Association between *Candida species* and caries index in children

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Abstract

Background: *candida species* is one of the most important opportunistic oral fungal flora that contributed to oral candidal infections. Recently some studies indicate the association between increased caries incidence in children with oral candidal carriage.

Objective: To candida species is one of the most important opportunistic oral fungal flora that contributed to oral candidal infections. Recently some studies indicate the association between increased caries incidence in children with oral candidal carriage.

Patients and Methods: Sixty-one children with ages 6 to 12 years , DMFT, dmft, and OHI-S were measured and oral swabs were tested on three different surfaces (lingual, occlusal, and buccal) then cultured on SDA as a primary medium, and selective medium on CHROMagar, also germ tube test was done and for more precision, pure colonies were identified up to species based on their carbohydrate assimilation pattern by the VITEK 2 compact

Results: 22 (36.1%) children with candida carriage, C.albicans 12, (54%), C.dubliniensis 4,(18.18%), C.krusei 4, (18.18%), C. tropicalis 2, (9.1%). There was no association between candidal species in the oral cavity and caries index (DMFT, dmft).

Conclusion: Non-significant weak negative correlation appeared between candidal species in the oral cavity and caries index in children. The most predominant candidal species was *C.albicans.*

Keywords: *Candida, species*, DMFT, dmft. OHI-S, pedodontics

Introduction

The oral microbial flora is described as all microorganisms (bacteria, yeasts, viruses, and protozoa) that are found on or in the human oral cavity, and involve various separate habitats, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard palate, and soft palate with its contiguous extensions such as those on the tonsils and pharynx [1] these microorganisms live as commensal or as competitors [2]. In addition to bacteria, *Candida species* are also common oral cavity colonizers of healthy people with nearly 50% this commensal genus, Candida carriage rate observed in this population [3]. Oral *Candida species* have ability to connect to a different of host cell receptors through lectin-like and protein-protein form interactions and able to coaggregate with oral streptococci denoting that candidal cell participate to the growth, constancy, and embalmment of oral mixed microbial communities, and have an

important role in maintaining the balance of the oral microbial environment [4]. Candida species are included in the main opportunistic yeast infection in the world, but among the species of this genus, *C.albicans* continues to be the most common and although this yeast is responsible for approximately 50-90% of human yeast infections, is part of the commensal flora of more than half of the healthy population [5]. *C.albicans* is characterized by numerous virulence factors such as adherence, secretion of proteinases, the dimorphic transition from yeast-to-hyphal form, and production of biofilm [6]. Recently studies have focused on the presence of this fungus in individuals with oral diseases like periodontal disease [7]. In addition to dental caries [8]. But few studies have analyzed the diversity of candida species in children with tooth decay experiences, the interrelation between oral bacteria and fungi, and the roles they play in health and disease [9]. Dental caries or tooth decay is the most universal human chronic disease that advances slowly and is distinguished by localized and immutable demolition of the tooth [10] [11]. Dental caries is defined as a bacterial main cause, chronic, and multifactorial, that results from the imbalance between tooth mineral and cariogenic plaque bacteria in the physiologic equation, that when pH decreases, causes mineral loss over time [12]. The main cause of dental caries is plaque cariogenic microorganisms, which result in disturbance among acidogenic and aciduric populations with the oral environment and other plaque species, which leads to increased ensuing high-frequency carbohydrate disclosure [2]. Although dental caries is recognized as a bacterial cause (Streptococcus mutans and Lactobacillus), some studies have recently explained the association of candida species [13]. The coadhesion between C.albicans and oral bacteria in the oral cavity is crucial for C.albicans colonization and persistence [11]. Many studies focus on the role of Candida albicans coaggregation with *S. mutans* during adherence to the dental surface [14]. Several in vitro studies have pointed out that *C.albicans* enhance the adherence of *S. mutans* demonstrating a possible simplification mechanism during their relationship where the yeast cell could be used by the bacteria as support for adherence [15]. The purpose of this study was to find an association between *Candida species* and decayed, missing, and filled teeth in children and determination of most predominant Candida species in children

Patients and Methods

This cross-section study was approved by the scientific committee of the basic science department, college of dentistry, Hawler medical university. Sixty-one children (boys and girls) in the mixed dentition (age range 6 to 12 years), were tested for oral candidal carriage and they were referred to the department of POP (pedodontic-Orthodontic-Preventive), during three months period (March 2022- May 2022), and whose parents agreed to participate in the study. It was a blind study the microbiologist was uninformed of the identities of the samples, the samples were collected from children who visited the POP department by specialist prevention and then sent to the microbiology lab for culturing and identification. The inclusion criteria were healthy children aged between 6-12 in the mixed dentition stage.

the exclusion criteria were taking antimicrobial treatment permanently , chronic diseases like diabetes, thalassemia, and any condition that affect salivary flow.

