REVIEW ARTICLE

CANDIDA A PROBABLE ETIOLOGY IN EARLY CHILDHOOD CARIES- A LITERATURE REVIEW

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ABSTRACT

Early Childhood Caries, a rampant type of dental caries in children below 6 years of age; is a chronic childhood disease with a severe sequalae affecting the child and family. It is a multifactorial disease, with *S.mutans and Lactobacilli* being implicated as the main microorganisms in its etiopathogenesis. Recent literature suggests a probable role of *Candida*, fungal species, a normal commensal of the oral cavity, in its etiopathogenesis. Under particular predisposing physiological or pathological conditions, *Candida* is capable of provoking pathologies via endogenous infectious mechanism. In early childhood period, due to immature immune system and not fully established micro flora in the oral cavity, children are more susceptible to opportunistic microbial colonization. The present literature review provides compilations of previous studies implicating *Candida's* probable role in caries and an attempt is made to provide and update the current understanding of *Candida's* potential role in initiation of early childhood caries in order to help health providers to diversify their treatment modalities from antibacterial to anti-fungal.

Keywords: ECC, Caries, *Candida*, *C.Albicans*, Pre-school.

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INTRODUCTION

Early Childhood Caries (ECC) also known as early childhood tooth decay is a destructive form of dental caries that afflicts the young children. The older terminologies like "nursing caries" and "baby bottle tooth decay" have been replaced with broader term ECC. It is one of the most common chronic childhood diseases in this age group; and though not life-threatening, it affects normal health and well-being of the child. The prevalence of dental caries has reduced worldwide, yet that of ECC remains high and so it is currently a WHO concern. [1,2]

The difference between ECC and the dental caries is that, here the progression of caries is very rapid and widespread; and because of this rapid progression, its prevention and management is a challenge.

American Academy of Pediatric Dentistry (AAPD) defines early childhood caries (ECC) as the presence of one or more decayed (non cavitated or cavitated), missing (due to caries), or filled tooth surface in any primary tooth in a child 71 months of age or younger. Presence of any smooth surface caries in children younger than three years of age; one or more cavitated, missing due to caries or filled smooth surfaces of the primary maxillary anterior teeth in children from ages three to five; or a decayed, missing or filled score of >=4 (age 3), >=5 (age 4), >=6 (age 5) surfaces are termed as Severe-Early Childhood Caries (S-ECC) [3].

Early Childhood Caries is an infectious disease of bacterial origin[4]. The disease is the result of frequent sugar intake leading to changes in the oral microbial ecology to a cariogenic miroflora, leading to an imbalance between the demineralization and remineralisation process, favouring demineralisation of the teeth. Thus, acidity is a pre-requisite for caries formation, and acidogenic microflora plays an important role.

Oral bacteria like, *Streptococcus mutans* and *Lactobacillus spp.* are the main microorganism implicated for the initiation and progression of caries respectively.

But presently researchers are implicating the propable role of *Candida*, a fungi in caries etiopathogenesis. This article is a brief literature review on *Candidas* role in early childhood caries.

Candida species are fungi which are common inhabitants of the normal oral microbiota found in infants. *Candida* is an opportunistic pathogen and in immunocompromised individuals it has the ability to cause a variety of infections. For instance till date, oral thrush in infants and chronic atrophic candidiasis (denture induced stomatitis) in adult are the known most common clinical manifestations of oral candidiasis.[5,6,7] Among *Candida* species, the most prevalent in the oral cavity is *Candida albicans*.[3,1,2]

As a first colonizer

Among *Candida* species, the most prevalent in the oral cavity is *Candida albicans*. Reports suggest its presence in saliva, dental plaque and also infected dentin of children with early childhood caries. Craig reported that *Candida albicans* in the biofilm of ECC is twice more prevalent than caries free children[7,8,9,10]. Reports of candidal carriage range as high as 50%. [7]

