

# PRESENCE OF CANDIDA SPECIES IN SUPRA-GINGIVAL DENTAL PLAQUE AND CARIOUS DENTINE AT CHILDREN WITH EARLY CHILDHOOD CARIES

PRISUSTVO CANDIDA VRSTA U SUPRA-GINGIVALNOM DENTALNOM PLAKU I KARIOZNOG DENTINU KOD DJECE S KARIJESOM RANOG DJETINJSTVA

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## ABSTRACT

**Aim:** The aim of this study was to assess the presence of *Candida* spp. in different stages of early childhood caries development, with special emphasis on the dentin matrix invasion and destruction momentum.

**Methods:** The sample for this study comprised of sixty-one 3-6 years old children. Control group consisted of 30 caries free children, whereas experimental group consisted of 31 patients, diagnosed with advanced early childhood caries (ECC).

Plaque was collected from surfaces of intact enamel both from caries free and from children with caries. From the later group plaque was also collected from the surfaces of white-spot lesions. Two dentine samples were also obtained: sample of necrotic dentin from the central part of the lesion and a sample of partially demineralised dentin from the advancing front of lesion.

Microbiological analysis of samples was done by cultivation using Sabouraud agar and API identification systems (API *Candida*)

**Results:** *C. albicans* was predominant species; *C. krusei* and *C. galabrata* were also identified in samples of ECC affected children. In plaque samples, *Candida albicans* was found in 10% of caries free children. In children with ECC *Candida albicans* was found in 16,1% of intact enamel plaque samples, 22,6% of white spot lesion plaque samples, in 61,3% of necrotic dentin samples and 48,3% of partially demineralised dentin samples.

**Conclusion:** This study showed that there is an association of *Candida albicans* with the development of early childhood caries, which suggests that it has an important role in its aetiology.

**Key words:** *Candida albicans*, *Candida* species, early childhood caries, children

## SAŽETAK

**Cilj** istraživanja je bio ispitati učestalost *Candida* specijesa u različitim stadijima razvoja lezije karijesa ranog djetinjstva, s posebnim naglaskom na momenat invazije i destrukcije dentinskog matriksa.

**Metod:** Istraživanje je realizirano na uzorku od 61 (šezdeset jednog) ispitanika od tri do šest godina. Kontrolnu grupu činilo je 30 pacijenata s intaktnom denticijom, a eksperimentalnu grupu, koja je brojala 31 pacijenata, djeca sa uznapredovalim karijesom ranog djetinjstva.

Uzorci supragingivalnog plaka sakupljeni su sa intaktne cakleni kontrolne i eksperimentalne grupe, te sa kredasto-bijele lezije djece sa karijesom. Također su uzeti uzorci karioznog dentina centralne nekrotične zone i djelomično demineraliziranog dentina iz napredujućeg dijela lezije.

Mikrobiološka analiza uzoraka vršena je: kultivacijom na Sabouraud agaru, te identifikacijom specijesa na API *Candida* sistemu.

**Rezultati:** *C. albicans* je bila dominantan specijes u svim uzorcima, dok su *C. krusei* i *C. galabrata* izolirane samo u uzorcima karijesne lezije. U uzorcima plaka zdrave djece *C. albicans* je identificirana u 10% uzoraka, intaktne cakleni eksperimentalne grupe u 16,1%, a plaka bijele mrlje u 22,6% uzoraka. U uzorcima nekrotičnog dentina učestalost je bila 61,3%, te 48,3% u djelomično demineraliziranom dentinu.

**Zaključak:** Studija je pokazala da postoji asocijacija *Candida* specijesa, posebno *Candida albicans*, s razvojem karijesa ranog djetinjstva, što sugerira da ona ima važnu ulogu u njegovoj etiologiji.

**Glavne riječi:** *Candida albicans*, *Candida* species, karijes ranog djetinjstva, djeca

## Introduction

*Candida* species are common commensals of the mouth, which, under certain circumstances can opportunistically overgrow and cause acute or chronic forms of oral candidiasis, and furthermore, can spread through the bloodstream or upper gastrointestinal tract leading to systemic infection.

*Candida* spp. is able to colonize several surfaces of the oral cavity including the tongue, palate, buccal mucosa and hard surfaces of teeth. These species can be included among the components of dental plaque and are present in saliva [1].

