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Original Article

Oral Candidiasis Association with Dental Hygiene in Population of Islamabad - A Cross-Sectional Analysis

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Author`s Contribution

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ABSTRACT

Objective: To assess the prevalence and frequency of oral Candida species in dental patients in regions of twin cities of Pakistan by isolating and diagnosing the distinct species of Candida from oral samples of people visiting dental clinics in different areas of Islamabad and Rawalpindi. Also to Bridge the epidemiological gap present between oral candidiasis association with dental hygiene conditions in Pakistan

Methodology: This cross-sectional study was conducted in the department of biosciences COMSATS University Islamabad (CUI), Islamabad, Pakistan from August 2024 to October 2024. The samples were cultivated on HiCrome differential agar and Sabouraud Dextrose Agar (SDA) to identify the species. Surveys and manual records were used to collect the data, which was then analyzed using SPSS and MS Excel. Significant correlations between oral candidiasis and lifestyle or demographic characteristics were found using statistical studies, such as chi-square tests, with a significance threshold of $p<0.05$.

Results: Candida spp. colonization rates are 40–60% in healthy individuals, but 62–93% in immunocompromised patients, including those with HIV. The results indicated *C. albicans* as the most prevalent species (54%), followed by *C. krusei* (14%), *C. tropicalis* (11%), and *C. glabrata* (7%). The infection was most common in young adults (ages 21–30; 70%), married individuals (71%), and men (60.71%). Smoking and poor oral hygiene were significant risk factors, with a higher species diversity observed among smokers and 68% prevalence among non-mouthwash users.

Conclusion: The study emphasizes how risk variables like age, gender, lifestyle, and cleanliness habits are strongly linked to oral candidiasis. These results highlight the necessity of focused public health campaigns to raise awareness, encourage good oral hygiene, and address modifiable risk factors in order to lessen the prevalence of oral candidiasis in the general population.

Keywords: Candida spp., oral hygiene, non-albicans, denture infection

Introduction

Oral candidiasis (OC), also known as "thrush," is a fungal infection characterized by overgrowth and invasion of superficial tissues. The term "thrush" is derived from the bird's breast and the white flecks found in certain types of candidiasis. The disease's etymology dates back to Hippocrates, who defined OC as mouths affected with aphthous ulcerations. In 1846, French mycologist Charles Philippe Robin named Oidium albicans, which means "to whiten."

The AIDS epidemic led to increased interest in OC in the 1980s, and it remains the most common oral opportunistic infection in people with HIV and compromised immune systems.[1]

Dental caries, a bacterial infection, is the second most common infectious disease in human history, primarily caused by *Candida albicans*, a fungus found in the mucosal region. This fungus is responsible for widespread tooth decay and is primarily brought through the consumption of carbohydrates in the mouth.

The caries process involves demineralization and remineralization, with a complex and varied microbial population in the mouth cavity. The interaction between oral bacteria and Candida depends on cross-kingdom interactions, which can influence the severity and course of bacterial infections mediated by biofilms. *Candida albicans* has been found in polymicrobial biofilms linked to dental caries, periodontal disorders, peri-implantitis, denture

stomatitis, oral mucositis, and root canal infections.[2]

The high oral prevalence of *Candida glabrata* and *Candida albicans* among carriers and patients with oral candidosis may be partly caused by their comparatively strong resistance to lysozyme.[3] Oral epithelial tissues may be harmed by the combined colonization of *Candida albicans* and *Candida glabrata*, which can promote fungal invasion. It is necessary to possess additional risk factors along with colonization. Improper treatment of oral candidosis in immunocompromised patients can result in incurable candidosis or chronic persistent infection. Invasive candidosis can cause necrosis of the suppurative candidosis, ulcerative mucositis, oropharyngeal and esophageal candidosis inflammatory papillary hyperplasia of the hard palate, as well as potentially fatal systemic candidosis like *Candida* endocarditis.[4]

Poor oral hygiene (POH) is often linked to the development of oral cancer, with oral malignancies being significantly associated with POH. This is due to the carcinogenic properties of alcohol and tobacco, which can increase the risk of oral cancer even after controlling for factors like alcohol and tobacco consumption, education level, and socioeconomic status.[5]

Thrush, also known as pseudomembranous candidiasis, is typified by large white pseudo membranes made of fibrin, fungal hyphae, and desquamated epithelial cells. About one-third of cases of oropharyngeal candidiasis are of this type, making diagnosis simple. *Candida albicans* has the ability to change morphologically between yeast and hyphal forms, making it pathogenic.

