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The Role of *Candida* Species in the Occurrence of Dental Caries in Thi-Qar Governorate

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***Abstract*— Dental caries is a multifactorial disease associated with many factors , such as cariogenic microorganisms. *Candida* is associated with early colonization of cariogenic , leading to dental caries. The current study aims To isolate and diagnose *Candida* species from caries lesions and the surrounding dental plaque concerning concernin dental caries. This study was conducted from July 2021 to December 2022 in the Thi-Qar governorate. Samples were collected from 100 patients using sterile procedures for ages 4 to 65 years for both genders for various factors such as daily intake of sweets, daily brushing of teeth and smoking Eighty-three *Candida* isolates of six species were isolated, *C. albicans* was 44, *C. glabrata* 11, *C. dubliniensis* 14, *C. tropicalis* 10, *C. krusei* 3 , and *C. utilis* 1, in all age groups and for both genders. The current study proved the existence and role of different species of *Candida* in causing caries disease, not just *C. albicans*.**

Keywords— Dental caries, *Candida* species, *Candida albicans*.

I. INTRODUCTION

The oral cavity contains diverse microbiota harboring over 700 species of bacteria [1]. Although the role of bacteria in causing caries has been determined, the role of fungi is comparatively unknown[2]. Dental caries is a chronic, diet, microbial , and site-specific disease of dental hard tissues, caused by shifts from protective factors favoring tooth remineralization to destructive factors leading to demineralization[3]. When sugars or other fermentable carbohydrates are ingested, these dental plaque PH caused by organic acids increasing the solubility of calcium hydroxyl apatite in the dental hard tissues, and demineralization occurs as calcium is lost from the tooth surface [4]. *Candida* is a normal commensal in the oral cavity and participates in complex microbial oral biofilm formation .The percentage of *Candida* species colonization ranges from 20 to 40% in healthy individuals to about 60% in Immune- compromised people,
where it becomes the predominant flora [5]. *Candida* spp. is 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 [6]. *Candida* spp. has a higher prevalence of dental caries when compared to individuals without these microorganisms in the oral cavity [7]. *C. albicans* is associated with active decay and generalized gingivitis, but whether one or both are present depends on the structure of the co-existing microbial community [8]. The appearance of *Candida* was directly related to the caries status and inversely symmetrical to the age [9]. Samples of water from the water distribution network´s pipelines in residential areas in the Thi-Qar governorate contain 27% *Candida* species , and therefore it can transfer into the oral cavity of people in that area [10].
Most of the studies focused on the role of *C. albicans* in causing dental caries. However, there are also very few and limited studies isolating the other species of *Candida* from decayed teeth. Our current study focuses on isolating and diagnosing different species of *Candida* from caries lesions and the surrounding dental plaque in relation to caries for different ages and gender and their relationship to the daily intake of sugars, the number of times brushing teeth, in addition to smoking.

II. MATERIAL AND METHODS

A. *Collection of Samples*

Samples were collected under strict sterile conditions from 100 dental patients under the supervision of dentists in Thi-Qar Governorate from July 2021 to December 2022. A standardized questionnaire was completed for each patient , including cases age, gender, geographical location, sugar consumption , and daily use toothbrush. The samples of dental caries were collected from the carious lesions and dental plaque surrounding it depending on researchers [11], with modified using the following strategy:-
1-The patient's oral cavity was cleaned frequently with distilled water using a gargle to eliminate microorganisms on or around the decayed tooth.
2- Saliva is removed and dried from the caries lesion and the surrounding dental plaque using short air blows..
3- A sterile, gracey curette scraps all dental caries samples

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4- Samples of root caries were taken from the patients in the Endodontic department, where touch with any gingivitis or abscess if existen,was avoided.
5- Touching any part of the oral cavity was avoided when taking the sample.
6- For decayed teeth that are extracted, they should be washed with normal saline to remove the blood from the tooth as well as saliva, and then the sample is collected from the area of the tooth that contains the decay.
7- Transport swab media was used to collect the sample from the gracey curette.