A number of factors may cause the presence of *Candida* spp. in the mouth: birth infection, maternity hospital, bottle feeding, infected pacifiers, maternal skin, air, water and carious lesions [2]. Although *Candida albicans* is the most frequent fungal species in humans, other *Candida* species are also considered to be of clinical interest, such as: *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata* and *C. lusitaniae* [3]. In recent years, correlation between high prevalence of *Candida* spp. in supra-gingival plaque and the development of carious lesions became a subject of an increasing interest of clinical and microbiological research. A certain number of these studies have attempted to establish the significance of *Candida* spp. in the aetiology of early childhood caries (ECC), which is a chronic infectious oral disease with an early onset and rapid progression affecting toddlers and young preschool children.

A study by Marchant et al. demonstrated the frequency of *C. albicans* isolation of 89% in carious dentin of ECC, and at the same time, only 7% in biofilm of caries free children [4].

Similarly, a study of Akdeniz et al (2002) found that 69 % of children with caries and 5% of caries-free children were found to be *Candida* carriers [5].

According to the findings of de Carvalho et al., high frequencies of *Candida* spp. in supragingival plaque and dentine caries lesions in children with early childhood caries were recorded. In plaque samples *C. albicans* was present in 50% samples, *C. tropicalis* in 16.7% and *C. krusei* in 4,2%, whereas in dentine samples these species were identified in 70,8%, 4,2% and 8.3% samples respectively [6].

Ghasempour M et al, in their study performed in order to determine prevalence of *C. albicans* in dental

plaque and carious dentine of proximal and cervical lesions of early childhood caries, reported that this yeast was present in 20% of plaque samples and 60% of dentine samples of proximal lesions and in 80% plaque and 100% of dentine of cervical lesions as opposed to only 15% presence in plaque of caries free children [7].

In addition to studies reporting the frequency of occurrence of *Candida* spp. in plaque and infected dentine of ECC, some in vitro studies are revealing other possible cariogenic properties of these yeasts.

Back in 1986, results of the study of Samaranayake LP et al. showed large amount of acid production of oral isolates of *Candida albicans* and *Candida glabrata* in glucose supplemented saliva, mainly pyruvates and acetates, which lowered the pH of saliva down to a value of 3.2 [8]. Nikawa et. Al. demonstrated that *C. albicans* possesses the ability to dissolve HAP to a greater extent (approximately 20-fold) when compared with *S. Mutans* [9]. Additionally, in the study of Kinke et al., *Candida albicans* showed high acid tolerance secreting acid in significant quantities at pH 4.0, in contrast to acidification by *S. Mutans* which ceased at pH 4.2 [10].

Also there is strong evidence that *C. albicans* might favour the in vitro adherence of *S. mutants* in the dental biofilms, thus favouring its colonization [11].

The aim of this study was to assess the presence of *Candida* spp. in different stages of early childhood caries development, with special emphasis on the dentin matrix invasion and destruction momentum.

## Materials and methods

The sample in this study consists of 61 (sixty-one) children, aged three to six years, regular patients of the Clinic of Children and Preventive Dentistry, Faculty of Dentistry Sarajevo. Of the total number of patients control group comprised 30 caries free children (dmft = 0). The experimental group consisted of 31 patients, diagnosed with early childhood caries according to AAPD criteria [12]. The parents of patient received information about the objectives of the investigation and signed information consent form.

Inclusion criteria were: good general health, cooperative patient and presence of at least one carious lesion extending to the inner half of the dentine layer.

Children did not take antibiotics at least two weeks before study, nor were they treated with fluoride during the same period. All clinical work and samplings were done by one investigator.

Clinical examination for each child was performed in order to establish caries status, after which 24-hour-old supra-gingival plaque was harvested. To be able to do that, parents were instructed not to brush their children's teeth the night before examination. Children additionally refrained from taking any food and drink in the morning prior to plaque samples collection. Samples were pooled from a minimum of three sites (occlusal, vestibular, palatal), including anterior and posterior teeth using sterilized excavator. Plaque was collected from surfaces of intact enamel, both from caries free and from children with caries. From the later group, plaque was also collected from surfaces of white-spot lesions. Each sample was suspended in a sterile tube containing 1000µl sterile saline solution.

For the experimental group of children, two samples of carious dentin were also obtained. First of them, necrotic dentin sample, was collected at the centre of the lesion with sterilized small excavator. The second sample of partially demineralised dentin from the advancing front of lesion was collected with another sterile excavator, after all necrotic dentine from the cavity walls and most of it over the pulp was excavated by a sterile burr. Following sampling, remaining infected dentin was removed; cavity was lined with calcium hydroxide and restored with glass ionomer cement. Prior to cavity preparation local anaesthesia was administered.

