

Original Article

## Incidence of *Candida* Species Biofilms in Pediatric Cancer Patients Undergoing Chemotherapy Treatment

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**Article Info**

Received: May 14, 2023

Revised: June 10, 2023

Accepted: June 18, 2023

### Abstract

The oral thrush due to biofilms of *Candida* species is a common infection for the oral cavity of cancer patients undergoing chemotherapy, leading to oral infections. These biofilms, composed of *Candida* organisms, pose challenges in terms of diagnosis and treatment due to their increased resistance and ability to persist in the oral environment. This study aimed to determine the frequency of *Candida* species biofilm colonization in the oral cavity of pediatric cancer patients receiving chemotherapy. By examining the incidence of *Candida* sp. biofilms, this research provides valuable insights into the oral health challenges specific to children with cancer, emphasizing the need for targeted preventive and management strategies to address these infections effectively. In this study, a total of 100 oral cavity swabs were collected, with 50 swabs obtained from cancer patients undergoing chemotherapy and suspected to have oral candidiasis, and the other 50 swabs collected from non-cancer healthy individuals serving as control. All swabs were cultured on Sabouraud Dextrose agar (SDA) media, followed by microscopic examination of positive samples. The API identification candida system was utilized for the identification of *Candida* species, along with the implementation of biochemical tests to further study the characteristics of the identified species. The findings demonstrated that out of the 50 cancer patients, approximately 47% (23 patients) exhibited positive yeast growth on SDA agar. Among the 50 non-cancer control cases, approximately 30% (15 cases) showed positive growth on SDA agar. Furthermore, using the API candida system, we confirmed the presence of candida species in 25 cases. *Candida albicans* was identified as the most prevalent species, followed by *Candida parapsilosis*, *Candida krusei*, and *Saccharomyces cerevisiae*, which were detected less frequently.

In conclusion, there were a significant increase in *Candida* species among cancer patients undergoing chemotherapy treatment in comparison to non-cancer control individuals. This finding highlights the impact of chemotherapy on the prevalence of *Candida* infections in cancer patients, emphasizing the need for heightened attention and appropriate management strategies in this vulnerable population.

**Keywords:** Oral candidiasis, Fungal, *Candida* sp., Biofilms, Cancer.

### INTRODUCTION

Cancer patients undergoing chemotherapy are highly susceptible to fungal infections, particularly those caused by *Candida* species, due to their compromised immune system. *Candida* yeasts are typically present as commensal organisms in the oral cavity, with a prevalence of approximately 20-

60% in the general population. However, in immunocompromised individuals, these yeasts can transition into opportunistic pathogens, and their ability to cause infection is influenced by both the virulence factors of the organism and the host's immune response.<sup>1-3</sup> When there is an imbalance

in the normal oral microbiota or when the host's immune system defenses are compromised, *Candida* species seize the opportunity to proliferate. This can lead to recurrent infections in the oral mucosal cavity. Factors such as dimorphism, phenotypic switching, extracellular enzyme production, adhesive properties, and biofilm formation contribute to the colonization and persistence of *Candida* in the oral cavity.<sup>4-5</sup> Oral candidiasis (OC) is a fungal disease caused by the overgrowth of yeast-like fungi known as *Candida* in the oral cavity. It is an opportunistic infection that frequently affects the oral mucosa.<sup>6-8</sup>, the common important sorts of *Candida* are *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. pseudotropicalis*, *C. guilliermondii*, *C. krusei*, *C. lusitanae*, *C. parapsilosis*, and *C. stellariae*. approximately 80% isolated from clinical infection<sup>9</sup>. Oral candidiasis, also known as oral thrush or candidiasis, refers to infections that occur in the mouth, primarily on the tongue, inner cheeks, and other areas of the oral mucosa. It is a condition caused by *Candida* overgrowth in the oral cavity.<sup>10</sup>. Moreover, that oral candidiasis is a *mycosis* (yeast spherical & dimorphic, fungal infection)<sup>11</sup> of *Candida sp.* on the mucous membranes of the mouth, correspondingly it is commonly present as a superficial infection of the vaginal mucous<sup>12</sup>. OC is commonly in human fungal infection<sup>13,14</sup>, predominately in early and later life<sup>13</sup>. Oral candidiasis can be influenced by various predisposing factors, including systemic conditions such as impaired systemic defense mechanisms, immunodeficiency, immunosuppressive medications, malignancies, diabetes, congenital conditions, and the use of broad-spectrum antibiotics. Local factors such as impaired local defense mechanisms, smoking, poor oral hygiene, mucosal diseases, and reduced saliva production also contribute to the development of oral candidiasis.<sup>15</sup>

