Original Article

Incidence and prevalence of oral candidal colonization in patients with cleft lip and palate

ABSTRACT

Aims and Objectives: This study aims to assess and compare the incidence and rate of oral candidal colonization in patients with cleft lip and palate and collate it with healthy individuals. The objectives of this study were to evaluate the factors that may affect the incidence, rate, and polycolonity of colonization of oral *Candida* species in patients with cleft lip and palate (CLP) as compared to healthy individuals.

Materials and Methods: The current prospective study involved the collection and assessment of oral samples from 50 CLP patients and compared it with oral samples from 50 healthy individuals. Colonies of *Candida albicans* and other *Candida* species were identified by using germ tube test and differential chromogenic media. Assessment of the distribution trend of various *Candida* species and their variation based on age, gender, type of cleft, number of surgeries, and oral hygiene status were recorded.

Results: A highly significant (*P* value < 0.001) statistical difference was seen in the oral carriage rate of *Candida* species between the two groups with values more in the study group (60.75 ± 71.43) than in controls (1.62 ± 2.22). Individually comparing the different *Candida* species, significant difference in presence of C albicans (*P* value = 0.004), C parapsilosis (*P* value = 0.015), and C dubliensis (*P* value = 0.027) was seen as compared to other species. In the context of the type of cleft and the presence of *Candida* species *Candida* albicans was the most prevalent in patients with the cleft alveolus (60.0% *n* = 15), unilateral CLP (66.7% *n* = 8), and bilateral CLP (100.0% *n* = 3) respectively. Nonsignificant variation in prevalence was seen in the various fungal species based on an individual's gender and oral hygiene and the number of surgeries status was observed.

Conclusion: The anatomical alteration in the oral cavity of patients with CLP contributes to the high incidence and prevalence of *Candida* species.

Keywords: Candida, cleft lip, cleft palate, oral cavity

INTRODUCTION

Prenatal life hosts a sterile oral cavity, where the inhabitation of organisms begins from birth as the child traverses through the vaginal canal (in neonates born naturally).[1] The resident microbiota of each neonate depends on externally influenced factors like gestational age, delivery mode, type of feeding, postpartum length of hospital stay, and general environment conditions.[2] The oral cavity consists of various topographic anatomical structures like recesses, mucosal folds, and invaginations which present different pH due to variations in local factors like oxygen concentration, ionic composition, access to

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.njms_127_22	

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Received: 21 July 2022, **Revised:** 12 August 2022, **Accepted:** 06 October 2022, **Published:** 14 April 2023

How to cite this article: Khan I, Ahmedi S, Ahmad T, Rizvi MM, Manzoor N. Incidence and prevalence of oral candidal colonization in patients with cleft lip and palate. Natl J Maxillofac Surg 2023;14:72-8.

saliva, presence or absence of teeth, and mechanical interactions, thus dictating the quality and quantity of oral microbiome.[3]

The oral cavity houses numerous commensal organisms among which *Candida* species, a form of yeast, are ubiquitously found with a wide range oral prevalence of 2–71% in healthy individuals.[4] This variation can be possibly due to age, oral health status, diet, ethnicity, subject selection criteria during assessment, organism sampling method, and an individual's socioeconomic and disease status.[5,6] Oral *Candida* infections are frequently seen when there is a lapse in host's defenses which can be due to local factors like prosthesis irritation, xerostomia, medications like antibiotics, immunosuppressants, treatment regimens like chemotherapy, radiation therapy, and systemic disorders like physical debilitation, malnutrition, endocrine or immune disturbances and often take a form of invasive infection with high mortality rate.[7,8]

Cleft lip and palate (CLP) is the most common congenital craniofacial anatomical defect wherein communication between the nasopharynx and oral cavity is present.[9] This communication leads to an ecological alteration in the microflora of the oral cavity, which can be due to repeated hospitalizations and surgical interventions a CLP patient is subjected to since birth, up to 20 years of age and beyond.[10,11] However, little information is available regarding the colonization and incidence of *Candida* species in these individuals.