The prevalence of the *Candida* in the oral cavity varies with age: 4% in 4-5 days old babies, 24% in premature newborns, and 30% in children aged 3-12 years. [6] Higher frequencies of *Candida* were also found in children using pacifiers. Its presence in the oral cavity may be related to many factors such as birth infection, nurse fingers, hospital maternity ward [11,12,13], baby's feeding bottles, infected pacifiers, maternal skin, air, water and carious teeth.[7]

Candidal carriage in children

Candida counts were significantly associated with caries prevalence with increase in their DMFS score.[14] The carriage was approximately 3 times greater in females than in males. The yeast isolates were 71% *Candida albicans*; 19.7% *Saccharomyces spp*. and 8.6% *Candida* tropicalis.[15] Studies explains that poor oral hygiene, in children consuming unrestricted diets, may influence the salivary levels of yeasts irrespective of the frequency or amount of sugar consumed.[16,17,18,19] Children with oral *C. albicans* frequently maintained carriage over time, even with regular dental care.[20,21] Carious teeth

may constitute an ecological niche for *C.albicans* and, potentially responsible for recurrent oral and non-oral candidiasis.[22] Oral environment stabilization procedures were found to be efficient in reducing *Candida spp.* counts, especially when the zinc oxide-eugenol cement was employed.[23]

Candida albicans was observed in the oral cavity of healthy school and preschool children compared to adolescents.[24,25] The frequency of *C. albicans* in ECC was higher when compared to caries and caries-free groups.[26] *Candida albicans* was more frequently isolated in all studies, and it was the only species present in caries-free children.[27,28]

Post chlorhexidine treatment, decreased for mutans *streptococci* and *lactobacilli*, but large numbers of *Candida spp.* still remained in the saliva of several children. *Candida spp*. reduced only after post antifungal therapy. And few researchers suggested the use of antifungal drug nystatin (oral rinses) to control caries.[29]

Candida - free individuals significantly inhibited the blastoconidial growth more than *Candida*-carriers suggesting that saliva may play a role in modulating oral candidal populations in health.[30,31,32]

Recent studies concluded that prevalence of *Candida albicans* in dental plaque and carious lesions of children with ECC were relatively high and prevalence was higher in cervical group of caries.[33,34] Further studies revealed that *Candida albicans* genotype A was dominant among SECC Children.[35]

Greater acid potential

C. Albicans is acidogenic and has the ability to ferment many carbohydrates (hetero -fermentative). It is also acid-tolerant, and increased presence is seen in the oral cavity due to great number of retentive sites.[36]

Under favourable physiological or pathological conditions, it is capable of provoking pathologies. It is endowed with dimorphism i.e; it is able to exist both in yeast and pseudohyphal/hyphal form, and this property is referred to as a major virulence determinant.[37] Yeast form are normal commensal of the oral cavity. Pseudohyphal (budding shape) has been associated with a fungal (saprophytic) condition while the presence of hyphal forms has been associated with active symptomatic infections. *Candida albicans* displays many pathogenic forms, because of which it is capable of adhering to various surfaces, interfering with the immunological system of host organisms and producing several catabolytes.[38]

Some studies have shown that in an environment with a pH below 5.5, which is relevant for early childhood caries formation, acidification by *Mutans streptococcus* decreases considerably and ceases around pH 4.2 (deSoet et al.1991), whereas *C.albicans* can still secrete acid at pH 4.0 (Kl inke et al., 2009)[39,40]. Furthermore, the study by Nikawa et al. has revealed that *C.albicans* was capable of dissolving the hydroxyapatite at an approximately 20-fold rate higher than *Mutans streptococcus*, despite a lower number of yeast cells in the culture (Nikawa et al., 2003) studies above may indicate that *C. albicans* is capable of producing acids and demineralizing dental tissue in vivo.