Study design

An information form was used to record data on each child including age, gender, and dental examination. Caries experience was determined by using (DMFT; Decay, Missing, and Filling Teeth for permanent and dmft; for primary teeth) [16].The participants were evaluated by the oral hygiene index surface (OHI-S) [17] and divided into three groups (good, fair, and poor oral hygiene with OHI-S scores of 0-0.9, 1-1.9, 2-6 respectively), a specialized dentist examined the children for OHI-S and dental caries using WHO criteria, which is measured as the sum of the number of decayed, missing due to caries, and filled, then divided by the sum of the population, and also parents were asked about nutrition (either were breastfeeding or bottle feeding).

Sampling and culturing

The sampling collection was done for each child by gently rubbing (three times across each site) a sterile cotton swab (using three disposable swabs) over the buccal, occlusal, and lingual surface of the mandible, and each swab was replaced in its tube [18] , within one hour all samples were inoculated on Sabourauds dextrose agar (SDA) (Lab M Limited, Lancashire, United Kingdom) as a primary medium and incubated under the aerobic condition at 37℃ for 48-72 hrs. [13]. After the incubation, a diagnosis of Candidal growth as creamy convex colonies were done. The germ tube formation test was tested by culturing a single candidal colony with human serum at 37℃ for 2 h, as primary

identification for *C.albicans* and C. dubliniensis. A presumptive identification based on the characteristic colony colors of candida colonies was done by subculturing aerobically at 37℃ for 24 h on CHROMagar Candida (75006 Paris, France) [19,20]. For more precision pure colonies were identified up to species based on their carbohydrate assimilation pattern by the VITEK 2 compact (biomerieux, France) yeast identification system [21,22].

Statistical Analysis

The sample size was chosen by G-power V3, the power was set at %80, the effect size at 0.2, and the alpha 0.05 . The data were tested for normality tests and nonparametric data set spearman, correlation test was applied between year class and caries indices. Descriptive statistical outputs were expressed as mean and stander deviation. The statistical analysis was done by using Spss version 25, and the P<0.05 was set for statistical differences [23].

Results

Sixty-one (children 31 males and 30 females 6 to 12 years of age) visited the Dentistry college, and POP department for three months. The oral candidal growth was as follows: 63.9% (39 children) with no growth and 36.1% (22 children) with candidal species positive. The results of Candida spp oral swabs on SDA agar were as follows,14 (45.2%) males and 8 (26.7%) females were in candida carriages. *Candida colonies* were showed white to creamy, smooth, and convex colonies Figure (1a). Germ tube formation after 2 h incubation of candidal colony with serum Figure (1b). Growth in CHROMagar was shown in Figure (2).
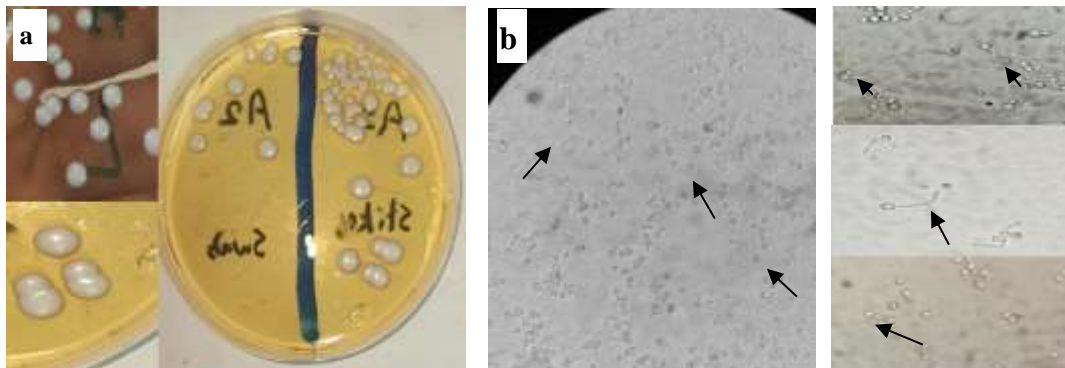
Figure(1): *Candida spp* **(a)** colonies of *Candida sp.* on (SDA) Sabourauds dextrose agar at 37℃ for 48-72 hrs. **(b):**(The arrows) Germ tubes of *C.albicans* in serum after two hours at 37℃. X 400
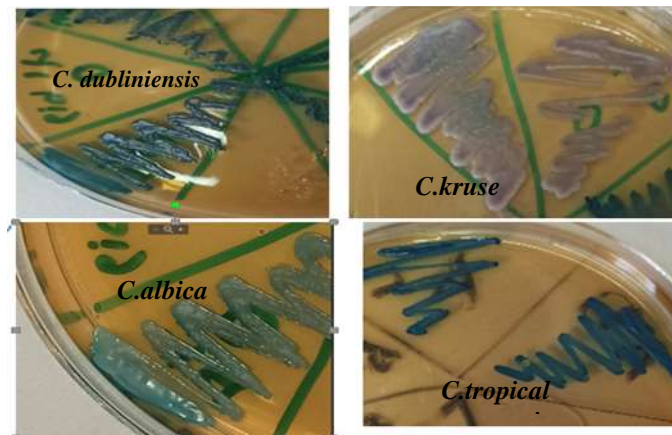