Candida is an opportunistic pathogen in humans whose quantity and quality in an individual depends on their age, health status, and geographic area, thus posing a difficulty in determining the precise oral carriage rate.[12] However, there are varied thoughts regarding their colonization variation with age.[10] Candidal colonization in CLP patients is an unexplored area that holds larger importance for the Indian subcontinent considering the environmental conditions and high country-specific CLP prevalence.[8] Thus, the present study was undertaken to assess the incidence of oral *Candida* colonization in relation to the type of cleft, age of the patient, gender, and the number of surgeries the patient has undergone.

AIM

To assess and compare the incidence and rate of oral *Candida* colonization in patients of CLP and healthy individuals.

OBJECTIVES

To assess:

- Factors that may affect the incidence and rate of colonization such as type of CLP, patient's age and gender, number of surgeries the patient has undergone, and oral health status.
- Different *Candida* species and their correlation to the specific cleft. (if any)

MATERIALS AND METHODS

Ethical Clearance was obtained by Institutional ethics Committee, vide reference number: 1/10/293/JMI/IEC/2020 dated:-27/10.2020; the study was cleared by the institutional bio-safety committee via letter number: P1/42-21.12.2020 and the trial was registered prospectively in the Clinical Trial Registry of India (CTRI) (registration number: CTRI/2020/12/029821. The study was conducted collaboratively in the departments of oral and maxillofacial surgery, faculty of dentistry and medical mycology lab, department of biosciences from December 2020 till May 2022. Without gender bias, consecutive 50 CLP (study group) and 50 age and sex-matched nonCLP (control group) patients meeting the set inclusion and exclusion criteria were enrolled to be a part of the study. Informed sign consent was taken from patient/patient's guardian in their vernacular language, to be a part of the study.

Inclusion criteria

- Patients willing to be a part of the study
- Nonsyndromic cleft patients
- Absence of systemic diseases
- Absence of clinical signs of candidiasis
- Absence of oral premalignant disorders and other mucosal lesions like lichen planus, leukoplakia, etc.

Exclusion criteria

- Patients with deleterious habits like smoking, betel nut, gutka, or tobacco consumption habits
- Patients with a history of taking antimicrobial, antifungal, immunosuppressive or corticosteroid therapy in the last four weeks prior to sample collection.
- Edentulous patients especially in case of young patients less than 1 year of age

All the enrolled patients were divided into the following three groups based on their age.

- Group I: 5 years or younger
- Group II: Between 6 and 16 years
- Group III: 17 years and above

Each patient was allotted a unique identification number which was shared with the microbiologist and statistician, who were blinded to further patient details to avoid assessment bias.

Clinical assessment of patients

Oral examination of all patients was done by making the patient sit on a dental chair such that the inferior border

of the mandible was parallel to the floor in an open mouth position. Under adequate lighting oral cavity was examined using a mouth mirror, explorer, and William's marking periodontal probe. In case of patients younger than 5 years who could not sit on a dental chair by themselves were made to sit in the laps of their guardians to conduct the desired examination. The oral hygiene status of all patients was assessed by recording of gingival index (GI),[13] plaque index (PI),[13] and decayed, missing, filled teeth (DMFT/dmft) index using the World Health Organization criteria.[14]

Sample collection and transport

A sterile swab wetted with normal saline was used to collect oral smear samples from participating patients. In a twisting motion, the swab was wiped along the mucosal surface of the dorsum of the tongue, buccal mucosa, palatal mucosa, and floor of the mouth to collect the desired sample. The swab was immediately stored in a sterile empty tube and transported to the Medical Mycology Laboratory, Department of Biosciences, Faculty of Natural Sciences, for microbial analysis.

Microbial culture

The collected samples were inoculated on yeast extract, peptone, and dextrose (YPD) 2.5% agar plate having a YPD ratio of 1:2:2 (HiMedia Laboratories Pvt. Ltd., Mumbai) and incubated at 37°C for 2–3 days before reporting it as a negative culture. A positive result was indicated by the detection of characteristic *Candida* colonies using the germ tube test and observing blastospores on a direct wet mount using a light microscope (Nikon Eclipse 90I inverted microscope with magnification of 40×). Other species of *Candida* were identified using chrome agar differential media (HiMedia Laboratories Pvt. Ltd., Mumbai).