B. Microorganisms' Activation

All samples were cultured in brain heart infusion broth (BHI) separately at 16- 24 hours for activation of the microorganisms in samples. Sabouraud Dextrose broth (SDB) was used to activate *Candida* species that may be found and it is done by incoulating millitter from growth of each BHI broth in SDB at 37◦C for 16- 24 hours.

C. Isolation Of Yeast Species

Two selective culture media were used to isolate, diagnose, and purify different *Candida* species, where tenfold dilution of the SDB growth and 0.1 ml are cultured on Sabouraud Dextrose agar (SDA) aerobically at 37◦C for 24 to 48 hours at 30 °C [12]. Identification of specific yeast species based on the examination of the morphological features of the colonies, including the shape, color, diameter and height of the colony after its growth on SDA[13]. Part of the yeast grown on SDA was cultured on HiCrome *Candida* Differential Agar and incubated for 24-48 hours at temperature 37 ◦C [14]. This medium used to distinguish between the species of *Candida*, dependent on the color and appearances of the surface of the colony different colors. The colonies of *C. albicans* appeared in light green color, the color of the colonies of *C. tropicalis* was in blue color, while the colonies of *C. dubliniensis* gave a pale green color, the colonies of *C. krusei* grew in light red, the colonies of *C. glabrata* was cream to white while the *C. utilis* appeared in pale pink. These results were derived based on the instructions given by the manufacturer for this agar medium [15]. To confirm the identification and differentiating Candida spp., the *Candida* identification kit (Himedia-KB006) was applied. It is contain on 12 standard biochemical assays. A loopful of colonies from the SDA medium were used to prepare the inoculum, which was then seeded into sterile saline, each well of the kit received 50 µ of inoculum, which was incubated at 25°C for 24-48 hours. Results were interpreted in accordance with the criteria listed in the identification index.

III. RESULTS

In this study, different species of *Candida* were isolated and diagnosed from the caries lesions and the dental plaque surrounding them. All items and groups of patients showed varying proportions of *Candida* species isolates, indicating an essential role for most types of *Candida* in the occurrence of caries. The results of the current study may change the prevailing idea that only *Candida albicans* from the rest of the *Candida* species have a role in the appearance of caries. A total of 100 patients took part in this study, their ages ranging from 4 to 65 years , with a gender distribution of 51% male and 49% female, Their average age was 35 years. The Their ranged from (4-12) years, and their number was 42 (42%). The ages of adolescents ranged between (13-17) years with a percentage of 22 (22%), the ages of young people ranged between (18-45) years, and their number reached 27 (27%). The patients were distributed from urban areas (63/100), while from rural areas (37/100). All patients were questioned about the number of times they intake sweets per day, and it was found that the number of those who never intake sugars daily was (13/100), and those who intake sweets once (30/100), twice (27/100), three times (30/100). A total of (52/100) patients reported not brushing their teeth daily, once (37/100), twice (7/100), and three (7/100). Most patients were nonsmokers (91/100), while (9/100) were smokers **(Table I).**

Table I: Characteristics of the Survey Participants with the Candida Species Isolated.

Items			Candida isolates					
		Numbers	C.alb.	C.glabrata	C.dubl.	C.tropicalis	C.krusei.	C.utilis
Gender	female	51	25	6	10	9	2	1
	male	49	19	5	4	1	1	0
Age	4-12	42	25	9	3	2	2	1
	13-17	22	9	1	3	3	0	0
	18-45	27	9	0	7	4	1	0
	46-65	9	1	1	1	1	0	0
Location	Urban	63	28	6	8	6	1	1
	Rural	37	16	5	6	4	2	0
Sugar intake (daily)	0	13	2	1	1	1	0	0
	1	30	7	0	4	2	1	1
	2	27	14	2	5	2	0	0
	3	30	21	8	4	5	2	0
TBT (daily)	0	52	27	9	7	4	1	0
	1	37	13	2	6	5	1	1
	2	7	1	0	1	1	1	0
	3	4	3	0	0	0	0	0
Smoking	Yes	9	3	0	2	2	0	0
	No	91	41	11	12	8	3	1