Figure (2): Different Candida sp. growth on CHROMagar at 37℃ for 24 hrs

The results of different oral Candidal species were *C.albicans* 12, (54%), *C.dubliniensis* 4,(18.18%), C.krusei 4, (18.18%), *C. tropicalis* 2, (9.1%) as shown in Figure (3). *C.albicans* was the most prevalent yeast and found in all age classes, significantly more boys had a positive growth of *C.albicans* than girls, 9(75%) males, and 3(25%) females. A large number of *C.albicans* were found in 8-9 years an average of three males and one female. The highest DMFT mean±SD was 3.60 ± 3.977 found in the 10-11 years' average and the highest dmft mean was 9.40 ± 6.931 found in the 6-7 years' average, Table (1) provides an overview of the relationships among age, gender, DMFT and dmft (mean± SD) and candidal species distribution.
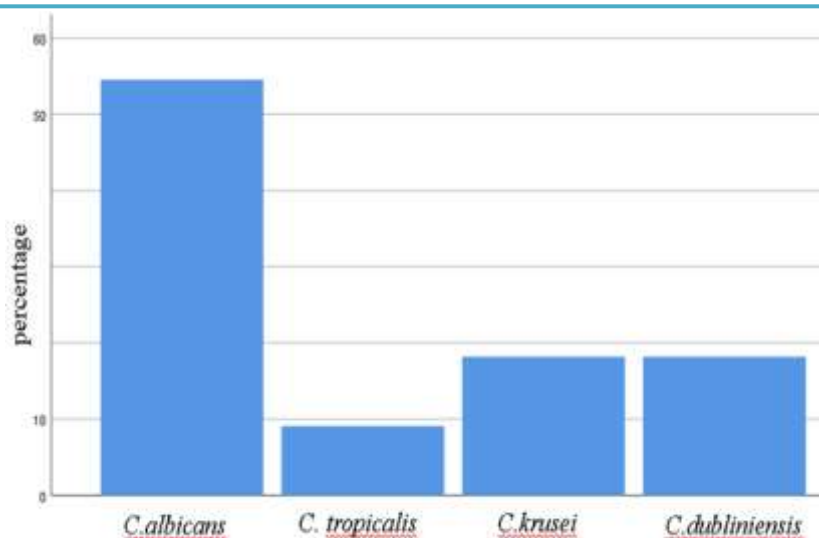
Figure (2): The percentage distribution of Different Candida sp. isolated from oral cavity

Total negative and positive results of Candidal species in the male and female oral cavity Table (2). Figure (3) shows a relationship between age classes and means of (DMFT, dmft). r=o.95 indicate a strong correlation between the age group with dmft but a non-significant correlation (r=0.6) with DMFT. C.albicans was isolated from all locations (50% buccal, 41.7% lingual, 8.3% occlusal), and all species were isolated from lingual Table (3). Non-significant weak negative correlation appeared between candidal species in the oral cavity and caries index in children as noted in Table (4). 21 children were sorted as good by OHI-S, 47.6% of them with positive candida carriage, 30 children were sorted as fair, 23.3% of them with positive candida carriage and only 10 children were sorted as poor by OHI-S but 50% of them with positive candida carriage as shown in Table (5). Table (6) Reveals the relationship between a child's nutrition during infancy and isolates of Candida species which were 22 breastfeeding, 16 bottle feeding, and 23 mixes (breastfeeding, bottle feeding). A large amount of Candida carriage was found in children with a mix feeding 10 children, then breastfeeding 9 children, and finally bottle feeding 3 children.

