

Pediatric oropharyngeal candidiasis: A comprehensive study on risk factors and most prevalent species of *Candida*

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Abstract: Oropharyngeal candidiasis is caused by opportunistic fungal pathogen *Candida*. Decreased immune level whether in immunocompromised patients or in extreme age groups facilitate the progression of disease in vulnerable individuals. Current study focusses on evaluation of most prevalent species of *Candida* in pediatric patients and also assesses risk factors causing oropharyngeal candidiasis. Total of 1152 pediatric patients previously diagnosed with oropharyngeal candidiasis from rural areas of District Bahawalpur were included in this study. Oral clinical swab samples were collected from these patients. From collected clinical swab samples, isolation, characterization and identification of *Candida* spp. was carried out to assess most prevalent species. Questionnaire was filled, with prior patients consent, for assessment of risk factors associated with oropharyngeal candidiasis. *Candida albicans* was the most prevalent species with 68.6% prevalence, while *C. lusitanae* (a nonalbicans) was the least prevalent with 0.4% prevalence. Among other non-albicans, *C. glabrata* counted 12.9%, *C. tropicalis* 5.6%, *C. krusei* 8.5%, *C. parapsilosis* 3.1% and *C. dublineiensis* 1.1%. Associated risk factors included hygienic status of mother, patient's cleanliness and economic condition of the parents. Being disease of infancy, oropharyngeal candidiasis, is the oral infection with many episodes of relapse in pediatric patients and if not addressed properly may lead to severe invasive and noninvasive infections. There is dire need of integrated efforts to reduce the burden of infections in pediatrics specially and common population in general, by adopting various steps including more awareness programs, increasing economic status of the people, patient counselling and many other steps in rural areas.

Keywords: Oral fungal infection, pediatric oral infection, oropharyngeal candidiasis, oral infection, fungal infections.

INTRODUCTION

Oropharyngeal candidiasis, a fungal disease caused by *Candida* species, especially *Candida albicans*, can develop at any stage of life but is considered as infant disease (Kozinn *et al.*, 1958). Human oral microbial colonization starts in few hours after the birth of neonate (Xu *et al.*, 2015). Some researchers have demonstrated development of oral microbiome before delivery. Oral microbiota development is maximum during first month and stabilizes in first year of child (Gritz and Bhandari, 2015). Among other predominant predisposing factors controlling the oral microbial development, mode of nutrition i.e. breast fed and formula fed also control the oral microbial species growth and development (Gritz and Bhandari, 2015). Other key oral microbial species growth and development detrimental factors include mode of delivery (Dominguez-Bello *et al.*, 2010), breast feeding, formula feeding (Sampaio-Maia and Monteiro-Silva, 2014, Mueller *et al.*, 2015, Holgerson *et al.*, 2013), oral health of mother during pregnancy (Silk *et al.*, 2008), mothers vaginal microflora (Mändar and Mikelsaar, 1996)

and others.

As oral cavity is harbored by variety of microbes including fungal species like *Candida albicans* and other non-albicans species. Approximately 40%-60% of the healthy individuals harbor *Candida* in oral cavity. *Candida*, an opportunistic pathogen, may cause variety of infections which may be local or systemic (Berberi *et al.*, 2015). Oropharyngeal candidiasis in addition to esopharyngeal candidiasis is the most diagnosed oral fungal infection in the patients with compromised immunity. Both diseases not only are the severe but also chances of relapse are very high. Above 4 fold increase in oral thrush infection has been reported during 1980s (Pons *et al.*, 1997). In addition to immunocompromised patients, the individuals who are receiving head and neck radiotherapy may develop oropharyngeal candidiasis (Zaura *et al.*, 2014, Redding *et al.*, 1999).

Current study is the first study in Pakistan which focusses the oropharyngeal candidiasis prevalence in neonates and children along with assessment of predominant predisposing factors which are associated with oropharyngeal candidiasis. This study is also unique in targeting rural areas instead of including urban areas.

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MATERIALS AND METHODS

Present study was conducted in Tehsil Ahmad Pur East, District Bahawalpur, densely populated tehsil in southern Punjab, Pakistan. Total of 1152 pediatric patients, previously diagnosed with oropharyngeal candidiasis, from zero to four years age groups and of both genders were included in the current study. But those patients who were above the age of four years and were healthy or had not infected with oropharyngeal candidiasis were excluded from the study. Before collecting clinical swab samples, patient's mothers or father or guardian (in case of absence of parents) were briefed about the study and patient consent was taken verbally. A questionnaire was also filled for risk factor assessment. This was a two-step study in which in the first step isolation and characterization of *Candida* species from swab samples collected from pediatric patients was carried out and in the second step risk factors were evaluated from the questionnaire which was filled from patients.