Statistical analysis

The collected data was analyzed using IBM SPSS Statistics for Windows version 16.0 (Armonk, NY: IBM Corp). Descriptive statistics were undertaken for patient details including type of cleft, gender, and age. Mann–Whitney U-test was done to compare DMFT/dmft, PI, and GI scores and colony forming units (CFU) between the study group and controls. Chi-square test was done for intergroup comparison of the presence of overall *Candida* growth and according to different *Candida* species and colonization according to the number of surgeries. The significance level was set at *P* value <0.05 with a confidence interval of 95%. Results are presented as mean ± standard deviation.

RESULTS

The age distribution of CLP and control patients is depicted in Figure 1. Most patients in the control group were over 17 years of age ($n = 30$), while most patients in the CLP group were aged between 7 and 17 years ($n = 20$). Among the study group, 29 were males while 21 were females with the cleft of the alveolus being the most common entity in both groups [Figure 2]. Among the study group, the greatest number of patients ($n = 18$) were subjected to two surgeries previously, while 16, 9, and 7 patients underwent 0, 1, and 3 surgeries, respectively. None of the patients in both groups exhibited any clinical signs and symptoms of oral candidiasis. A nonsignificant correlation was seen between the two groups for DMFT, PI, and GI scores [Table 1]. A highly significant (*P* value < 0.001) statistical difference was seen in the asymptomatic oral carriage rate of *Candida* species between the two groups with CFU values significantly more in the study group (60.75 ± 71.43) than in controls (1.62 ± 2.22).

Prevalence of *Candida* species based on study groups, age, gender, type of the cleft, and number of surgeries in cleft patients

A highly significant difference (*P* value 0.001) in the presence of *Candida* species was seen between the two groups. Individually comparing the different *Candida* species, significant difference in the presence of *C albicans* (*P* value = 0.004), *C parapsilosis* (*P* value = 0.015), and *C dubliensis* (*P* value = 0.027) was seen, while the presence of *C glabrata* (*P* value = 0.487), *C tropicalis* (*P* value = 0.269),

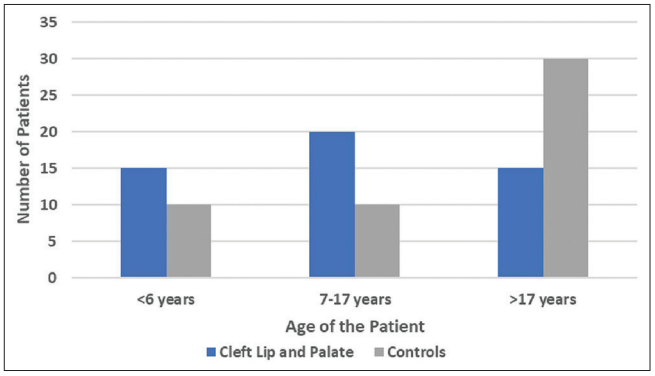

Figure 1: Age distribution of patients from study as well as control group

Table 1: Scores of various indices in the two groups

Index	Study group	Mean	Standard deviation	*P*
DMFT	Control	2.08	1.57	0.288
	Cases	2.40	1.76	
PI	Control	0.92	0.44	0.17
	Cases	1.08	0.72	
GI	Control	0.92	0.40	0.62
	Cases	0.88	0.48	

