealants, antiplaque agents and sugar substitutes (49). Oral environment stabilization (using glass ionomer and zinc oxide-eugenol cements) also helps to reduce the number of pathogenic microorganisms, reducing the number of sites for *Candida* colonization and consequently reduces the risk of fungal infections (13, 17, 50, 51). HIV-infected children who had positive growth for *Candida spp.* also had a greater number of dentinal carious lesions suggesting a potential relationship between these two factors (52).

The high isolation frequency (32,1%) of *Candida albicans* from root caries lesions of adult subjects in Zaremba et al. study, may suggest that carious foci are reservoirs of yeasts too (39).

Subjects with hyposalivation have an aciduric supragengival plaque with high numbers of *S. mutans*, *Lactobacillus spp.* and *C. albicans*, which constitute a risk factor for caries (53).

Yeasts are microorganisms normally present in the oral cavity (13) of healthy individuals, and according to several studies, the percentage of *Candida* spp. colonization ranges from 20% to 75% (24, 40, 54-56) of healthy individuals and become predominant genus of oral microbiota in more than 60% of immunocompromised subjects (8).

Candida spp. are able to colonize several surfaces of the oral cavity including the tongue, palate, cheek and hard surfaces of teeth. They are also present in saliva as a consequence of oral surface colonization (8, 15, 19, 57-59). This microorganism is dimorphic (3), existing both as yeast and in pseudohyphal or hyphal form (60). This property is referred to as the major virulence determinant. *Candida albicans* displays other pathogenic factors, such as interfering with the immunological system of the host organism, and producing several metabolites, such as pyruvates and acetates (11).

Given the above stated the main goal of the present work is to gather literature relating *Candida* oral colonization and the development of carious lesions.

4. Materials and Methods

An extensive bibliography search was performed through the search engine "PubMed®", using the query: "Candida" and "Dental caries" or "Caries". The query was performed in April 2012. A total of 207 references were initially retrieved. Exclusion criterion was papers written in languages other than English, Portuguese or Spanish. The resulting sample included 136 references. On a second phase, two different readers read all the papers titles and selected them for inclusion or exclusion according to their relevance for the present review. On a third phase the abstracts of the references resulting from the second phase were read and classified for inclusion or exclusion according to their relevance for the present review. The final set of references used for this review included 44 papers. Additional 52 references were used for contextualization of the subject.

5. *Candida spp.*

Candida species are common colonizers of mucosal surfaces in oral cavity (21) and they are capable to adhere to tooth surfaces, participate in biofilm formation and proceede to carbohydrate fermentation (3, 61). *Candida albicans* was present in a higher frequency in occlusal tooth surfaces when compared to buccal mucosa and tongue, because it has much more affinity to carious tooth structure. (54) Colonization of the oral cavity by this yeast occurs through commensalism (51, 62, 63), without causing disease (24). The most dominant specie, *Candida albicans* (21), an opportunistic dimorphic fungi (3) has been suggested to have an acidogenic and cariogenic potential (14, 47). It also possesses adhesive and proteolytic properties, which plays an important role for dentinal caries progression (17, 64). The transformation from the saprophytic to the parasitary form is related not only with its virulence but also with host-related factors (42), including, the most important one, the modification of the oral microbiota balance (13). This modification may occur due to such as orthodontic appliances (65, 66) or mouthbreathing (67). *Candida albicans* can alternate between growth as single yeast cell, pseudohyphae or polarized filaments (60). This three morphotypes and the switch between them its important for the establishment of the disease (68). However, hyphae are considered the most virulent morphotype (69). It has been shown that this morphotype are capable to penetrate enamel fractures and dentinal tubules from outside the tooth (64) as well as from the root canal (48).

As mentioned before, several factors, such as adherence, persistence, dimorphism and/or germ tube formation, phenotypic switching, interference with host defense system, synergism with bacteria, and production of hydrolases or other metabolites, have been proposed to be virulence factors of this fungus (70).

6. Mechanisms related to *Candida albicans* that can lead to dental caries

The mechanisms underling this pathogenic process are still unclear. It is thought that *Candida albicans* may utilize the dentinal structure, especially collagen, for growth, but as it grows, may degrade the dentinal collagen, probably leading to dentinal lesions (28, 29). Also, *Candida* is able to colonize hard tooth surfaces, invade dentinal tubules and produce a large amount of acids, because the yeast metabolizes dietary sugars, provoking desmineralization of the dental enamel (71) and dissolution of hydroxyapatite (8, 14).