Ethical approval

Current study was approved by the Ethics Committee of Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

RESULTS

Demographic and risk factors associated and aggravating the oropharyngeal candidiasis infection include various aspects of patients. Gender distribution showed that 70.8% were females and 29.2% were male (table 1). Majority of the pediatric patients were less than one year of age and 2% were above one year of age (Fig. 1). The most recorded weight in patients was 8kg with frequency

of 34.98% of the total 1152 patients and 10kg was the rarely recorded weight among patients (table 2). As anticipated, due to the economic situation of this locality, babies were mostly fed by their mothers as high as 83.33% followed by both with mother's milk and feeder fed was 14.06% and feeder fed alone counted 2.60% (fig. 2). Maximum people were unaware of the importance of boiling milk. They used milk as such or fresh milk instead of boiling it (fig. 2). Majority of people were unwilling to change feeder (fig. 2). Similar pattern was observed for changing of nipple of feeder. Majority of people considered it as unimportant to change nipple before it was destroyed. As given in fig. 2, Nipple of the feeder was used for maximum time and after it was torn out or non-usable then they changed it. This behavior is represented in fig. 2. Majority of families had three to five children i.e. 51.7%, then 44.6% families had two or one number of kids and least count i.e. 3.6% of families had six to ten children. Economic stability of the family was determined by their monthly income per family. 89% families had 6000 to 15000 Pak. Rupee monthly income, 8% had 16000 or above and very less number had below 5000 Pak. Rupee monthly income. Majority of people were working as a labor worker hence they earned average monthly wages. 77% of the family's heads were illiterate and just 23% were literate as given in fig. 2. It was observed that very less percentage i.e. 0.2% were graduates, 0.9% were under graduates, 2.4% were under matriculates, 5.8% had passed their primary level of education and 14.1% were just literate people and rest of 76.6% were illiterate. Similar to father literacy rate, mother literacy rate was also very poor i.e. 93.1% women were illiterate while only 6.9% were literate. Role of mother health in progression of disease was insignificant i.e. 91.7% were healthy while 8.3% patients' mothers were ill. Villagers view about patient counseling, oral hygiene, its importance and procedure, seminars, workshops, group meetings, village campaigns about oral

Table 1: Demographics of the patients

Demographics of patients			
Age		Gender	
less than 1 year	Above 1 year	Male	Female
1128(98%)	24(2%)	336(29.2%)	816(70.8%)

Table 2: Weight variation and distribution

Weight	Average patient	%age
<5kg	0.33	0.09
6kg	109.67	28.56
7kg	80.33	20.92
8kg	134.33	34.98
9kg	40.67	10.59
10kg	1.67	0.43
11-15kg	8.33	2.17
16kg and above	8.67	2.26

health and its importance, or any other activity on the body hygiene were recorded. Unanimously the answer was in negative that meant that they were unaware of such things.

Candida albicans was the most prevalent specie when clinical samples were cultured on CHROMAgar media as well as on Corn Meal media. *C. albicans* was the most prevalent *Candida* spp. with 68.6% of prevalence and *C. tropicalis* was the least prevalent specie on CHROMAgar media (table 3) while *C. lusitaniae* on Corn Meal media with 5.6% and 0.4% respectively (table 4). Other species isolated on CHROMAgar media were *C. glabrata* 12.9%, and *C. krusei* 8.5%. On Corn Meal Media *C. glabrata* counted 12.9%, *C. tropicalis* 5.6%, *C. krusei* 8.5%, *C. parapsilosis* 3.1%, and *C. dublineiensis* 1.1%.

Table 3: *Candida* species prevalence growth on CHROMAgar media

Name of <i>Candida</i>	% prevalence
<i>C. albicans</i>	68.6
<i>C. glabrata</i>	12.9
<i>C. tropicalis</i>	5.6
<i>C. krusei</i>	8.5
<i>Candida</i> spp.	4.4

Table 4: *Candida* species prevalence growth on Corn Meal media

Name of <i>Candida</i>	% prevalence
<i>C. albicans</i>	68.6
<i>C. glabrata</i>	12.9
<i>C. tropicalis</i>	5.6
<i>C. krusei</i>	8.5
<i>C. parapsilosis</i>	3.1
<i>C. dubliniensis</i>	1.1
<i>C. lusitaniae</i>	0.4