Candida albicans, a naturally diploid species, has a genome size of 14.3 megabases, eight chromosomes, and a GC concentration of 33.5%. The common laboratory isolates, SC5314, contain 43,665 heterozygous sites, with one heterozygous single nucleotide polymorphism per 330 base pairs. However, these sites are not evenly distributed across the genome and vary greatly among isolates.[6] A distinct parasexual cycle has been discovered in the lab, despite Candida albicans being traditionally thought to be an asexual species.. Candida virulence and pathogenicity are immediately adapted and promoted when these constraints are positively addressed. In addition to its resistance to antifungal drugs, Candida can spread through various channels, including sexual activity, childbirth, and healthcare-associated channels. Infectious candidiasis can occur through the hands of medical personnel or tainted medical equipment, exposing patients to sterile bodily parts. Contact with infected surfaces or objects can also result in transmission, particularly in patients with impaired immune systems.[7]

Oral candidiasis (OC) is a common form of oral candidiasis, characterized by the invasion of the oropharyngeal cell lining. The disease can be classified into acute, chronic, and chronic manifestations, with oral symptoms being the same for various non-albicans Candida species. [8] Some species form biofilms, such as *Candida glabrata*, rather than hyphae before infection happens. When mucosal and epithelial surfaces are destroyed by cytolytic proteins and enzymes, many types of candidiasis develop.[9] The human oral cavity is invaded by a diverse range of microorganisms, Candida species use to exacerbate periodontal disease, but they have identified detrimental characteristics such as adhesion, invasion, dimorphism, and biofilm development. Candida species can co-aggregate with the periodontal pathogen, exhibiting commensal or pathogenic traits depending on the location of the microbial communities.[8] Bacteria like Tannerella forsythia, Porphyromonas gingivali, and Aggregatibacter actinomycetemcomitans are frequently linked to periodontal disease. Currently, there is minimal evidence that yeast involvement is a factor in periodontal disease. [10] Fungi may act alone, in combination with subgingival bacterial infections, or both. By generating pro-inflammatory cytokines, they can also function as cofactors, increasing the likelihood of periodontal attachment loss and ultimately causing periodontal disease.

Over the past 20 years, invasive infections caused by non-albicans Candida species have increased, causing *Candida albicans* to lose ground. *Candida parapsilosis*, a complex of three cryptic species, is particularly significant. Candida species are common asymptomatic oral cavity colonizers that can cause opportunistic infections in vulnerable individuals.[11] Symptoms of candidiasis include loss of taste, bleeding, burning or pain, and stomatitis. Candida levels in the mouth may rise in cases of weakened immune

systems, extreme ages, steroid usage, cancer, and HIV/AIDS. Factors such as injuries, inadequate oral hygiene, radiation therapy, chemotherapy treatments, iron deficiency, poor nutrition, dental prostheses presence, and tooth decay can also increase the likelihood of colonization and infection development. It can induce invasive infections that spread throughout the body, including respiratory, gastrointestinal, urinary tract, and esophageal infections. Children, the elderly, and patients receiving immunosuppressive or systemic corticoid medication due to organ transplantation are at high risk of developing oral candidiasis. The incidence of candidiasis and the variety of yeast species found may be significantly impacted by the use of inhaled corticosteroids by adults and children with bronchial asthma. [12] Candida biofilm development is crucial for Candida pathogenicity in mucosal candidiasis, causing persistent infection, recurrence, and antifungal resistance compared to planktonic Candida. [13]

Methodology

This study explores the prevalence of oral candidiasis in Pakistan's regions of Islamabad and Rawalpindi, using ArcGIS software to map the region. The study aimed to check prevalence of oral candidiasis, using online and manual surveys from August 2024 to October 2024. Participants over 10 were included, while those under 10 and those who had taken antibiotics within two weeks were excluded. Sterile swab samples from 50 dental caries patients were collected and cultured in Brain Heart Infusion Broth and Sabouraud Dextrose broth. The study analyzed oral candidiasis prevalence using SPSS statistics and Microsoft Excel, with chi-square tests and GraphPad software for visualization. Ethical approval was obtained from the Institutional Review Board of COMSATS University Islamabad. (CUI-Reg/Notif/632/24/685)

The prevalence of oral candidiasis among individuals in specific regions of Pakistan is determined. This cross sectional study was conducted in the department of biosciences COMSATS University Islamabad (CUI), Islamabad, Pakistan from August 2024 to October 2024.