It is supposed that there are at least two processes that can be predicted in vivo which involve in the acid production of a surrounding environment by *Candida albicans*

1) It produces several organic acids including pyruvic acid and acetate (Samaranayakeet al., 1986; Collingset al., 1991)[41,42]

2) Abundant H +ATPase on the plasma membrane of yeasts pumping out proteins from the cell is induced by glucose and makes a contribution to the acidification (Bowman et al., 1986; Manavathuet al., 1999).[43] Since acidification causing demineralization of dental tissues plays the most important role in the progression of early childhood caries.[44,45] These, the acid production of microbes has been considered as one of the typical characters of caries pathogen.[46,47] According to the viewpoint of Marsh involved in dental caries[48], there are undoubtedly other acidogenic microorganisms. Considering the potential of *Candida albicans* to adherence to saliva proteins and *S.mutans*, its acid producing capability, its

ability to penetrate into dentinal canals and its enzymatic activity to degrade collagen; indicates that it may be having greater cariogenic ability and may be having a possible role in the progression of early childhood caries.

Samaranayake LP et al in 1986, conducted a invitro study to determine the growth and acid production of *Candida* species in human saliva supplemented with glucose/ glucose-free. The results of the study showed that the growth of *Candida* in saliva was accompanied by rapid decline in pH from 7.2 to 3.2 over 48 hours. And major acidic components initiating and sustaining this pH drop were pyruvates and acetates. The study concluded that acidic metabolites may play an important role in pathogenesis of oral *Candida* infections.[49]

J. Verrant et al in 1991 conducted an invitro study to determine the effect of different pH on *Candida* strains ability to adhere and form hyphae. Hefound that *Candida* were capable of adhering to buccal epithelial cells at all the pH values studied; (7.3,6.0,2.6). and the adhering strains at pH 2.6 showed hyphal forms.[50]

Pathogenicity

Studies have shown that *C.albicans* adheres to saliva-coated surfaces with assistance of salivary proteins, acrylic[51,52], dental hard tissues.[53,54,55,56] It can readily adhere to mucosal epithelial cells[57,58] and collagen[59] with the Proline-rich proteins and provide receptors for adhesion of *C.albicans* to enamel pellicles, and also help in its adhesion to *streptococcal* surfaces[60]. Thus, salivary proteins may act as a bridge (ligand) for the interactions between *C. albicans* and oral bacteria, which may aid *C. albicans* to participate in the early childhood caries development. The secreted aspartyl proteinases (Saps) are among the most important virulence factors of *C. albicans*, and are related to the adhesion of *C. albicans* to tooth surfaces and the degradation of extracellular matrix and proteins. Wengiung L et al in 2014, hypothesized that Saps are higher in S-ECC groups and Saps 1 may play a role in development of S-ECC.

Sen B.H et al in 1997 conducted an invitro study to determine the colonization of *Candida albicans* on cleaned human dental hard tissues using scanning electron microscope. The results of the study showed that hyphae penetrated into cracks, followed the ridges of the cavities and migrated into dentinal tubules. The study concluded that dental hard tissues may be invaded by *C.albicans* and are potential reservoir for disseminating candida infections.[61,62]

Nikawa H et al in 2003 conducted a study to determine the in vitro cariogenic potential of *Candida albicans*. The adherence and dissociation of *Candida albicans, Candida tropicalis, Streptococcus mutans and Streptococcus sanguinis* to six substrates including hydroxyapatite which exhibit hydrophobicity, was examined using bioluminescent adenosine triphosphate assay and spectrophotometrical method. The results shows that *Candida albicans* adherence to hydroxyapatite was extraordinary high through electrostatic interaction, but in small number. They concluded that *Candida albicans* possesses the ability to dissolve HAP to a greater extent (approximately 20-fold) when compared with *S.mutans*.[63,64]

***Candida* in association with Bacteria.**

Thein Zm et al; showed that co-culture with highest concentration of each of foregoing bacteria resulted in a consistent reduction in the yeast counts in candida biofilm, except for *Lactobacillus, S.mutans* and *S.intermedius* co-cultures indicating quantitative and qualitative nature of the bacteria modulating *C.albicans* biofilm formation in mixed species environment like our oral cavity.[65] Invitro studies using artificial biofilm homogenously inoculated with *Streptococcus mutans* and *Candida*; simultaneously confirmed the adherence capacity of *Streptococcus mutans* to *Candida* when in association.[66]