*Candida albicans* is the main causal agent of oral candidiasis in up to 95% of cases, *C. albicans* is commonly in the mouth or oral mucosa, moreover it can isolate from mouth cavities of healthy cases<sup>16-18</sup>. *C. albicans* is a commensal and versatile organism that can lead to good adaptation to the

human host, the change in the microenvironment of the host causing the proliferation of pathogen and increasing the opportunity to invade any site<sup>16,19,20</sup>.

*Candida* has several virulence features that participate in oral candidiasis pathogenesis<sup>21</sup>. Most species of *Candida* in vivo form hyphal structures that assist the yeast to penetrate the oral epithelium as well as help to adhere to the mucosal surface<sup>22</sup>. The oral mucosal cavity is an optimal environment for the lifestyle of microbial. Extrapolymeric material and hyphae are essential for biofilm growth as a result of the maintenance of the integrity of the structure, the capacity of adhesive, and their participation in the resistance of antifungals. Moreover, the release of extracellular lipolytic and proteolytic enzymes is associated with their pathogenicity<sup>23,24</sup>. This research aimed to examine the occurrence and epidemiology of *Candida sp.* biofilms in the oral mucosal cavity of cancer patients undergoing chemotherapy, in comparison to a healthy control group, within the city of Erbil. The study focused on identifying the incidence and characteristics of biofilm formation by *Candida* species in these two patient populations.

## MATERIALS AND METHODS

### Sample Collection

Between 10th January and 1st March 2018, samples were collected from pediatric patients aged 1-9 years with different types of cancer undergoing chemotherapy at Nanakali Hospital, specialized in cancer treatment. Additionally, samples were collected from healthy non-cancer cases as control from Raparin Hospital in Erbil-city. A total of 100 oral swabs were obtained, with 50 swabs taken from cancer patients suspected of having oral candidiasis, and the other 50 swabs collected from non-cancer healthy individuals for comparative purposes. A questionnaire was administered to gather medical and clinical histories from all participants. Sterile swabs were used for sampling, with the swabs employed for both microscopic examinations to assess their characteristics and for culture to isolate *Candida* species.

### Identification and Isolation of *Candida* species

Sabouraud Dextrose (SDA) agar (microexpress, Accumix, India) was used to isolate *Candida*

*sp.*, SDA agar was prepared according to manufactures protocol via mixing 65.0 gm of SAD powder with 1 liter of water, followed by heating and sterilized by autoclave. Oral thrush swabs were inoculated or grown on the surface of SDA agar, then incubated at 37°C for 24-48 hours. The API Candida system from (bioMérieux, France) is a combined system utilized to identify yeasts within 18-24 hours. The API candida strip contains 10 well consisting dehydrated substrates, which can be used to identify 12 tests (enzymatic reactions or sugar acidification), which consisting GLU: D-glucose, GAL: D-galactose, SAC: D-saccharose, TRE: D-trehalose, RAF: D-raffinose,  $\beta$ MAL: 4-nitrophenyl  $\beta$ Dmaltopyranoside,  $\alpha$ AMY: 2-chloro-4-nitrophenyl- $\alpha$ D maltotrioxide,  $\beta$ XYL: 4-nitrophenyl- $\beta$ Dxylopyranoside,  $\beta$ GUR: 4-nitrophenyl- $\beta$ Dglucuronide, URE: urea,  $\beta$ NAG: 5-bromo-4-chloro-3-indoxyl-N-acetyl $\beta$ D-glucosaminide,  $\beta$ GAL: 5-bromo-4-chloro-3-indolyl- $\beta$ Dgalactopyranoside. The reactions produced results after incubation is detected by changing in colour spontaneously. The inoculum was prepared by using NaCl 0.85 % Medium which provided with API candida strip, identical and well-isolated colonies of yeast was mixed with NaCl medium to prepare suspension with turbidity equal to 3 McFarland, suspension was homogenized and used directly with API candida strip. Then distributing the prepared yeast suspension into the strip, and the tubes were covered by parafilm. The bottom of the box was felt with distilled water to avoid evaporation, closing the incubation box, an aerobic condition was used to incubate at 36°C  $\pm$  2°C for 18-24 hours. The reaction code is obtained into a numerical profile: on the sheet result provided with the kit, the tests are divided into three groups and a number 1, 2 or 4 is appropriated to each one. Together adding it, the numbers identical to positive reactions within each group, we obtained a 4-digit numerical profile.