The total study duration ranged from August 2024 to January 2025, depending on the sample size and complexity of analysis. The sample size calculated by Rao soft calculator by assuming 95% CI, 5% margin of error, Z (critical value for confidence level) of 1.96. The population of Islamabad is 1.01 million and the sample size is 384 and total population of Rawalpindi is 2.10 million and sample size is 383. Here is the link for Rao soft calculator from which the sample sizes have been calculated.

For the purpose of knowledge and awareness of oral candidiasis in Islamabad and Rawalpindi, a quantitative cross-sectional study was designed, and online and manual anonymous survey forms were prepared utilizing online Survey Forms from August 2024 to October 2024. There were seven sections of questions in the questionnaire, which were closed-ended. The questionnaire was completed both online and manually. The questionnaire was designed about the participant's socio-demographics, knowledge, attitude and behavior, perception, one's health, practices and interventions and risk factors related to oral candidiasis. Questions were generated after reviewing guidelines issued by World Health Organization, different articles on PubMed, Google scholar and NCBI. [14]

The occurrence and profusion of Oral candidiasis was investigated to determine the extent of its spread in Pakistan. Considering the previous epidemiological studies, the current impact and prevalence of Oral candidiasis was investigated. In epidemiology age, gender along with demographic data was collected.[15] Participants over the age of 10 are included in the study and participants under the age of 10 and those who had taken any antibiotic within 2 weeks are excluded from study.

Sterile swab samples of 50 dental caries patients from various dental clinics of Islamabad and Rawalpindi were collected under strict sterile conditions. The samples were mostly taken from carious lesions and dental plague surrounding it.

The oral cavity of patient was cleaned with distilled water by gargling to get rid of microorganisms on or around the decayed tooth or lesions. By using short blows saliva was removed from lesions. Contamination free oral swabs samples were taken. Touching the oral cavity during sample collection was avoided. Charcoal swab media was used to collect the

sample. For the activation of microorganisms, the samples collected were cultured in Brain Heart Infusion Broth (BHI) for 16-24 hours. By inoculating the growth of each BHI broth in Sabouraud Dextrose broth (SDB) for 16-24 hours at 37°C the Candida species were activated. [16]

To isolate, diagnose and purify the Candida species two selective culture media were used. Tenfold dilution of the SDB growth and 0.1ml was cultured on Sabouraud Dextrose agar (SDA) at 37°C for 24-48 hours. Specific yeast species determination was done based on morphological features of colonies, shape, color, and diameter after its growth on SDA. The growth from SDA was taken and cultured on HiCrome Candida differential Agar and was incubated at 37°C for 24-48 hours. This medium was used to distinguish the varied species of Candida based on color and appearance of surface of the colony distinct colors. The colony of *C. albicans* appeared light green in color, *C. tropicalis* exhibit colonies of blue color, *C. krusei* colonies was of light red in color, whereas the colonies of *C. glabrata* appeared creamy white.[17]

This study analyzed the Oral candidiasis prevalence in selected areas of Pakistan. SPSS statistics and Microsoft Excel were used to analyze the data in order to examine the results. Data was entered into Microsoft Excel, and for the statistical analysis, data was analyzed on the SPSS software by chi-square test and the statistical significance level was set at $p < 0.05$.

Ethical approval was attained by the Institutional Review Board (IRB) of the Department of Biosciences, COMSATS University Islamabad.

Results

Out of the total dental caries patients sampled, 56% tested positive for Candida species, with Candida albicans being the most prevalent isolate. Non-albicans species, including C. krusei, C. tropicalis, and C. glabrata, were also identified, with a notable percentage of mixed infections observed. Detailed distributions of these species and their relative frequencies are presented in Table I.