Lucja M.Jarosz et al; revealed that CSP (Competence-Stimulating peptide), an *Streptococcus mutans* quorum sensing molecule secreted during the early stages of growth, inhibits the *Candida albicans* morphological switch.[67] Further; S.Gregoire et al; revealed that glucan coated yeast

cells significantly increased the accumulation of *Streptococcus mutans* on Hydroxapatite surfaces. And this Glucan-mediated fungal- bacterial interaction represents novel cross-kingdom interaction that is involved in development of virulent biofilms associated with ECC.[68] The ability of *S.mutans* and *C.albicans* together to form biofilms is enhanced in vivo due to presence of *C. albicans* augments exopolysaccharides (EPS) production, such that co-species biofilms accrue more biomass and harbor more viable *S. mutans* cells than single-species biofilms. Glucosyltransferase-derived EPS was proved to be a key mediator of co-species biofilm development, and that co-existence with *C. albicans* induces the expression of virulence genes in *S.mutans*. Altogether, these studies demonstrate a novel mutualistic bacterial-fungal relationship that occurs at a clinically relevant site to amplify the severity of this ubiquitous infectious disease.[69]

Conclusion

This literature review reinforce those of the previous scientific literature, implying that *Candida spp* are not merely passively associated with the caries process. It remains unclear whether *Candida species* are causative agents in early childhood caries initiation or progression, or whether *Candida* colonization are merely a consequence of severe early childhood caries activity. Further studies are required to elucidate the real role of this microorganism in the etiology of ECC, which may aid in management and prevention of this chronic childhood disease.

REFERENCES

1. Peterson P. Continuous Improvement of oral health in the 21st Century - the approach of the WHO Global Oral Health Programme; World Oral Health Report 2003, WHO/NMH/NPH/ORH/03.2 (downloaded on 03.04.2014).
2. Low W, Tan S, Schwartz S. The effect of severe caries on quality of life in young children. Pediatric Dent.1999;21:325-6.
3. American Academy of Pediatric Dentistry. Early childhood caries: Unique challenges and treatment options. Pediatr Dent. 2000;22:21.
4. Gibbons RJ, Amstrong WD. Bacteriology of Dental Caries. Journal of Dental Research 1964;43:1021-8.
5. Signoretto C, Burlacchin G, Fiorenzo F. Support for the role of *Candida spp* in extensive caries lesion of children. New Microbiologica, 2009;32(1):101-7.
6. Peretz B, Mazor Y, Daon N, Bar-Ness Greenstein R. *Candida Mutans, Streptococci*, Oral Hygiene and caries in children. Journal of Clinical Pediatric Dentistry. 2011;36(2).
7. De Carvalho FG, Parisotto TM, Hebling J, Spolidorio LC, Spolidorio DMP. Presence of *Candida spp.* in infants oral cavity and its association with early childhood caries. Brazilian J Oral Sci 2007;6:1249-53.
8. Thaweboon S, Thaweboon B, Nakornchai S, Jitmaitree S. Salivary secretory IgA,pH, flow rates, *mutans streptococci and Candida* in children with rampant caries. Southeast Asian J Trop Med Public Health 2008;39:893-9.
9. Molaic E, Gestalin A, Quinio D, etal. The extent of oral fungal flora in 353 students and possible relationship with dental caries. Caries Res. 2001;35:149-55.
10. Wetzel WE, Hanisch S, Sziegoleit A. The germ colonization of the oral cavity in small children with the nursing bottle syndrome. Schweiz Monatsschr Zahnmed 1993;103:1107-12.
11. Lay KM, Russel C. *Candida* species and yeasts in mouths of infants from a special care unit of a maternity hospital. Arch Dis Child. 1977 Oct;52(10):794-6
12. Young G, Krasner RI, Yudkofsky PL. Interaction of oral strains of *Candida albicans* and *Lactobacilli*. J Bacteriol.1956;72(4):525-9
13. Ollila P, Niemela M, Uhari M, Larmas M. Risk factors for colonization of salivary lactobacilli and *Candida* in children. ActaOdontol Scand. 1997Jan;55(1):9-13.
14. Russell JI, MacFarlane TW, Aitchison TC, Stephen KW, Burchell CK. Caries prevalence and microbiological and salivary caries activity tests in Scottish adolescents. Community

Dent Oral Epidemiol. 1990 Jun;18(3):120-5.