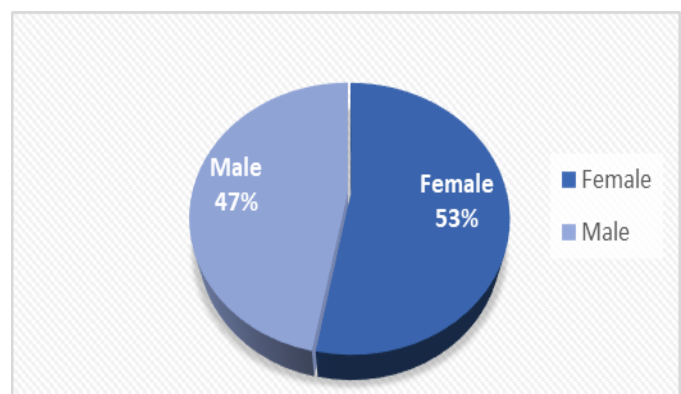
**Statistical Analysis**

The data were analyzed using Microsoft Office Excel 2016 and Statistical Package for the Social Sciences (SPSS, version 25).

**RESULTS**

**Participants' characteristics**

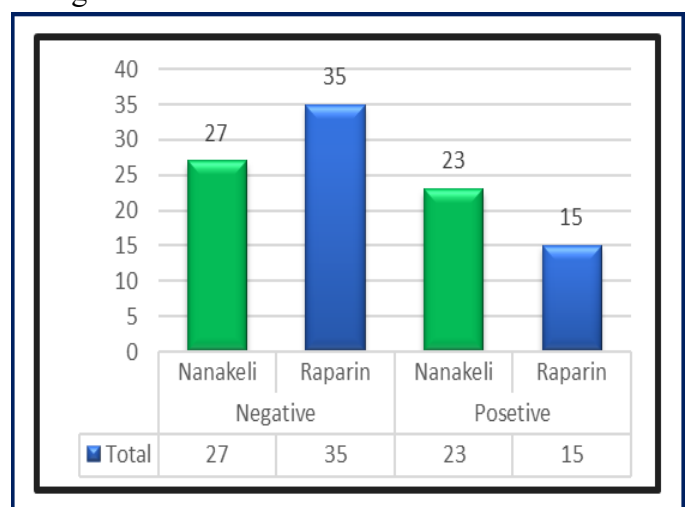
A total of 100 participants were included in this study, with 50 patients having different types of cancer collected from Nanakali Hospital and 50 non-cancer healthy individuals serving as controls from Raparin Hospital. Among the participants, approximately 53% were female and 47% were male. The mean  $\pm$  standard deviation (SD) age of the participants was 1.8 years, as shown in Figure 1, which illustrates the gender differences.



**Figure 1.** The percentage of two gender that participated in the study.

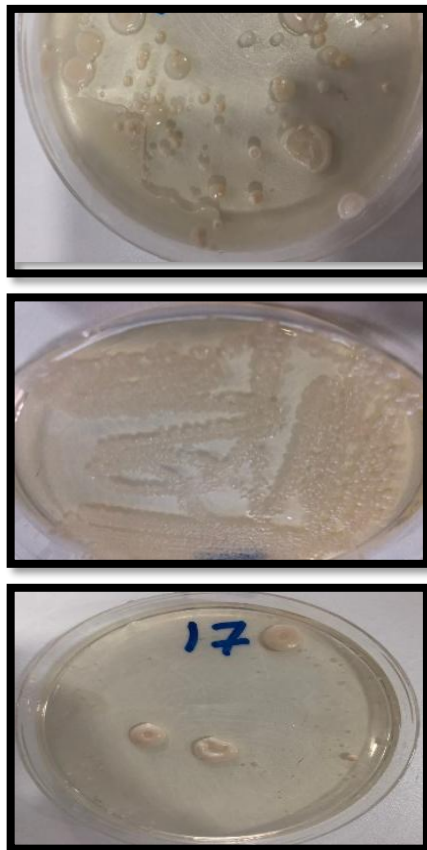
**Colonies characteristics**

The colonies of *Candida sp.* grown on Sabouraud Dextrose agar (SDA) were observed to have a creamy to white color, soft consistency, smooth texture, and a fermented smell, as depicted in Figure 2.