Dentine samples were suspended in sterile tubes containing 5ml of Thioglycollate Broth and together with the plaque samples transported immediately to laboratory for microbiology analysis. Sabouraud agar was used as the primary culture medium.

Plaque samples in sterile saline solution were stirred using vortex mixer and spread on Sabouraud agar plates the same day, whereas dentine samples were incubated in Thioglycollate Broth at 37°C for 24 h prior to inoculation [6].

Inoculated plates were incubated for 48 hours at 37°C. Further species identification was done by means of Api Candida identification system (API 20C AUX, bioMérieux). The Api Candida consists of 10 tubes containing dehydrated substrates which enab-

le performance of 12 identification tests mostly relying on sugar acidification or enzymatic reactions. The strips are read after 24 and 48 hours of incubation at 30°C. A four digit numerical profile is obtained which is compared with those in a database with 26 yeast species [13].

## Results

A total of 61 children were included in the study. Out of them, control group (caries free children) consisted of 30 children, 14 females and 16 males, mean age of  $60,4 \pm 12,0$  months. Experimental group (ECC) comprised 31 subjects, 15 females and 16 males. Their mean age was  $54,5 \pm 12,7$ . There was no significant difference between the groups in terms of age (T test = 1.936; p = 0,058).

The most prevalent species identified in all of the sampling sites was *Candida albicans*. *C. krusei* and *C. galabrata* were not detected in any of the plaque samples of intact enamel both in control and experimental group, and, additionally, none of the white spot lesion plaque sample harboured *C. galabrata*. (Figure 1.)

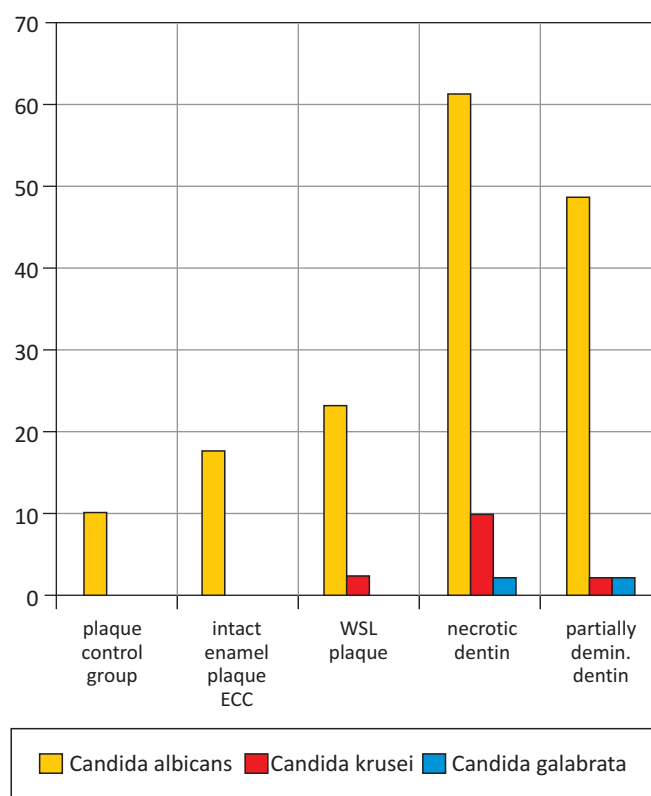


Figure 1.

Frequency of *Candida* species isolation according to sampling site

	GROUP			
	caries free (n=30)		early childhood caries (n=31)	
	%	n	%	n
Candida albicans	10	3	16,1	5 <sup>ns</sup>
Candida krusei	nd	nd	nd	nd
Candida galabrata	nd	nd	nd	nd

<sup>ns</sup> : values did not differ significantly (t test, p>0,05)  
 nd: not detected

**Table 1.**  
 Frequency of Candida species in supragingival plaque of intact enamel

	GROUP					
	white spot lesion (n=31)		necrotic dentin (n=31)		partially demineralised dentin (n=31)	
	%	n	%	n	%	n
Candida albicans	22,6	7	61,3	19*	48,3	15 <sup>ns</sup>
Candida krusei	3,2	1	9,7	3 <sup>ns</sup>	3,2	1
Candida galabrata	nd	nd	3,2	1	3,2	1

<sup>ns</sup> : values did not differ significantly (t test, p>0,05)  
 nd: not detected  
 \*: values differed significantly (t test, p<0,05)

**Table 2.**  
 Frequency of Candida species in plaque and dentin samples of carious lesion

C. albicans was isolated in 10% plaque samples of the control group and in 16,1% plaque samples of the intact enamel of the experimental group. (Table 1.)