15. Martin MV, Wilkinson GR.The oral yeast flora of 10-year-old schoolchildren. Sabouraudia. 1983 Jun;21(2):129-35.
16. Beighton D, Adamson A, Rugg-gunn R. Associations between dietary intake, dental Caries experience and salivary bacterial Levels in 12-year-old english school children. Archs Oral Biol 1996;41(3):271-80.
17. Molaic E, Gestalin A, Quinio D, etal. The extent of oral fungal flora in 353 students and possible relationship with dental caries. Caries Res. 2001;35:149-55.
18. Moreira D, Spolidorio DM, Rodrigues JA, Boriollo MF, Pereira CV, Rosa EA, Hofling JF. *Candida spp.* biotypes in the oral cavity of school children from different socioeconomic categories in Piracicaba-SP, Brazil. Pesqui Odontol Bras. 2001;15(3):187-95.
19. Akdeniz BG, Koparal E, Sen BH, Ateş M, Denizci AA. Prevalence of *Candida albicans* in oral cavities and root canals of children. ASDC J Dent Child. 2002;69(3):289-92.
20. Krishnakumar R, Singh S, Reddy VV. Comparison of levels of *mutans streptococci* and *lactobacilli* in children with nursing bottle caries, rampant caries, healthy children with 3-5 DMFT and healthy caries free children. J Indian Soc Pedod Prev Dent. 2002;20(1):1-5.
21. Starr JR, White TC, Leroux BG, Luis HS, Bernardo M, Leitao J, Roberts MC. Persistence of oral Candida albicans carriage in healthy Portuguese schoolchildren followed for 3 years. Oral Microbiol Immunol. 2002;17(5):304-10.
22. Hossain H, Ansari F, Schulz-Weidner N, Wetzel WE, Chakraborty T, Domann E. Clonal identity of *Candida albicans* in the oral cavity and the gastrointestinal tract of pre-school children.Oral MicrobiolImmunol. 2003;18(5):302-8.
23. doRego MA, Koga-Ito CY, JorgeAO. Effects of oral environment stabilization procedures on counts of *Candida spp.* in children. Pesqui Odontol Bras. 2003;17(4):332-6. Epub 2004 Apr 19.
24. Cortelli SC, Junqueira JC, Faria I, Koga C, Cortelli JR. Correlation between *Candida spp*. and DMFT index in a rural population. Brazilian Journal of Oral Sciences, 2006;5(17):1007-11.
25. Rozkiewicz D, Daniluk T, Zaremba ML, Cylwikrokicka D, Stokowska W, Pawinska M, Dabrowska E, Marczuk-Kolada G, Waszkiel D. Oral *Candida albicans* carriage in healthy preschool and school children. Adv Med Sci 2006;51:187-90.
26. Carvalho FG, Parisotto T, Barbieri DSV, Vicente VA, Fraiz FC, Lavoranti OJ, Estivalet STI, Lameira PR. Analysis of in vitro adherence of Streptococcus mutans and *Candida albicans*. Braz J Microbiol. 2007;38:624-31.
27. Jorn A, Ann L, Sara R, Alice M, Ingar O, Floyd E, Eugene J, Bruce J. Bacteria of Dental Caries in Primary and Permanent Teeth in Children and Young Adults.J Clin Microbiol. 2008;46(4):1407-17.
28. Ge Y., Caufield PW, Fisch GS, Li Y. *Streptococcus mutans* and *Streptococcus sanguinis* Colonization Correlated with Caries Experience in Children. Caries Res. 2008;42(6):444-8.
29. Hibino K, Samaranayake LP, Hagg U, Wong RW, Lee W. The role of salivary factors in persistent oral carriage of *Candida* in humans. Arch Oral Biol. 2009;54(7):678-83.
30. Luja M, Dong M,Henny C, Crielaard W, Bastiaan PK. Streptococcus mutans Competence-Stimulating Peptide Inhibits *Candida albicans* Hypha Formation. Eukaryotic Cell. Nov 2009;8(11):1658-64.
31. Palmer CA, Kent R Jr, Loo CY, Hughes CV, Stutius E, Pradhan N, Dahlan M, Kanasi E, Arevalo Vasquez SS, Tanner AC. Diet and caries-associated bacteria in severe early childhood caries. J Dent Res. 2010;89(11):1224-9.
32. Peretz B, Mayor Y, Daon N, Bar - Ness Greenstein R. *Candida, Mutans Streptococci*, Oral Hygiene and caries in children. Journal of Clinical Pediatric Dentistry Vol 36(2): Winter 2011.
33. Gregoire S, Xiao J, Silva BB,Onzalez I, Agidi