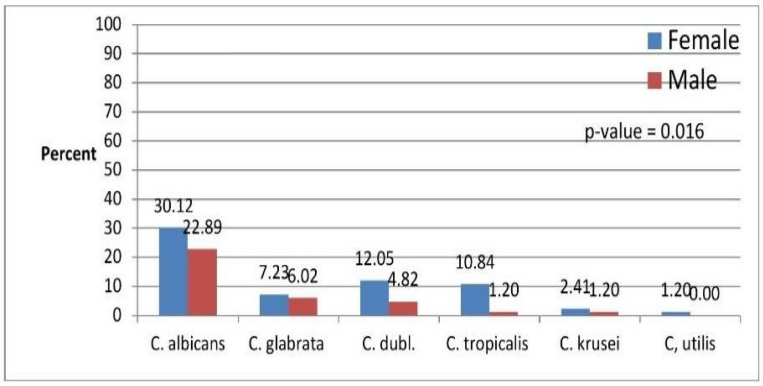

Fig.1. Relationship of oral Candida species growth with gender (individual's chi-square, *P*=0.016).

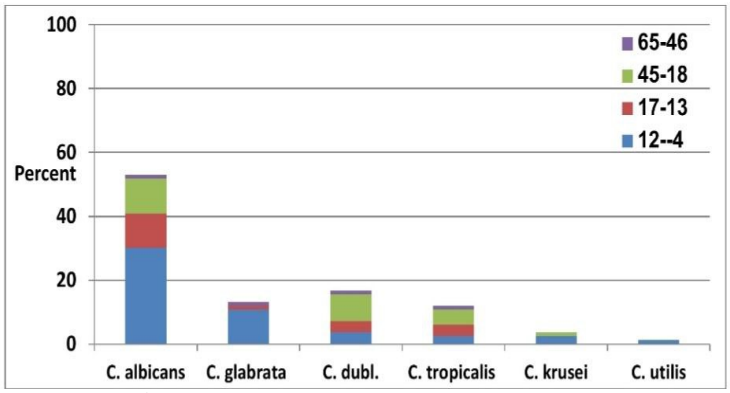

Fig.2. Relationship of oral Candida species growth with age (individual's chi-square, *P*=0.03).

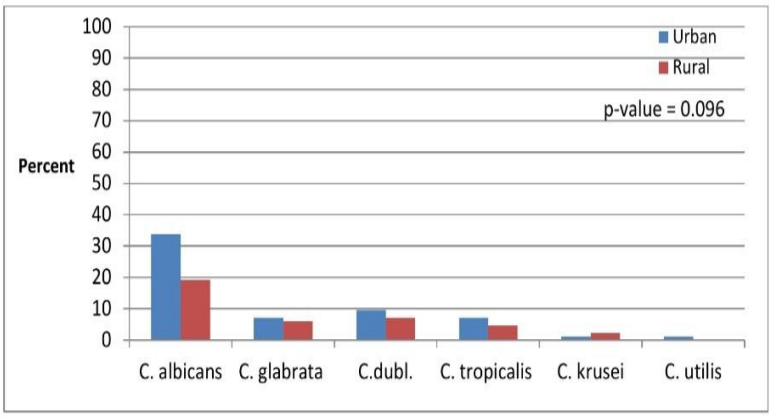

Fig.3. Relationship of oral Candida species growth with location (individual's chi-square, *P*=0.096).

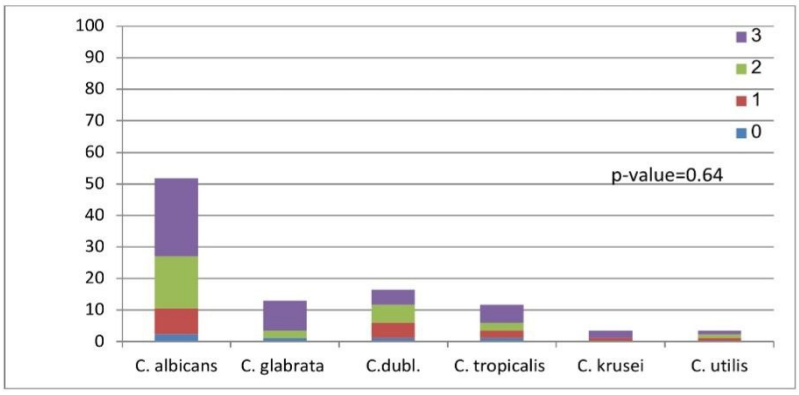

Fig.4:Relationship of oral Candida species growth with sugar intake frequency (individual's chi-square, *P*=0.64).