DMFT: Decayed, missing, filled teeth; PI: Plaque index; GI: Gingival index

C krusei (*P* value = 0.117), and *C utilis* (*P* value = 0.678) showed nonsignificant variation [Table 2]. The prevalence of *Candida* species based on gender saw nonsignificant distribution for all the species [Table 3]. A statistically significant difference in the prevalence of *C albicans*, *C tropicalis*, *C parapsilosis*, and *C glabrata* was seen in the three age groups for cleft and noncleft patients, while the presence of other *Candida* species showed a nonsignificant difference in prevalence [Table 4]. The asymptomatic oral carriage and prevalence of *Candida* species based on the type of cleft saw maximum presence in the cleft alveolus group (50.0%) followed by patients of unilateral CLP (24.0%) and least prevalence seen in the bilateral CLP group (6.0%). Checking the presence of individual species in each type of the cleft group, *C albicans* was the most prevalent species in patients with cleft alveolus (60.0%, *n* = 15), unilateral CLP (66.7%, *n* = 8), and bilateral CLP (100.0%, *n* = 3). In patients with cleft palate and cleft of soft palate, *C parapsiosis* and *C krusei* were reported to be 75.0 and 50.0%, respectively. A significant difference was seen in the distribution of *C krusei*, *C parasilosis*, *C glabrata*, and *C utilis* over the various types of clefts [Table 5]. A nonsignificant correlation (*P* value = 0.759) was seen in existence of *Candida* growth according to the number of surgeries in the cases group. With respect to *Candida* subspecies, a statistically significant variation was seen only with *C dubliensis* with all other species showing statistically nonsignificant prevalence when correlated with the number of cleft-related surgeries [Table 6].

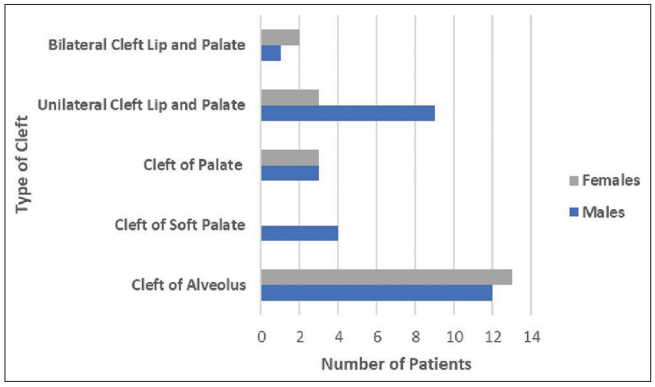

Figure 2: Distribution of Patients from the study group on the basis of type of cleft and gender

Table 2: Incidence of *Candida* species in the two groups

Study group	*Candida* species	Number of patients with the presence of species (percentage)	Number of patients with the absence of species (percentage)	*P*
Control	*Candida albicans*	14 (28%)	36 (72%)	0.004*
Cases		29 (58%)	21 (42%)	
Control	*Candida tropicalis*	2 (4%)	48 (96%)	0.269
Cases		6 (12%)	44 (88%)	
Control	*Candida krusei*	0 (0%)	50 (100%)	0.117
Cases		4 (8%)	46 (92%)	
Control	*Candida parapsilosis*	2 (4%)	48 (96%)	0.015*
Cases		11 (22%)	39 (78%)	
Control	*Candida dubliensis*	0 (0%)	50 (100%)	0.027*
Cases		6 (12%)	44 (88%)	
Control	*Candida glabrata*	3 (6%)	47 (94%)	0.487
Cases		6 (12%)	44 (88%)	
Control	*Candida utilis*	2 (4%)	48 (96%)	0.678
Cases		4 (8%)	46 (92%)	

*: Significant values

Table 3: Distribution of *Candida* species based on gender

Gender	*Candida albicans*	*Candida tropicalis*	*Candida krusei*	*Candida parapsilosis*	*Candida dubliensis*	*Candida glabrata*	*Candida utilis*
Males							
n	22	7	3	7	3	6	6
%	45.8%	14.6%	6.3%	14.6%	6.3%	12.5%	12.5%
Females							
n	21	1	1	6	3	3	0
%	40.4%	1.9%	1.9%	11.5%	5.8%	5.8%	0.0%
Total							
n	43	8	4	13	6	9	6
%	43.0%	8.0%	4.0%	13.0%	6.0%	9.0%	6.0%
P	0.687	0.027	0.348	0.769	0.999	0.305	0.01