PS, Klein MI, Ambatipudi KS, Rosalen PL, Auserman R, Waugh RE, Koo H. Role of Glucosyltransferase B in interaction of Candida albicans with Streptococcus mutans and with an Experimental Pellicle on Hydroxyapatite Surfaces. Applied and Environmental microbiology, Sept 2011; 77(18):6357-67.

34. Yang XQ, Zhang Q, Ying L, Yuan L, Zou J. Genotype distribution of *Candida albicans* in dental biofilm of Chinese children associated with Severe Early Childhood caries. Archives of Oral Biology2012; 57:1048-53.
35. Loesche W J, Schork A, Terpenning MS, Chen YM, Stoll J. Factors which influence levels of selected organisms in saliva of older individuals. Journal of clinical microbiology 1995;33 (10):2550-7.
36. Sullivan JM, Jenkinson HF, Cannon RD. Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. Microbiol. 2000;146:41-8.
37. Featherstone JD. The science and practice of caries prevention. J. Am. Dent. Assoc. 2000;131:887-99.
38. Hagihara Y, Kaminishi H, Cho T, Tanaka M, Kaita H. Degradation of human dentine collagen by an enzyme produced by the yeast *Candida albicans*. Arch Oral Biol.1988;33: 617-9.
39. de Soet JJ, van Loveren C, Lammens AJ. Differences in cariogenicity between fresh isolates of *Streptococcus sobrinus* and *Streptococcus mutans*. Caries Res. 1991;25: 116.
40. Manavathu EK, Dimmock JR, Vashishtha SC, Chandrasekar PH. Proton-Pumping-ATPase-Targeted Antifungal Activity of a Novel Conjugated Styryl Ketone. Antimicrob. Agent Chemother 1999;43:2950-9.
41. Samaranayake LP, Hughes A, Weetman DA, MacFarlane TW. Growth and acid production of *candida species* in human saliva supplemented glucose. J. Oral Pathol. 1986;15:251-4.
42. Collings A, Holmes AR, Shepherd MG. Secretion of end-products of metabolism by *Candida species.* Biomed. Lett. 1991;46:285-96.
43. Bowman BJ, Bowman EJ. H+-ATPases from mitochondria, plasma membranes and vacuoles of fungal cells. J. Membr. Biol. 1986;94: 83-97.
44. Kaminishi H, Hagihara Y, Hayashi S, Cho T. Isolation and Characteristics of Collagenolytic Enzyme Produced by *Candida albicans*. Infect Immun. 1986;53:312-6.
45. Nikawa H, Yamashiro H, Makihira S. In vitro cariogenic potential of *Candida albicans*. Mycoses 2003;46:471-8.
46. Maijala M, Rautemaa R, Jarvensivu A, Richardson M, Salo T, Tjaderhane L. *Candida albicans* does not invade carious human dentine. Oral Dis. 2007;13:279-84.
47. Klinke T, Kneist S, de Soet JJ. Acid Production by Oral Strains of *Candida albicans* and *Lactobacilli*. Caries Res. 2009;43:83-91.
48. Marsh PD. Dental plaque as a biofilm and a microbial community implications for health and disease. BMC Oral Health 2006 Suppl 1: S14.
49. Samaranayake LP, Hughes A, Weetman DA, MacFarlane TW. Growth and acid production of *Candida species* in human saliva supplemented glucose. J. Oral Pathol. 1986;15:251-4.
50. Verrant J, Shakespearet AP, Willcox MDP, Knox KW. The effect of pH on adhesion and hyphal formation by Strains of *Candida albicans*. Microbial ecology in health and disease 1991;4:73-80.
51. Damm DD, Neville BW, Geissler RH Jr, White DK, Drummond JF, Ferretti GA Dentinal candidiasis in cancer patients. Oral Surg. Oral Med. Oral Pathol.1988;65:56-60.
52. Makihira S, Nikawa H, Tamagami M. Bacterial and *Candida* adhesion to intact and denatured collagen in vitro. Mycoses. 2002;45: 389-92.