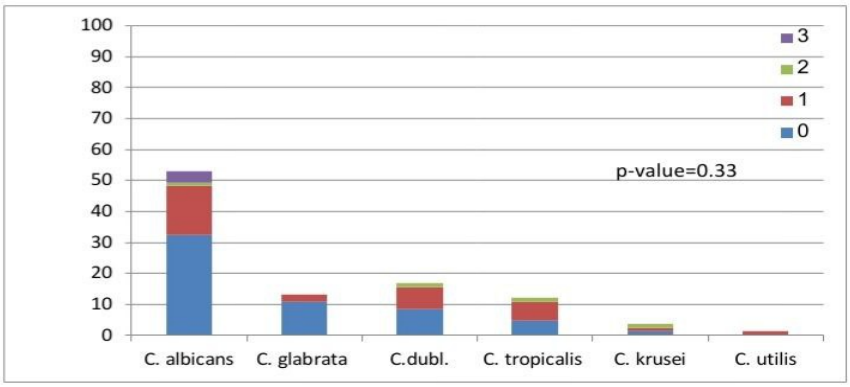

Fig.5. Relationship of oral Candida species growth with daily tooth brusher (individual's chi-square, *P*=0.33).

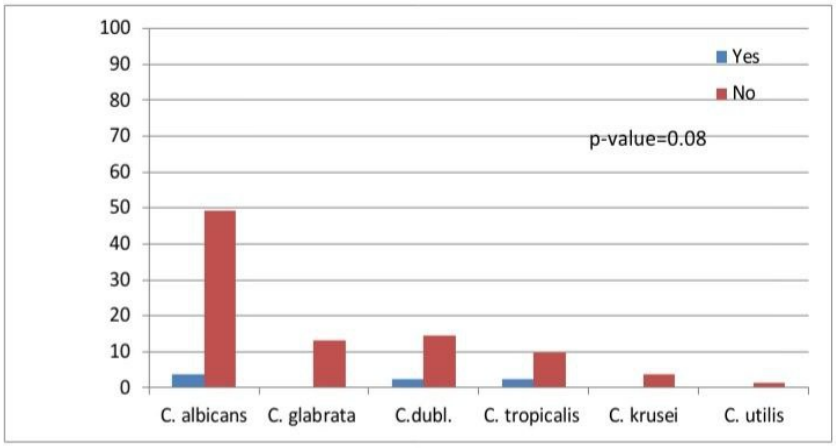

Fig.6. Relationship of oral Candida species growth with smoking

The differentiation between *Candida* species depended on the color of the colonies that grow on HiCrome *Candida* Differential agar. In this survey, six morphotypes of *Candida* species were collected from ~~the~~ dental caries patient. The total number of *Candida* isolates was 83, distributed to *Candida albicans* was 44, *Candida glabrata* 11, *Candida dubliniensis 14*, and C*andida tropicalis* 10, *Candida krusei* 3 and *Candida utilis* table 1. The difference in the distribution of *Candida* species across gender and age of patients was statistically significant (individual's chi-square test, *P*=0.016 , and *P*=0.03, respectively). Overall, the frequency of *Candida* species was higher in females than males. The highest frequency of species was recorded to *C. albicans* in both genders followed by *C. glabrata*, *C. dubliniensis*, *C. tropicalis*, *C. krusei* shown in Fig.1. The percentage of *Candida* isolates in children was high compared to the other of age groups, *C. albicans* and *C. glabrata* recorded the highest percentages (30.12%, 10.34%), respectively, while the percentage of the other types of *Candida* was low compared to the *C. albicans* and *C. glabrata*. It is worth noting that the percentage of *C. dubliniensis* appearance in the youth category increased significantly to 8.43% as in Fig.2. Urban group include (63 patients) and the rural group include (37 patients), however, *C. albicans* isolates were the highest in the two groups (33.7%, 19.28%), respectively, followed by *C. dubliniensis*, then *C. tropicalis*, *C. glabrata*, and finally *C. utilis* shown in Fig. 3. The extravagant and frequent intake of sweets leads to dental caries occurrence, as shown in Fig. 4. The percentage of *C. albicans* isolates increased when eating sweets three times and twice daily (24.71%, 16.47%) respectively. The percentage of *C. albicans* isolates were (8.24%, 2.35%) when intake sweets once or absence .The results of *C. glabrata*, showed that when sweets intake daily from (0, 1, and 3) the percentages were clearly increasing (1.18%, 0.00%, 2.35%, 9.4%) respectively. *C. dubliniensis*, *C. tropicalis*, *C. krusei* and *C. utilis* did not show a clear effect of this factor. The ratios of *C. albicans* isolates were gradually decreasing (% 32.53, % 15.66, % 1.20, % 3.61) when brushing teeth of (Not once, twice or thrice daily) respectively. The percentage of *C. glabrata* and *C. dubliniensis* were (% 10.84,% 2.41,% 0.00,% 0.00) and (% 8.43, % 7.23, % 1.20, % 0.00) respectively. The percentages of isolates of other species were not affected by tooth brushing like in Fig. 5. The results obtained in this research did not significantly in term of smoking. Non-smokers patients with a rate of 91% As illustrated in Figure 6, a minimal proportion of merely 9% of the participants were smokers.