**Figure 2.** The *Candida sp.* growth on SDA agar

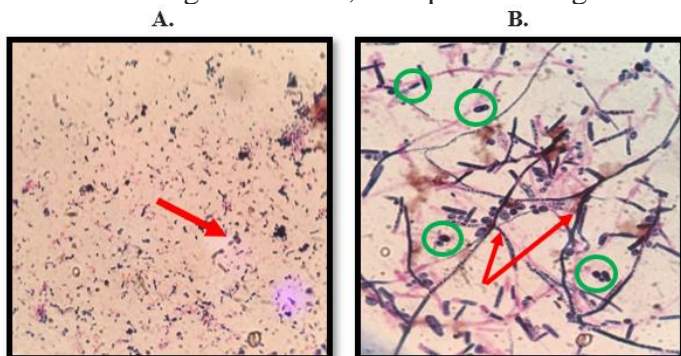
A total of 38 samples showed growth on SDA agar, with 23 samples obtained from Nanakali Hospital and 15 samples obtained from Raparin Hospital, as illustrated in Figure 3.



**Figure 3.** The number of positive and negative *Candida sp.* growth on SDA agar

**Microscopic examination of candida Sp.**

Microscopic examination of the 38 isolated samples that showed positive growth on SDA agar revealed the presence of *Candida sp.* in 30 samples. Under the microscope, *Candida sp.* appeared as oval to spherical or elongated oval cells. Additionally, hyphae and pseudohyphae with long branching filaments or tube-like structures were observed using Gram stain, as depicted in Figure 4.

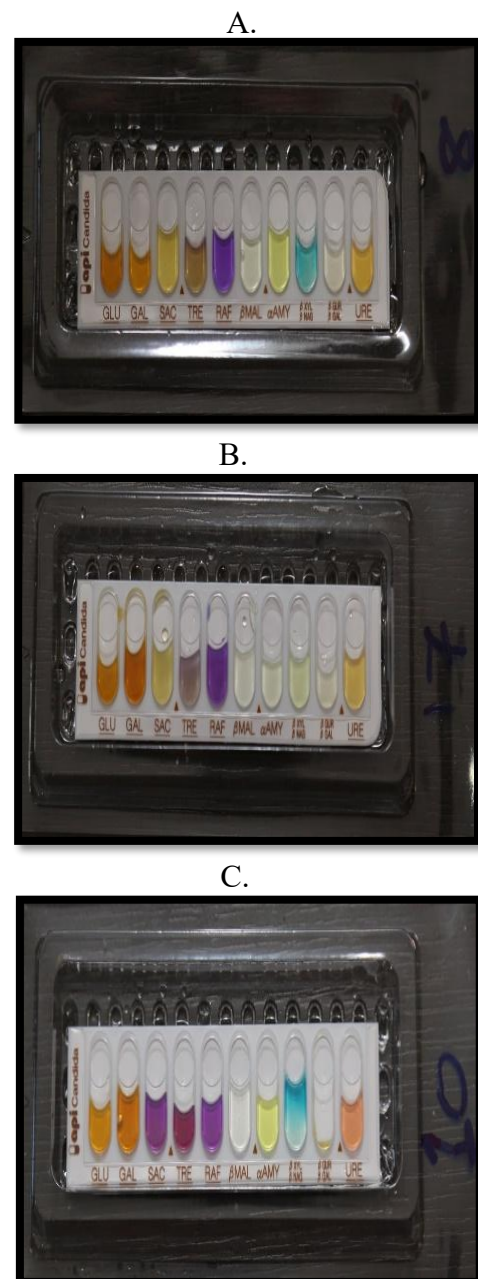


**Figure 4.** Microscopic examination of *Candida sp.* (A) the yeast body of *Candida sp.* (B) red pointer showing the hyphae and green circles representing *Candida Krusei*

**API Candida System (Biochemical characteristics)**

The identification of positive yeast growth on SDA agar was performed using the API candida system. Out of the 38 samples, 25 were confirmed

as positive using this system. Among the positive samples, 20 were identified as *Candida albicans*, *Candida parapsilosis*, or *Candida krusei* based on the code numbers obtained from the positive and negative biochemical tests provided by the manufacturer. Figure 5 illustrates the API candida system strips used for identifying positive *Candida* species. Furthermore, we detected 5 samples to be *Saccharomyces cerevisiae*.