*: Significant values

Table 4: Prevalence of *Candida* species according to age

Candida species	No cleft			Cleft alveolus			Cleft palate including the soft palate			Unilateral lip and palate			Bilateral lip and palate			*P*
	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	
C albicans	1	1	12		6	9	3		0	1	4	3	2	1		<0.001*
C tropicalis	0		2			4	2		0	0		0	0			0.018*
C krusei	0		0			1	3		0	0		0	0			0.250
C parapsilosis	0	0	2		4	4	3		0	0	0	0	0	0		0.004*
C dubliensis	0	0			2		2			0	1		0	0		0.155
C glabrata	0	0	3		0	0	0		0	0	2	3	1	0		0.029*
C utilis	0	1	1		0	2	2		0	0	0	0	0	0		0.092
No growth	9	8	12		5	1	1		1	3	1	0	0	0		0.054
Total	10	10	32		17	21	16		1	4	8	6	3	1		

G1: 5 years and younger; G2: 6–16 years old; G3: 17 years and older. *: Significant values

Table 5: Distribution of *Candida* species based on the type of cleft

	Candida albicans	*Candida tropicalis*	*Candida krusei*	*Candida parapsilosis*	*Candida dubliensis*	*Candida glabrata*	*Candida utilis*
Cleft alveolus							
n	15	4	1	8	3	0	2
%	60.0%	16.0%	4.0%	32.0%	12.0%	0.0%	8.0%
Cleft palate							
n	1	2	0	3	0	0	2
%	25.0%	50.0%	0.0%	75.0%	0.0%	0.0%	50.0%
Cleft of the soft palate							
n	2	0	3	0	2	0	0
%	33.3%	0.0%	50.0%	0.0%	33.3%	0.0%	0.0%
Unilateral cleft lip and palate							
n	8	0	0	0	1	5	0
%	66.7%	0.0%	0.0%	0.0%	8.3%	41.7%	0.0%
Bilateral cleft lip and palate							
n	3	0	0	0	0	1	0
%	100.0%	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%
Total							
n	29	6	4	11	6	6	4
%	58.0%	12.0%	8.0%	22.0%	12.0%	12.0%	8.0%
P	0.209	0.069	0.002*	0.008*	0.449	0.003*	0.022*

*: Significant values

Table 6: Prevalence of *Candida* species based on the number of surgeries in cleft patients

No of surgeries	*C albicans*		*C tropicalis*		*C krusei*		*C parapsilosis*		*C dubliensis*		*C glabrata*		*C utilis*		No growth	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	8	14	3	1	3	0	3	2	0	2	3	2	4		12	20
1	2	2					0	2			2	1			0	3
2	9	3	3	0			2	1	3	0			1		2	3
≥3	3	2	1	0	0	1	2	1	0	1	1	0	1		1	0
P	0.186		0.565		0.25		0.422		0.05*		0.741		0.076		0.321	

M: Male; F: Female; ≥: More than. *: Significant values

DISCUSSION

In this study, the authors have attempted to address the lacunae in literature concerning the incidence and/or prevalence of various *Candida* species seen in cleft individuals compared to noncleft counterparts. Knowing the oral carriage rate and variation from noncleft individuals of the various candidal species in cleft patients will help provide targeted therapy to cleft patients if need be. CLP patients can be at a higher risk of developing oral fungal infections because of altered oral microflora, hard and soft tissue anatomic variations, immunocompromised state, and high number of frequent hospitalizations.[8]

In our study, there was no significant difference in the mean values of the three assessed indices, i.e. DMFT, PI, and GI. This was partially like the results presented by Rawashdeh *et al*.[15] who reported statistically nonsignificant differences in PI values (*P* value = 0.09); however, they reported a significant difference in DMFT, and GI scores for the two patient groups (*P* value = 0.0001 and 0.0002, respectively). A significant difference in the scores of the DMFT index for cleft and noncleft patients has also been reported by Nair *et al*.[16] and Chopra *et al*.[17]