II. DISCUSSION

Dental caries is one of an essential common human infectious disease that can lead to loss of tooth structure, and it occurs due to the metabolic activation of the plague microorganisms [16]. Strict measures were taken when sampling in order to avoid the microorganisms present in the other parts of the mouth or saliva fluid. The oral microbiome may be in contact with the caries area and adjacent dental plaque. As well as to avoid the microorganisms present in the food remains that could interfere with the caries site. In addition, when samples were collected from decayed roots, contact with the gums contact was avoided. Gums contain normal flora or microorganisms that cause inflammation. All of these microorganisms may not be associated with caries. All of the above precautions have been taken because the oral microbiome of healthy humans contains 74 cultivable and 11 non-cultivable fungus species [17]. Also, the human mouth is home to the most varied microbial community in the body, with about 700 species of bacteria inhabiting the soft tissues of the oral mucosa and the hard surfaces of teeth [18]. On SDA, *Candida* forms cream and convex with a smooth surface; this agree with researcher [19], who suggests that it is rarely possible to distinguish between distinct species of *Candida* on SDA. Our results are consistent with researcher Okamo *et al.,* [20] who mentioned that, *Candida* species have a major role in the occurrence of caries in adults. Our study was also relatively consistent with researcher [21], which proved the role of *C. albicans* in children dental caries, while our study proved that different types of *Candida* have a role in causing caries in all age groups for 53% of patients from 4 to 65 years old. These results are consistent with what was reached the researchers [22] and Sheiham and James, [23]. in state of *C. albicans* only. Tooth brushing is very important to keep oral health. Our current study non significantly showed that daily brushing leads to a decrease in the appearance of *Candida* species in the carious lesion, which leads to a lower probability of dental caries. Also, regarding *C. albicans*, our results are consistent with the results of the researcher [24]. In spite of smoking and *Candida* infection are risk factors for many oral diseases [25].Since most of the patients participating in this research were non-smokers with a rate of 91% and only 9% of them were smokers, but it should be noted that the incidence of *C. albicans* was high in non-smokers (40.49%). This is due to the fact that the percentage of infection with oral Candida species, especially *C. albicans*, is shared by several reasons and cannot be limited to only one factor.

II. CONCLUSION

The involvement of Candida in the development of dental caries was observed in this research, with 83 Candida species isolates identified from both the carious region and the adjacent dental plaque.

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