Dental adherence

Cariogenecity depends not only on the terminal pH, but also on several other factors, including the ability of the organism to adhere or colonize the tooth surface. (72)

Among the virulence factors, adherence of yeasts to oral surfaces is thought to be one of the most important factors, as there are several ways in which the adherence contributes to candidal persistence. (14)

The adherence and dissociation of *Candida albicans* to six substrates including hydroxylapatite (HAP), which exhibit various hydrophobicity, was study by Nikawa et al. (14). It was showed that the adherence between the yeast and HAP was extraordinary high (14, 73). This association was effectively removed by high concentration of either phosphate or calcium ions, suggesting that either electrostatic interactions or ionic bonds between proteinaceous components of fungal cell wall and HAP may play an important role. The results suggest that *C. albicans* adhere to HAP through electrostatic interaction (74, 75), although little is known about this adherence mechanism. In addition, it is still unclear whether *C. albicans* dissolves HAP or not. (14) One of the most important properties that contribute to the initial adherence to the solid surfaces is the hydrophobic interaction (14).

The findings that the adherence of both isolates of *C. albicans* to HAP was particularly high was consistent with the observations of Sen et al. who showed that *C. albicans* easily colonized on enamel, dentine or cementum, in the absence or presence of a smear layer. (64)

Also was demonstrated that the presence of the yeast enhance the adherence of *Mutans streptococcus* to the oral biofilm, one of the main aetiological agent of dental carie (76). Zijnge et al. identified, for the first time *in vivo,* that *Streptococcus* sp. adhere to a central axis of *Candida* cells or hyphae, forming corncob structures in supragingival plaque (77).

Acid production

The rate of dental hard tissue demineralization does not only depend on the extent of microbial acid formation but also on its duration. Fermentable carbohydrates rapidly decrease in concentration after an oral intake due to ingestion and fermentation. Acid formation continues undimished until the sugar concentration falls below the threshold of saturation that is relevant to the enzymatic processes involved. This means that caries pathogenicity of microorganisms may be influenced by substantital differences in this threshold. The exceedingly high acid tolerance may favor *lactobacilli* and *Candida albicans* in microbial competition, particularly when they are resident in deep dentine caries lesions with small openings. (78)

Candida is dependent on high concentration of dietary sugars to produce acid (28), which leads to the dissolution of hydroxyapatite crystals in enamel and dentin (14, 29).

There are at least three different processes that participate in the acidification of a surrounding environment by yeast cells: extrusion of several organic acids (the predominant pyruvic acid is even more potent to reduce the pH of an already intensely acidified environment than lactic acid); the presence of H^+-ATPase in the plasma membrane of yeasts, which actively pumps out protons from the cell to generate an electrochemical gradient which is used in the co-transport of nutrients (79, 80); the excretion of carbon dioxide resulting from oxidative or fermentative glucose metabolism (78).

Candida and *Lactobacillus* are known acidogenic microorganisms that colonize oral cavity. Acid formation rates of both species proved to be similar at both neutral and low pH, while in a moderately acidic environment the yeast produced less acid than the lactobacilli (78). At every condition, in Klinke et al. study, that include different pH levels and concentrations of glucose, pyruvate revealed to be the predominant organic acid anion secreted by *C. albicans*, except at low initial glucose concentration at pH 7.0, where acetic acid was dominant (78). Acetate was also observed as the predominant acid anion in resting plaque fluid (81) and in

arrested caries lesions (82). Acid production by *Candida* was higher at pH 4.0 but required a higher concentration of glucose than *lactobacilli*. (78) In agreement with these studies are the work of Samaranayake (83) showing that *Candida* lower the pH of glucose-supplemented saliva down by secreting organic acids demonstrating extreme acid tolerance. Interestingly in a study by Nikawa et *al.* (14), *C. albicans* dissolved hydroxyapatite in a liquid culture.

In adults with type 2 diabetes mellitus was reported a significantly higher prevalence of root surface caries than in non-diabetic individuals as well as a higher colonization by *Candida*. A high blood glucose level may enhance candidal adhesion to epithelial cells *in vivo*. (43)

Candida albicans contribution to caries progression may therefore depend on both the amount of fermentable carbohydrates and the proportion of sucrose and glucose in the diet (28, 84).