Sociodemographic and behavioral factors showed clear associations with oral candidiasis. The highest prevalence was recorded among young adults (particularly ages 21–30), males, and married individuals. Lifestyle-related risk factors such as smoking and poor oral hygiene significantly correlated with both higher prevalence and greater diversity of Candida isolates. Specifically, non-users of mouthwash or floss exhibited higher infection rates.

The results show that males had a higher prevalence of oral candidiasis (17 cases, 60.71%) than females (11 cases, 39.28%). The most frequent isolate in both sexes was *Candida albicans;* nevertheless, mixed growth was more common in males (23.52%) than in females (18.18%). In patients with dental caries, there was a statistically significant correlation between oral *Candida* infection and gender. While $p < 0.005$, $p = 0.000$.

The significance of taking marital status into account while analyzing and treating oral *Candida* infections is highlighted by the statistically significant correlation ($p = 0.000$, $p < 0.005$).

The increased frequency of mixed growth in both groups raises the possibility that smoking contributes to the diversity of fungi in the oral cavity. Because smoking is a statistically significant risk factor for oral *Candida* infections ($p = 0.000$, $p < 0.005$), smokers should receive tailored therapies to reduce their risk.

A comprehensive breakdown of species distribution in relation to age, gender, marital status, smoking, and hygiene practices is provided in Table II.

Table I: Prevalence of Oral Candidiasis in Dental Caries Patient

Candida species	(n)	%	Percentage of Isolates
No growth	22	44%	-
Candida albicans	15	30%	54%
Candida krusei	4	8%	14%
Candida glabrata	2	4%	7%
Candida tropicalis	3	6%	11%
Mixed growth	4	8%	14%
Total	28	100%	100%

Table II: Candida sp. Isolates and total in relation to Age, Gender, Martial Status, Smoking Status, Oral Hygiene Status of the Participants.

Factors		*Candida ablicans*	*Candida krusei*	*Candida galbarata*	*Candida tropicalis*	Oral candida N(%)	Mixed growth
Age (Years) Number (n)	10-20 (5)	3 (60%)	1 (20%)	0 (0%)	1 (20%)	13 (26%)	0 (0%)
	31-40 (08)	4 (50%)	0 (0%)	1 (12%)	0 (0%)	05 (10%)	3 (38%)
	41-50 (3)	1 (33.3%)	2 (66.6%)	0 (0%)	0 (0%)	06 (12%)	0 (0%)
	51-60 (2)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	04 (8%)	1 (50%)
	Total = 28	16 (57%)	5 (18%)	1 (3%)	2 (7%)	50 (100%)	4 (15%)
Gender	Male (17)	10(58.82%)	3 (17.64%)	0 (0%)	0 (0%)	27 (54%)	4(23.52%)
	Female (11)	5 (45.45%)	1 (9.09%)	0 (0%)	3(27.27%)	23(46%)	2 18.18%)
	Total	15 (53.6%)	4 (14.28%)	0 (0%)	3 10.71%)	-	6 21.42%)
Marital Status	Married (20)	10 (50%)	5 (25%)	2 (10%)	0 (0%)	27 (54%)	3 (15%)
	Single (8)	3 (38%)	1 (12%)	0 (0%)	2 (25%)	23(46%)	2 (25%)
	Widow(0)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Divorced(0)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Total= 28	13 (47%)	6(21%)	2(7%)	2(7%)	-	5(18%)
Smoking status	smoker (18)	10 (55%)	2 (11%)	1 (6%)	1 (6%)	17(34%)	4 (22%)
	Non-smoker(10)	6(60%)	0(0%)	0(0%)	1(10%)	33(66%)	3(30%)
	total=28	16(57%)	2(7%)	1(4%)	2(7%)	-	7(25%)
Oral Hygiene Status		4(44.4%)	2(22.2%)	0(0%)	2(22.2%)	43(86%)	1(11.1%)
	Non-users(19)	11(58%)	2(11%)	0(0%)	1(5%)	07(14%)	5(26%)
	Total=28	15(54%)	4(14%)	0(0%)	3(11%)	-	6(21%)