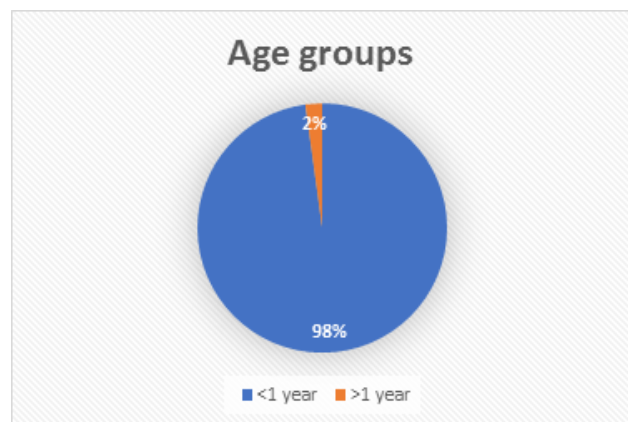


Fig. 1: Age groups.

Protocol for Clinical swab sample collection adopted by Binita 2012 (Srivastava *et al.*, 2012) was followed. Swab samples culture enrichment was done on Sabouraud's

dextrose agar media containing 10% chloramphenicol. Culture plates were incubated for 24-48 hours. Plates having no yeast growth were incubated further for 72 hours. Colony forming units were determined. Morphology of the colony was examined by the protocol adopted by Kausar Fatima 2014 (Fathima and Rajendran, 2014). Smear was prepared then gram staining was done. Presence of pseudo-hyphae and budding cells were observed to confirm the *Candida* presence. 2-3 colonies were inoculated in human serum taken in test tube. Incubated on water bath for 2-3 hours at a constant temperature maintained at 37°C. After incubation, glass slide was prepared by placing serum on glass slide and placed coverslip on it. Observations were made under low and high-power objectives sequentially. Presence of germ tube in this incubation time was taken as a positive indication for *C. albicans* presence (Fathima and Rajendran, 2014).

STATISTICAL ANALYSIS

MS Excel 365 was used for the calculation of percentage prevalence of *Candida* strains presented in tables 1 and 2 while SPSS 16 was used in the estimation of frequency of risk factors given in figs. 1 and 2.

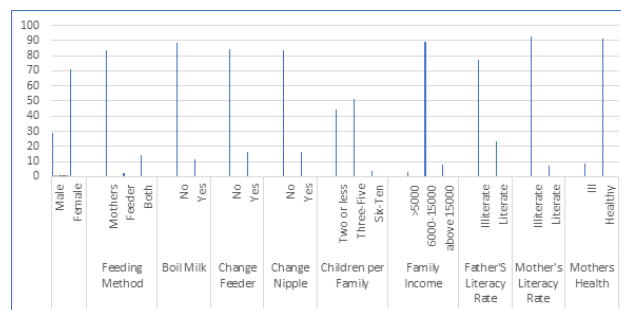


Fig. 2: Correlation of predisposing factors of oropharyngeal candidiasis.

DISCUSSION

Current study had two threads. In one strand risk factors associated with oropharyngeal candidiasis were assessed and prevalence of *Candida* species on the other thread was estimated. Numerous studies has been conducted for the assessment of predisposing risk factors of oropharyngeal candidiasis in adults but little is known about the oropharyngeal candidiasis of neonatal age and the age onwards (Hoppe, 1997, Hope *et al.*, 2012). A few studies have been conducted to evaluate gender specificity of oropharyngeal candidiasis. In current study female patients were in higher count from male patients. This high count might be due to the overall high female population as compared to male population. Unluckily we cannot confirm this phenomenon because of unavailability of data showing gender wise population of Tehsil Ahmad Pur East in this age group to confirm high female count in this locality. Although census held on