**Figure 5.** API candida identification system referred to the positive and negative biochemical tests for *candida sp.* (A) *Candida albicans* (B) *Candida parapsilosis* (C) *Candida krusei*.

The changes observed in the biochemical tests using the API candida system are summarized in Table 1.

**Table 1.** Biochemical characteristics (API candida system) of yeast species isolated from oral thrush samples

API candida	GLU	GAL	SAC	TRE	RAF	βMAL	αAML	βXYL	βGUR	URE
<i>Candida albicans</i>	+	+	+	+	-	+	-	-	-	-
<i>Candida parapsilosis</i>	+	+	+	-	-	-	-	-	-	-
<i>Candida krusei</i>	+	-	-	-	-	-	-	-	-	-
<i>Saccharomyces cerevisiae</i>	+	+	+	-	+	-	-	-	-	-

(+) positive reaction, (-) negative reaction

**DISCUSSION**

Y-chromosomal Oral thrush, also known as candidiasis, is a prevalent infection of the oral mucosal cavity caused by opportunistic microorganisms. Candida species, which normally exist as commensal organisms, can become pathogenic when there is a breakdown in the host immune system's ability to inhibit their overgrowth<sup>25</sup>. Various systemic conditions can result in compromised immune systems, including HIV, chemotherapy treatment, organ transplantation, and diabetes mellitus. These conditions weaken the body's ability to effectively combat infections, making individuals more susceptible to opportunistic pathogens such as Candida species.<sup>26</sup> Cancer and chemotherapy treatment induce immunosuppression, creating a conducive environment for the proliferation of Candida infections. This immunocompromised state, coupled with mucosal damage, disrupts immune function and contributes to the development of yeast infections like candidiasis.<sup>27,28</sup> In a previous study, it was found that cancer patients exhibit a significantly higher susceptibility to Candida sp. colonization in the oral mucosal cavity, with an incidence of approximately 77.8%, compared to 37.5% in healthy control individuals. This increased susceptibility can be attributed to various factors, including the use of radiation, chemotherapy, and certain medications that suppress the host immune system, ultimately leading to the development of candidiasis infections.<sup>29,30</sup> Our study revealed a significant increase in oral Candida sp. infections among patients undergoing chemotherapy compared to healthy non-cancer control individuals. We isolated a total of 38 positive yeast growth samples on SDA agar, with 23 cases (46%) from Nanakali Hospital representing cancer patients and 15 cases (30%)

from Raparin Hospital representing non-cancer cases. The higher prevalence in cancer patients may be attributed to the underdeveloped immune system in children at these ages. Furthermore, the API candida system confirmed that 25 cases were positive for Candida sp. In this study, using the API Candida system, we identified four Candida species, including Candida albicans, Candida parapsilosis, Candida krusei, and Saccharomyces cerevisiae. Among these species, Candida albicans was the most frequently detected. Our findings are consistent with the research conducted by González,<sup>31</sup> who identified *Candida species* causing candidiasis in chemotherapy patients. Similarly, Bashir's study on adult cases indicated a higher susceptibility to *Candida sp.* infection in patients undergoing chemotherapy<sup>32</sup>, Aldossary's research also reported Candida infection in the oral cavity of cancer patients.<sup>2</sup> Furthermore, our results align with the previous work conducted by Ramírez.<sup>33</sup>

**CONCLUSION**

Oral candidiasis (thrush) is a prevalent fungal infection observed in cancer patients undergoing chemotherapy. Our study revealed a notable increase in the colonization of Candida species in the oral mucosal cavity of cancer patients receiving anti-cancer treatments. These findings highlight the association between Candida species and the oral mucosal cavity in immunocompromised cancer patients. Early detection and isolation of Candida species are crucial for determining appropriate and effective antifungal treatment strategies.

**Acknowledgment**

The authors express deep thanks to all participants and allowing us to take oral swab despite his/her suffering.

## Conflict of Interest

immunity. *Microb Infect.* 2011;13(12-13):963-976.

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