



Oropharyngeal Candida Colonization in Patients with Acute Myeloid Leukemia

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ABSTRACT

Objectives: Oral candidiasis has increased in recent years because of the increasing number of high-risk populations. The prevalence of *Candida* species is different worldwide because of the difference between population characteristics, sampling protocols, and geographic regions. Therefore, determining the more prevalent *Candida* species in different geographic regions seems essential. This study aimed to determine the more prevalent *Candida* species in acute myeloid leukemia (AML) patients in comparison with healthy individuals in Iran in 2016.

Materials and Methods: Fifty-one patients with AML and 62 healthy controls participated in this cross-sectional study. Samples were collected using a swab rubbed softly on the dorsal surface of the tongue and the oropharynx. The samples were cultured on CHROMagar *Candida* for 2 to 4 days. For differentiation between albicans and non-albicans species, positive samples were linearly inoculated on Corn Meal Agar with Tween-80. *Candida* species were identified using a microscope. Data were analyzed using chi-square and Fisher's exact tests.

Results: *Candida* colonization was more frequent in AML patients (41.2%) in comparison with healthy participants (38.7%). *Candida glabrata* (*C. glabrata*; 27.5%) and *Candida albicans* (*C. albicans*; 32.3%) were the most common isolated species in the AML patients and the controls, respectively. There was a significant decrease in the frequency of *C. albicans* ($P=0.022$) and a significant increase in the frequency of *C. glabrata* ($P=0.002$) in the AML patients in comparison with the controls.

Conclusion: AML patients are more susceptible to candidiasis. *C. glabrata* is the dominant *Candida* species in AML patients.

Keywords: Acute Myeloid Leukemia; *Candida*; Oral Candidiasis; Oropharynx

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INTRODUCTION

Candida species are part of the healthy microflora of the oropharynx and are present in

the oral cavity of 15.2% to 75% of the healthy population. Candidiasis is the most prevalent opportunistic fungal infection in the oral cavity.

Although *Candida albicans* (*C. albicans*) is a weak pathogen in healthy individuals, it is the cause of candidiasis in about 85% of patients [1]. *C. albicans* can convert from healthy microflora to a pathogenic microorganism in conditions such as diabetes mellitus, organ transplant, acquired immunodeficiency syndrome (AIDS), and antineoplastic chemotherapy [2-5].

Oral candidiasis has increased in recent years because of the increasing number of high-risk populations [6]. Oral *Candida* species can cause infection in the gastrointestinal (GI) tract, can spread to the bloodstream, and can even be life-threatening [7]. Invasive fungal infections are most common in patients with positive fungal colonization. An increasing rate of oral fungal colonization leads to the development of severe clinical complications and invasive infections [8]. Although recent studies have shown that *C. albicans* is the most frequently detected strain in both healthy individuals and patients, non-*albicans* species show an increased rate, especially in patients with a compromised immune system [8-10]. Recently, the significance of non-*albicans* species has increased because of the resistance of some species, like *C. tropicalis* and *C. glabrata*, to antifungal drugs [11].

Patients with acute myeloid leukemia (AML) are more susceptible to Candidal infections because of deficiencies in the immune system [12]. The distinction of different species is critical due to variable susceptibility to antifungal drugs and the diversity of the virulence of *Candida* species [13]. The antifungal prophylactic protocol is recommended in some high-risk and immunocompromised cases, like cancer patients, and it reduces the mortality rate due to yeast infections [14].

The prevalence of *Candida* species is different worldwide because of the difference between population characteristics, sampling protocols, and geographic regions [15,16]. Therefore, it seems essential to have a valuable source of data about the most prevalent *Candida* species in Iran.

This study aimed to determine the more prevalent *Candida* species in AML patients, who are susceptible to candidiasis, in comparison with healthy individuals in Iran to

gain essential information for effective treatment.

MATERIALS AND METHODS

This cross-sectional study was conducted from January 2016 to January 2017. Type 1, type 2 errors and effect size were considered 0.05, 0.2, and 0.2, respectively. Fifty-one patients (group 1) with AML hospitalized in the hematology-oncology ward of Valiasr Hospital of Tehran University of Medical Sciences, Tehran, Iran, participated in this study. The sampling was carried out before the initiation of antineoplastic treatments.

Sixty-two healthy controls (group 2) were selected from among individuals who referred to the School of Dentistry of Tehran University of Medical Sciences. The Ethical Committee of Tehran University of Medical Sciences approved this study (IR.TUMS.REC.1395.2510).

Patients with possible risk factors of candidiasis, such as human immunodeficiency virus (HIV) / hepatitis B virus (HBV) / hepatitis C virus (HCV) positive, diabetes, Sjogren's syndrome and other autoimmune diseases, dentures, oral *Candida* lesions, maxillofacial radiotherapy, and those already taking antifungal drugs were excluded from the study.

In the control group, the exclusion criteria were systemic diseases and taking antifungal drugs for three months previously. Unconscious patients and those with severe clinical conditions were excluded. Pregnant and lactating women were excluded as well.

The aim of the study was explained to both groups, and written consent was received. The baseline data, including age ($P=0.772$), gender ($P=0.584$), and smoking habits ($P=0.884$), were matched between the two groups.

Samples were collected between 9 a.m. and 12 a.m. using a dry sponge swab in a test tube filled with normal saline (pH=7.2) by softly rubbing the swab on the dorsal surface of the tongue and the oropharynx.

The samples were cultured on CHROMagar *Candida* and Sabouraud dextrose agar and were incubated at 35°C for 2 to 4 days. The growth of any *Candida* colonies was recorded as a positive case.