1998 shows that male count was high as compared to female count with growth rate of 2.87. (<http://www.pbscensus.gov.pk/sites/default/files/DCR/Punjab/Bahwalpur.pdf>) Another possible cause may be decreased immunity in female neonates as compared to male neonates of the same age. Previous studies have confirmed differences in immune response related to gender (Imahara *et al.*, 2005). Generally females are more prone to infections (Ruggieri *et al.*, 2016). Hence our observations confirm previous studies findings. Different risk factors are associated with pediatric oral fungal infection. It is estimated that human body has microbial mass which accounts 1-3% of ones total body mass. Apparently it seems very little percentage but it represents a huge microbial mass which is above 100 trillion microbial cell count (Whitman *et al.*, 1998). This complex microbial communal development and stabilization is the sequence of a series of microbial interactions which starts from birth to whole life but first year of neonatal age is very critical in this sequela (Palmer *et al.*, 2007, Torrazza and Neu, 2011). In current study, age of 98% of patients was lower than one year. Previous literature confirmed that neonates having no stabilized oral microbial community on one side and underdeveloped immune system on the other, are prone to infection. In previous studies oropharyngeal candidiasis has been marked as the disease of infancy. Hence our study results are in line with the previous studies (Butler and Baker, 1988). Weight of the neonates at the time of birth is predictor of their forthcoming anomalies. Subnormal weight measures result in congenital malformations. Low body weight means below 1.5 kg of body weight at the time of birth (Hack *et al.*, 1983). In current study no child had been reported to have subnormal weight. All participants were in normal body weight range. 83.33% of the pediatric patients were fed by their mother, while 2.60% were fed by feeder and rest of 14.06% were fed by both. Hence there was direct correlation of mother feeding and propagation of oropharyngeal candidiasis. Previous studies has confirmed that there is direct relationship with high carriage rates of *C. albicans* and breast feeding (Johnstone and Marcinak, 1990). Current study is consistent with the results of previous studies. Milk can be source of pathogens especially in neonates and children (Dhanashekar *et al.*, 2012). In current study, count of neonates who were fed by their mothers was greater than those who were fed by their mothers and feeder. Hence use of boiled milk was of low significance in current study as majority of the patients having oropharyngeal candidiasis were fed by their mothers. Use of bottles for feeding of infants, duration of use of these bottles and extended use of nipples of these bottles might be contributing factor of oropharyngeal candidiasis. As previously reported by Clavano, chances of oropharyngeal candidiasis was high in formula fed infants fed by bottles as compared to those who were fed by breast feeding (Clavano, 1982). In current study, bottle

fed infants count very less, hence this factor has insignificant role in contributing to oropharyngeal candidiasis. No previous data was available to show effect of increased number of children in family and their relationship to oropharyngeal candidiasis. In our study majority of patient's families had three to five children in their family. No data was collected for presence of oropharyngeal candidiasis in other family members. Hence it was not confirmed that whether horizontal transfer was the cause of oropharyngeal candidiasis in infants. Economy is very important which results in high purchase power. In our study, low income families were present as well as families with average income. Maximum number of families had average monthly income. To maintain good hygienic conditions, they do not find ways to maintain it without good expenses allocated for this purpose. It may contribute to low hygiene and ultimately in development of oropharyngeal candidiasis, as prevalence of microbes increases in poor hygienic conditions (Olaechea *et al.*, 2004). In current study a serious issue was recognized in the form of low literacy rate of parents. High count of illiteracy with its significance in oropharyngeal candidiasis was observed. Awareness, perception and practice is directly related to literacy rate. It is unfortunate that our rural areas had poor literacy rate. Oral hygiene of the mother (Mueller *et al.*, 2015) is very important as it may be source of first inoculation in the oral cavity of the neonates (Tamburini *et al.*, 2016, Rodríguez *et al.*, 2015). In current study, majority of the patient's mothers were healthy. But no data was collected about the oral hygiene of the mothers. It is evident that mother oral microbial community determines the oral microbial flora development in the newly forming community in the neonates. Also, others oral microbial is transferred to neonates directly. We cannot confirm that mother's oral microflora has not been transferred via oral contact of mother to neonates. In previous studies, it has been confirmed that mother's oral microbiome is the initial source of neonatal oral microbial communities (Jiménez *et al.*, 2005, Mueller *et al.*, 2015)

In current study *C. albicans* was the most prevalent with 68.6% of prevalence among all *Candida* species. *C. lusitanae* was least prevalent among non-albicans. Previous studies on prevalence of *Candida* species showed that *C. albicans* was highly prevalent *Candida* specie and other non-albicans species were less prevalent (Ahmad and Khan, 2009, Phillips and Karłowicz, 1997). Current studies results are consistent with previous studies findings.

CONCLUSION

Pediatric oral fungal infection characterized by oropharyngeal candidiasis is multifactorial in its cause. Propagation and spread of this disease can be stopped by improving local level awareness of this disease,

increasing awareness programs to improve body hygiene in general and oral hygiene in specific will lead to decreased prevalence of oropharyngeal candidiasis. As mentioned in the result section, no workshops, seminars, campaigns or activities on local level were held hence there is dire need to do same for improving health status of the local folks.

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