53. Douglas LJ. Adhesion of pathogenic *Candida species* to host surfaces. Microbiol. Sci.1985; 2:243-7.

54. Edgerton M, Scannapieco FA, Reddy MS, Levine MJ. Human submandibular-sublingual saliva promotes adhesion of *Candida albicans* to polymethylmethacrylate. Infect Immun.1993;61:2644-52.

55. Cannon RD, Nand AK, Jenkinson HF. Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite. Microbiol. 1995;141:213-9.

56. OSullivan JM, Cannon RD, Sullivan PA, Jenkinson HF. Identification of salivary basic proline-rich proteins as receptors for *Candida albicans* adhesion. Microbiol. 1997;143:341-8.

57. Moreira D, Spolidório DM, Rodrigues JA, Boriollo MF, Pereira CV, Rosa EA, Hofling JF. *Candida spp.* biotypes in the oral cavity of school children from different socioeconomic categories in Piracicaba-SP, Brazil. Pesqui Odontol Bras. 2001;15(3):187-95.

58. Berman J, Sudbery PE. *Candida albicans*: a molecular revolution build on lessons from budding yeasts. Nat.Rev. Genet. 2002;3:913-30.

59. Yang YL. Virulence factors of *Candida species.* J. Microbiol Immunol Infec. 36,223-228.

60. Mariana H, Joana A, Rosário O. Adhesion of *Candida albicans* and *Candida* dubliniensis to acrylic and hydroxyapatite. Colloids Surf. B Biointerfaces 2004;33:235-41.

61. Wetzel WE, Hanisch S, Sziegoleit A. The germ colonization of the oral cavity in small children with the nursing bottle syndrome. Schweiz Monatsschr Zahnmed 1993; 103:1107-12.

62. Sen BH, Safavi KE, Spångberg LS. Colonization of *Candida albicans* on cleaned human dental hard tissues. Arch oral Biol. 1997;42:513-20.

63. Nikawa H, Yamashiro H, Makihira S. In vitro cariogenic potential of *Candida albicans*. Mycoses 2003;46:471-8.

64. Ugun-Can B, Kadir T, Akyuz S: Oral *Candidal* Carriage in children with and without dental caries. Quintessence Int 2007;38:45-49.

65. Thein ZM, Samaranayake YH, Samaranayake LP. Effect of oral bacteria on growth and survival of *Candida albicans* biofilm. Arch Oral Biol 2006;51:672-80.

66. Maijala M, Rautemaa R, Jarvensivu A, Richardson M, Salo T, Tjaderhane L. *Candida albicans* does not invade carious human dentine. Oral Dis. 2007;13:279-84.

67. Raja M, Hannan A, Ali K. Association of oral *Candidal* carriage with dental caries in children. Caries research 2010;44(3):272-6.

68. Thylstrup A. How should we manage initial and secondary caries? Quintessence International.1998;29(9):594-8.

69. Megan LF, Marlise IK, Punsiri MC, Kathleen SA, Stacy G, Pai CH. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes the virulence of plaque-biofilms in vivo. Infection and Immunity 2014 Feb.