Discussion

The study revealed significant findings about the prevalence of *Candida* species and their association with oral candidiasis. *Candida albicans* was the most common isolate (54%), followed by non-albicans species such as *C. krusei* (14%), *C. tropicalis* (11%), and *C. glabrata* (7%). The prevalence was highest among younger age groups, with 70% in the 21–30 age group and 60% in the 10–20 age group.[1,2] Age has been noted as a usual factor in the probability of developing oral candidiasis in prior reports. Age, however, may be a contributory variable in co-infections, according to the current study. It was more common in men (60.71%) than in women (39.28%). Married individuals exhibited the highest prevalence (71%), possibly due to lifestyle or environmental factors. Smoking emerged as a significant risk factor, with smokers showing a higher diversity of *Candida* species and a greater proportion of isolates (64%). Poor oral hygiene was strongly associated with oral candidiasis, as 68% of non-users of mouthwash or floss were affected compared to 32% of users.[18,19]

Wearing dentures can raise the risk of developing a fungal infection called Candida albicans and cause mechanical discomfort and inflammation. The preservation and growth of germs are facilitated by dentures, which raises the possibility of Candida colonization and denture stomatitis.

Just 28.1% of respondents to a survey by Agrawal et al. reported maintaining adequate oral hygiene.[20]

Compared to the studies of Santiwongkarn et al. and Nabavi et al., it was lower. The prevalence of oral candidiasis in all participants was 7.94%, according to the South Iran study's findings. This was greater than some earlier research conducted in Slovenia, India, and northern Iran. Despite the fact that Faraz's study found that oral candidiasis was more common in men, other research suggests that oral candidiasis is more common in women.

According to Fragkou et al., children aged 3 to 13 who were caries-active had a considerably larger quantity of Candida and harbored the bacteria more frequently than children who were not caries-active. Children with dental cavities in Yemen had saliva with Candida levels of at least 1000 cfu/mL.[21]

Studies have indicated an increase in Candida, among individuals aged 80 and above with cavities or dental appliances low saliva acidity levels and reduced red blood cell counts.

These findings are supported by literature noted that individuals with Candida spp. had a 2.3-fold greater prevalence of dental caries, while Stoopler *et al*., 2024 reported a 64% prevalence of *Candida* species among individuals with dental conditions.[22,23] The inverse relationship between age and candidal carriage observed in this study aligns with, who reported a higher prevalence in younger individuals. Gender differences in oral candidiasis are consistent, which highlighted disparities in oral health knowledge and habits.

According to research from Iran, the prevalence of oral candidiasis was about three times higher in cigarette smokers and 1.5 times higher in ex-smokers than in non-smokers. These results were in line with those of other investigations. According to a tenable theory, smoking cigarettes lowers salivary flow rate, which in turn lowers saliva Ph.

Behavioral factors, such as smoking and poor oral hygiene, were strongly linked to Candida prevalence, consistent with findings from (24) and (25), who emphasized the role of oral hygiene and smoking in creating a cariogenic environment. Overall, these results underscore the critical need for targeted interventions focusing on age, gender, marital status, smoking cessation, and improved oral hygiene to effectively manage and prevent oral candidiasis.

Conclusion

Candida albicans is the leading cause of oral candidiasis, with non-albicans species such as *Candida krusei, Candida tropicalis*, and *Candida glabrata* also playing a growing role. Factors like poor oral hygiene, smoking, systemic diseases, cancer therapies, and weakened immunity contribute significantly to oral candidiasis prevalence. Oral candidiasis was more prevalent in young adults (21–30 years) compared to other age groups, in married individuals compared to single or divorced individuals, and in men compared to women. Smokers exhibited greater species diversity of Candida compared to non-smokers, while the prevalence of oral candidiasis was higher among non-mouthwash users (68%) compared to mouthwash users. To address these challenges, public health initiatives should focus on improving oral hygiene and reducing risk factors like smoking. Future research should explore interactions within the broader oral microbiome, including fungi, bacteria, viruses, and other microorganisms, to better understand disease mechanisms. Advanced in vitro models and artificial intelligence can enhance our understanding of fungal infections, enabling more accurate diagnosis and treatment. Finally, novel antifungal therapies are urgently needed to combat resistance and improve outcomes.

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