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Table (1): DMFT and dmft (mean ± SD) with age, gender, and Candida species distribution

Age classes	N	male	female	DMFT***	dmft**	Candida	Candida species	N*
6-7 years Average	10	5	5	0.60 ± 0.966	9.40 ± 6.931	2	*C.dubliniensis*	1
							C.albicans	1
7-8 years Average	12	7	5	0.50 ± 1.243	8.00 ± 5.862	4	*C.albicans*	1
							C.dubliniensis	1
							C.krusei	2
8-9 years Average	9	5	4	2.33 ± 1.732	4.67 ± 4.242	5	*C.albicans*	4
							C.dubliniensis	1
9-10 years Average	13	5	8	2.00 ± 1.732	4.85 ± 3.337	4	*C.albicans*	2
							C. tropicalis	2
10-11 years Average	10	7	3	3.60 ± 3.977	3.30 ± 3.529	5	*C.albicans*	2
							C.krusei	2
							C.dubliniensis	1
11-12 years Average	7	2	5	1.43 ± 1.397	3.00 ± 1.732	2	*C.albicans*	2
Total	61	31	30					

* represents the number of isolated species in each positive sample.
** Decay, Missing, and Filling Teeth for primary teeth.
*** Decay, Missing, and Filling Teeth for permanent teeth.
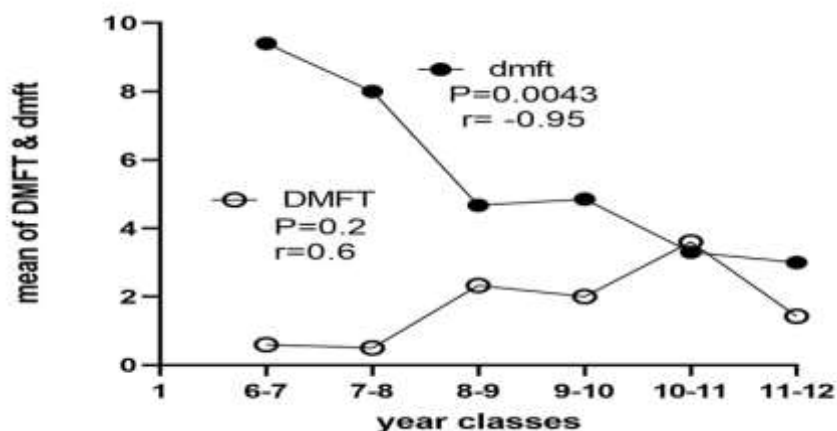
Figure (3): Spearman r correlation coefficient carves between age classes and means of (DMFT, dmft). r=o.95 indicate a strong correlation between the age group with dmft but a non-significant correlation (r=0.6) with DMFT

Table (2): Negative and positive results of Candidal species in the male and female children's oral cavity

		Gender		
		Male	Female	Total
Candida	negative	15	24	39
	positive	16	6	22
Total		31	30	61

Table (3): Isolation of Candidal sp. from different locations

Candidal sp.		buccal	lingual	occlusal
C.albicans	12 (54%)	6	5	1
C. tropicalis	2(9.09%)		2	
C.dubliniensis	4(18.18%)	1	3	
C.krusei	4(18.18%)	3	1	

Table (4): Spearman: non-significant correlation coefficient (r and p-value) between Candida sp. and DMFT, dmft

	r	P value
dmft	-0.23	ns
DMFT	-0.25	ns

Table (5): Candidal carriage results with the Oral Hygiene Index Surface (OHI-S) scores

		Candida		Total
		negative	positive	
OHI-S.	Good	11	10	21
	Fair	23	7	30
	Poor	5	5	10
Total		39	22	61

Table (6): the relationship between Candida prevalence and a child's nutrition during infancy

		nutrition			Total
		breast	bottle	mix	
Candida	negative	13	13	13	39
	positive	9	3	10	22
Total		22	16	23	61

Discussion

An initial objective of the current study was to identify the association between candida carriage in school children. However, the result of this study did not show a clinically significant association between the studied variables that augmented to accept the null hypothesis furthermore based on the selected sample size and choosing the criteria range for the case ages we see that these factors to some extent contribute to the study findings. In the current study, the higher frequency of Candida carriage was recorded in lingual surfaces (50%), and buccal surfaces (45.5%). This result may explain the strong bond of *Candida sp* with this surface, but just (4.5%) of the buccal surface, may be due to mastication processes that clean the surface of the occlusal. *C. albicans* was the most predominant strain. this result was similar to those recorded by other studies [13, 24, 25] the output of overall Candida sp was 36.1% this result is somewhat lower than found in a previous study, which showed a prevalence of 70.5% among 6 to 14.5 years [25] but it is