<u>*Candida albicans* hypha formation</u>

Saliva induces the germ tube formation of *C. albicans* although is a weaker inducer of hypha formation than serum (9).

A compound excreted by the caries bacterium *Streptococcus mutans* inhibits the morphological transition from yeast to hyphae, an important virulence trait, in the opportunistic fungus *Candida albicans* (68). It was named *Streptococcus* diffusible signal factor (SDSF).

N-3-oxo-C_{12} homoserine lactone (HSL), a signaling molecule involved in bacterial quorum sensing, completely represses *C.albicans* hypha formation without altering the growth rate. Although many gram-negative bacteria produce HSLs with shorter acyl chains, the inhibition of *C.albicans* hypha formation is caused specifically by long-chained HSL molecules. In addition, related, non-HSL molecules with long acyl chains, such as dodecanol and farnesol, also inhibit the hypha formation of the yeast (85). It was shown that *S.mutans* increased the growth of *C.albicans* by stimulating coadhesion while simultaneously suppressing the formation of hyphae. (9)

S.mutans can affect germ tube formation of *C.albicans* in cocultures even if the bacteria and the yeast are physically separated. These results indicate that *S.mutans* secretes one or more diffusible molecules that affect *C.albicans* hypha formation. This inhibition occurs through

several mechanisms, one of which involves the secreted quorum-sensing molecule CSP (competence-stimulating peptide).

The discovery of *S. mutans* CSP as a signaling molecule involved in interactions with *C. albicans* adds a structurally unrelated molecule to the list of known bacterial signaling molecules affecting the yeast (9).

Role of glucosyltransferases

Glucosyltransferases (Gtfs) secreted by *Streptococcus mutans* bind to saliva-coated apatite (sHA) and to bacterial surfaces that by synthesizing exopolymers *in situ*, promote cell clustering. Glucosyltransferases B (GtfB) adhered more effectively to the *C. albicans* yeast cell surface with glucans, than to yeast cells without surface glucans (uncoated), in an enzimatically active form, promoting the formation of a glucan-rich matrix *in situ*. So, the presence of glucan-coated yeast cells significantly increased the accumulation of *S. mutans* on the sHA surface.

Glucan-like structures that form between cocci and yeasts, suggesting that glucans may play a role in mediating their interactions (86). Therefore, Gregoire et al. (87) hypothesized that *S. mutans*-derived Gtfs would bind to the *C. albicans* cell surface to produce exopolysaccharides (EPS). The presence of surface-formed glucans would increase *S. mutans* binding to the yeast cell and concomitantly enhance the ability of *C. albicans* to adhere to the sHA surface. This relationship, in addition to enhanced acidity (78), may explain the detection of elevated amounts of *S. mutans*, along with *C. albicans* and EPS in the plaque from toddlers with early childhood caries (ECC), thereby modulating the development of virulent biofilms.

The exact identities of the GtfB binding sites on the yeast cell surface are unknown.

Collagenolytic enzyme produced by *Candida albicans*

An extracellular acid proteinase from *C. albicans* was characterized by Remold et al. (88) and Ruchel (89) and the relationship between the infectivity of mice and proteinase production was describe. The *C. albicans* enzyme degraded both the native acid-soluble collagen and the insoluble dentinal collagen (29, 90). It was though that the yeast may utilize the dentinal structure, especially collagen type I (represents more than 90% of the dentin organic matrix) (91-94). As a result, *C. albicans* colonization and proliferation is facilitated, which could help to

promote the carious process in dentin (95, 96).

Enzyme activity was not detected at pH 6.0, and incubation at a pH higher than 6.0 caused irreversible denaturation of the enzyme. Some substances like cysteine, urea and pepstatin inhibited enzyme activity.

Despite the fact that the features described above indicate *C. albicans* is a potent caries pathogen, its significance has been often denied since colony-forming units of fungi usually represent only a small percentage of the total microbiota (28). On the other hand, the biomass of *C. albicans* is 40 times larger than those of bacteria like *streptococci* (78).

7. Conclusion

Candida albicans is an opportunistic microorganism existing in oral cavity as commensal but may lead to pathogenic states, like candidosis. Most bibliography showed that this yeast is involved in the development of dental caries, although we had found some authors that don't consider *Candida* as a cariogenic microorganism.

As shown *Candida* possesses adhesive and proteolytic properties, that are fundamental for dentinal caries progression.

However, other mechanisms that may be involved in the settlement and development of caries are still unclear.

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