Our study showed *C albicans* as the most frequently encountered species in both the groups, i.e. in 58 and 28% of patients in the study group and control, respectively. This was in line with the study by Rawashdeh *et al*.[15] who reported *C albicans* incidence of 81.5 and 72.7% in cleft and noncleft patients, respectively. However, the presence of other species showed contrasting variations. In cleft patients, our results showed *C parapsilosis* (22%) as the next commonly found species followed by *C tropicalis*, *C dubliensis*, and *C glabrata*, each having an incidence of 12% with *C krusei* and *C utilis* as the least common species (8% each). In contrast, Rawashdeh *et al*.[15] found *C galbrata* (10.5%) as the next most common with *C kefyr* (7.9%) being the least common. For control patients, they reported the presence of only *C kefyr* in addition to *C albicans*, while our study also saw the presence of *C tropicalis*, *C parapsilosis*, *C glabrata*, and *C utilis* in control patients. The ubiquitous presence of *C albicans* during infancy and at different points of cleft surgeries has also been demonstrated by Boriollo *et al*.[18] and da Silva *et al*.[19] which was in line with our results.

Our results showed no significant difference was found in the distribution of various *Candida* species when compared with gender, which was similar to the findings of Rawashdeh *et al*.[15] Comparing the results of species distribution based on the type of cleft, our study saw the greatest colonization in patients with cleft alveolus, which was in contrast with the findings of Rawashdeh *et al*.[15] who found the maximum prevalence in patients with bilateral CLP.

Our findings showed a significant difference in the colonization of *C albicans*, *C parapsilosis*, and *C dubliensis* between cleft and noncleft individuals, while an insignificant difference was found for other species. To the best of our knowledge, this was the first of the kind analysis done, comparing the species variations in cleft and noncleft individuals supported by statistical values. Among the cleft patients subjected to surgeries, our results showed that patients with two surgeries showed the maximum *Candida* colonization. This contrasted with the results of Rawashdeb *et al*.[15] who found the maximum fungal prevalence in patients with three or more surgeries. No other work available in literature correlates the number of surgeries with the presence of *Candida* species in cleft patients. Though all of our patients in the study group were asymptomatic for oral candidiasis and none exhibited any signs or symptoms, still the significantly high rates of *Candidal* colonization found in the study group can always pose a risk of infection, especially when the host immune system is compromised.[19]

A commensal organism of the human body, *Candida* species are found in large numbers in the oral cavity and throughout the digestive and urinogenital tracts.[8,15] Human immune system prevents their overzealous growth, preventing them from taking the form of an infection; however, the infectious state is highly invasive and debilitating and is commonly seen in older individuals and those with endocrinopathies leading to an immunocompromised state.[8,20] Oral candidiasis is the most common human fungal infection seen with a wide carrier rate of as low as 2% to as high as 95% among various age groups, healthy and compromised individuals.[15,20] This variation in carrier rate and conversion to opportunistic infections depends on multiple factors like age, diet, use of oral prosthesis, duration of hospital stay, and general oral health status.[8,15] Oral cleft is a common, multifactorial congenital defect that can manifest in multiple combinations depending on the extent of anatomic nonunion. Pathological oronasal communications in these patients alter the oral microflora and require multiple surgical interventions and hospitalization for repair, thus making them prone to fungal infections.

With a population of more than a billion, the Indian subcontinent accounts for nearly 27,000 to 33,000 cleft cases per year.[21] The risk factors enumerated previously added by the tropical conditions in this part of the world put a cleft individual at greater risk of oral fungal infections. There is a scarcity of literature addressing the issue of asymptomatic oral carriage rate in patients with CLP as well as variation of *Candida* species with respect to specific oro-facial clefts, and the present study attempts to fill in this lacuna. Clinical studies in future may be planned to determine the virulence and polyclonality of *Candida* species obtained from asymptomatic CLP patients and correlating it with individual *Candida* species from symptomatic patients exhibiting oral candidasis.

CONCLUSION

Alteration in the oro-mucosal anatomy can significantly contribute in oral candidal colonization. Our study exhibits a significantly high asymptomatic oral carriage rate of oral *candida* species in patients with CLP. The type of cleft and number of previous surgeries that a cleft patient has

undergone may have a role in altering and modifying the oral candidal flora.

Declaration of patient consent

The authors declare that they have obtained consent from patients. Patients have given their consent for their images and other clinical information to be reported in the journal. Patients understand that their names will not be published and due efforts will be made to conceal their identity but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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