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same as found in another study, which demonstrated a prevalence of 30% to 50% from children 3 to 12 years old [26]. The results of our study show no statistically significant correlation between *Candida sp.* and caries index (dmft, DMFT), and the same results found in other studies [13, 25, 27] several reasons may explain this result: the children knew about visiting dentistry clinic, could have enhanced their tooth brushing capacity, which explains that 10 children with poor OHI-S. 50% of them with *Candida carriage*, also in some studies they explain that growth interaction between cariogenic bacteria and C.albicans during the early stages of biofilm formation by inhibiting hypha formation [15], maybe the sample's quality impact the results since children's family were in educated level and such studies need different types society levels, and also the sample quantity was not enough to obtain a proper result. more investigation is necessary to find the association between oral *Candida sp.* and dental caries in school children because most studies focus on preschool children as they found a strong relationship between *Candida sp.* and dental caries, especially in early childhood caries [3, 28, 29]. However, this role appears to be investigated. Recent studies in vitro have shown that C.albicans prevent caries [30, 31]. Although OHI-S showed no correlation with candida carriage in school children, a similar result was also done in preschool children [32].

Conclusions

Candida albicans was the most predominant candidal species that isolated from the oral cavity in the selected ages, with no statistical relation between *Candida sp.*

and caries index (dmft, DMFT). This research has thrown up many questions in need of further investigation, it seems to be clear that such association candida and caries index are multifactorial because of the cultural and social backgrounds of selected cases. It would be interesting to assess the effects of the educational, social, and cultural factors in developing dental caries in school children.

Recommendations

We recommend other investigations to be done in such ages (school children) and a large number of data for further understanding of the relationship between candida species and cariogenic bacteria in the oral cavity, to find the best way to prevent caries in children.

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Ethical clearance: Our study was accepted and obtained by the medical cure committee of the College of Dentistry, Hawler Medical University.

Conflict of interest: Nil

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الارتباط بين انواع خميرة المبيضات(الكانديدا) و مؤشر التسوس عند الاطفال

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الملخص

خلفية الدراسة: تعتبر فطر كانديدا (المبيضات) من أنواع آلفطر ألانتهازية الموجوده داخل فم الانسان حيث يسبب الاصابات الفم الفطريه. تشير بعض الدراسات مؤخراً إلى الارتباط بين زيادة حدوث التسوس عند الاطفال وزيادة وجود فطر كانديدا (المبيضات) الفموي.

اهداف الدراسة: لايجاد العلاقة بين انواع فطر كانديدا (المبيضات) الفموية عند الاطفال ؤعلاقتها مع تسوس الاسنان . ولتحديد اكثر انواع السائدة من اصناف المبيضات.

المرضى والطرائق: ٦١ طفل ضمن عمر (١٢-٦)سنة تم فحصهم في قسم الاطفال كلية طب الاسنان جامعة هولير الطبية في اربيل كوردستان العراق وتم قياس موشر التسوس من خلال موشر التسوس المفقودالحشوة من الاسنان الدائمية DMFT والاسنان اللبنية.dmft وتم قياس موشر صحة الفم البسيط OHI-S ، تم اخد مسحات فموية من اسطح مختلفة من جهة الاطباق وجهة الخد الفحوى وجهة اللسان وتم زرع العينة في وسط SDA كوسط اولي . ثم تم اختيار وسط اختياري وسطي فيCHROMagar اختبار انبوب الجرثومية واجرائه لاكثر دقة ونقاء للمستعمرات لتحديد العينات التي يعتمد عليها الكاربوهيدرات ونمط الاستيعاب عن طريق VITEKS 2 المدمج.

النتائج: : ٢٢ (٣٦,١) % (من الاطفال لديهم فطر كانديدا (المبيضات) 12 C.albicans و (٥٤%)4 C.dubliniensis ،(١٨,١٨%) ، (9.1%) C. tropicalis 2, ، C.krusei 4(18.18%)) لا يوجد علاقة بين انواع فطر كانديدا (المبيضات) في التجويف الفموي ومؤشر التسوس (DMFT, dmft)

الاستنتاجات: ظهر ارتباط ضعيف غير معنوي بين انواع المبيضات في تجويف الفموي ومؤشر تسوس الاسنان في الاطفال. الاكثر انتشارامن نوع فطر كانديدا (المبيضات) كان C.albicans .

الكلمات المفتاحية: فطر كانديدا (المبيضات).   DMFT, dmft مؤشر التسوس في الاسنان الدائمية واللبنية. ومؤشر صحة الفم البسيط OHI-S، طب اسنان الاطفال

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