The frequency of isolation of C.albicans in white spot lesion plaque, necrotic dentin from central part of the lesion and partially demineralised dentin from advancing part of the lesion was 22,6 %, 61,3% and 48,3% respectively. (Table 2)

The frequency of C.albicans in necrotic dentin was significantly greater than in the white spot lesion plaque (t= 3.303; p=0,002)

## Discussion

During the 80's and 90's of the last century, growing research interest on microflora of ECC was evident. Most of these studies focused on mutants Streptococci and Lactobacillus spp., although other microorganisms were isolated as well.

As previously mentioned, Marchant et al. found a high frequency (89%) of Candida albicans isolation in carious dentin of children affected with ECC [4]. Radford et al. showed in their study that Candida spp. were isolated more frequently from the saliva of infants with caries compared to those who were caries-free (23.7 vs. 10.4%) [14].

Study of de Carvalho et al. reported that Candida species frequency was significantly higher in plaque and dentine samples of children with ECC than in caries free children and in children with common

type of caries. Furthermore, C.albicans was the most prevalent Candida species in ECC group, in which C. krusei and C. tropicalis were also detected, but not in caries free and common caries type group [2]. Ghasempour et. al., determined the presence of C. albicans in 80% samples of plaque of cervical lesions in 2-5 year old children and only in 15% samples of caries free children of the same age. C. albicans was present in all of the dentine samples of cervical lesions (100%) [7]. Strong association of C. albicans and early childhood caries was seen in the study of Srivastava B. et al. Prevalence of the C. albicans was significantly higher in the group of caries affected children with deciduous dentition compared to permanent dentition group [15].

In our study, the frequency of isolation of Candida spp. in supra-gingival plaque and carious dentine samples in children suffering from early childhood caries has been analyzed and compared with those of caries-free children. In plaque samples of intact enamel of both ECC group and caries free children, C. albicans was isolated, but there was no statistical difference in frequency of isolation between groups. C. krusei and C.galabrata were not isolated in neither of the groups. As stated above, in the study of de Carvalho et al., the only Candida species isolated from plaque of caries free children (intact enamel) was C. albicans which is in accordance with our results [2].

Affected enamel plaque samples in our study harboured C. krusei and C. galabrata, whereas C. krusei and C. tropicalis were found in the same samples of de

Carvalho study [2]. This difference might be due to cultivation and identification method.

When analyzing isolation frequency in plaque and dentine samples of established lesion in ECC group (white spot lesion, necrotic dentin from central part of the lesion, partially demineralised dentin from advancing part of the lesion), it was found that the predominant species identified in all samples, again, was *C. albicans*. It is worth noting that significantly higher frequency of *C. albicans* presence was observed in necrotic dentine than in the plaque of the white spot lesion, which suggests that this yeast could have a more important role in the progression of caries lesion, rather than in its initiation.

This could be explained by the fact that *C. albicans* produces collagenolytic enzyme, i.e., has proteolytic activity for type I collagen and can adhere to the intact and denaturated collagen exposed from dentin through different mechanisms [9, 16, 17, 18].

Several other studies showed that *C. albicans* possesses additional capabilities, which may propose its significant role in dental caries pathogenesis.

Cannon RD et al. reported that *Candida* is able to adhere to saliva-coated hydroxyapatite. [19] Salivary component that contains the proline-rich proteins provides receptors for adhesion of *C. albicans* to enamel pellicles [20]. These proteins can be absorbed to streptococcal surfaces and thus enhance the adhesion of *C. Albicans* [21].

It has been shown that culture media that are rich in carbohydrates such as glucose, sucrose and particularly galactose, increase the adherence of *C. albicans* to surfaces [2]. This is clinically important because children affected with ECC are mostly fed with sucrose added milk formulas that contain lactose which degrades to galactose and glucose. This carbohydrate rich diet may lead to increased colonization of *C. albicans*. In addition, due to host defence factors, e.g. immature immune system and not completely established commensal microflora, the infants are more susceptible to opportunistic microorganism colonization in the oral cavity [2].

Taking into consideration these specificities of infant and early preschool age, and already mentioned facts that *Candida* is able to produce acids, dissolve hydroxyapatite and possesses high acid tolerance [8, 9, 10] it is reasonable to speculate that *Candida* can highly contribute to the caries disease process.

## Conclusion

The results of our study on the prevalence of *Candida* spp. in early childhood caries lesions, as well as data presented in recent literature, support the important role of *Candida albicans* in the progression of this disease. Since the role of *C. albicans* in carious process is for certain the result of contribution of the several factors, it is necessary that the association of these factors is investigated in future studies.

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