Candida species have chromogenic factors; the

change of color in the culture plates confirmed the initial fungal growth. To differentiate albicans and non-albicans species, positive samples were linearly inoculated on Cornmeal Agar with Tween-80 at 25°C for 3 to 5 days. Afterward, microscopic identification of Candida species was carried out by observing fungal morphology (formation of blastoconidia, chlamydoconidia, filamentous pseudohyphae, pseudomycelial, and mycelial) [17,18].

Different types of Candida species were recorded. Raw data were analyzed using chi-square and Fisher's exact tests in SPSS (version 20; SPSS Inc., Chicago, IL, USA). P-values of <0.05 were considered statistically significant.

RESULTS

Fifty-one patients with the mean age of 36.52±12.31 years and 62 healthy individuals with the mean age of 36.48±11.78 years participated in this study.

The demographic data of the participants are shown in Table 1. There was no significant difference in smoking between the patients and the controls (P=0.884).

Table 1. Mean±Standard Deviation and significance of population demographics

| | Patients | Healthy Controls | P-value |
|----------------|--------------------|------------------|---------|
| Age (y) | 36.52±12.31 | 36.48±11.78 | 0.772 |
| Sex | F 31(60.7%) | 37(59.6%) | 0.584 |
| | M 20(39.3%) | 25(40.4%) | |
| Smoking | 10(19.6%) | 12(19.4%) | 0.844 |

F: Female; M: Male

Overall, 21 and 24 positive samples were isolated from the patients and the controls, respectively. The oral colonization rate of Candida in the AML group (41.2%) was not significantly (P=0.21) higher than that in the control group (38.7%).

The frequency of *C. glabrata* was significantly

(P=0.002) higher in the AML group (27.5% versus 6.5%), whereas *C. albicans* was significantly more frequent (P=0.022) in the control group (32.3% versus 13.7%).

Multiple oral Candida colonization with two or more Candida species was more frequent in the AML group (7.9%). Other common oral Candida species isolated from the patients were *C. albicans* (13.7%), *C. tropicalis* (3.9%), *C. crusie* (2%), and *C. parapsilosis* (2%). *C. glabrata* (6.5%) and *C. tropicalis* (6.5%) were other common Candida species in the control group. There was no positive sample for *C. crusie* and *C. parapsilosis* in the control group (Table 2).

Table 2. The frequency of oral *Candida albicans* (*C. albicans*) and non-albicans species positive samples in patients and healthy controls

| Candida species | Patients N(%) | Controls N(%) |
|--|---------------|---------------|
| <i>C. albicans</i> | 6(11.7) | 17(27.5) |
| <i>C. glabrata</i> | 10(19.6) | 3(4.8) |
| <i>C. crusie</i> | 1(2) | 0(0) |
| <i>C. tropicalis</i> | 0(0) | 1(1.6) |
| <i>C. albicans-C. glabrata</i> | 1(2) | 0(0) |
| <i>C. albicans-C. tropicalis</i> | 0(0) | 2(3.2) |
| <i>C. glabrata-C. parapsilosis</i> | 1(2) | 0(0) |
| <i>C. glabrata-C. tropicalis</i> | 2(3.9) | 0(0) |
| <i>C. albicans-C. glabrata-C. tropicalis</i> | 0(0) | 1(1.6) |
| Total | 21(41.2) | 24(38.7) |

DISCUSSION

This study aimed to determine dominant Candida species in AML patients. The results showed that oral Candida colonization was higher in AML patients compared to healthy controls. According to previous studies, the risk of opportunistic infections, especially fungal infections, is increasing due to an increase in patients with suppressed immune systems [2,3,19,20].

The prevalence of Candida species in AML

patients is different in various geographic regions. Agarwal and Joyce [21] (2014) stated that fungal infections, especially candidiasis, increase the mortality rate of AML patients by 55% in Florida. In China, the frequency of *C. albicans* was 12.7% in patients with blood malignancy, 30.8% in lung cancer, 33.7% in patients with GI tumors, and 25.7% in the controls [8]. The patients used chlorhexidine and alkaline mouthwashes, which may have caused a lower prevalence of Candida in these patients. Non-albicans Candida was more prevalent in patients than in the controls [8]. In the United States of America (USA), *C. Kefyr* was reported in 83% of patients with blood malignancies at the Johns Hopkins Hospital [22].

Falagas et al [23] stated that *C. albicans* is more prevalent in hospitalized patients in Northern USA and Europe while non-albicans Candida is dominant in South Africa and Asia. *C. albicans* is more prevalent in Northern Europe and Switzerland (>60%) compared to other European countries and the USA (45-58%). This fungal infection is less prevalent in Asia and South America (40-42%). On the other hand, non-albicans species are found mainly in Asia. The highest prevalence of *C. glabrata* was reported in the USA (18.8-24%) and the United Kingdom (UK; 22.7%) while the lowest prevalence rate was reported in Brazil (4.9%) and Kuwait (5.6%). The prevalence of *C. parapsilosis* was reported to be 30.6% in Kuwait, 20.5-21.3% in South America, 23% in Spain, 19.9% in Australia, 1-5% in Switzerland, and 4.4% in Northern Europe. The prevalence of *C. tropicalis* was reported to be 20.9-24.2% in South America, 22.4% in Taiwan, 11-12% in the USA, and 4% in Central and Northern Europe. *C. krusei* was isolated only in Finland and France. *C. albicans* was the dominant species in Northern and Central Europe and the USA. Non-albicans species predominated in South America, South Europe, and Asia.

The highest prevalence of *C. albicans* was reported in Hungary (81.7%), Northern Europe (63.5-66.8%), Taiwan (63.7%), and

Switzerland (61.3%). The frequency of *C. parapsilosis* was 44% in Saudi Arabia, 35.5% in Italy, and 32% in Portugal. Lower rates have been reported in Switzerland and Brazil (4%) [23].

The frequency of candidiasis in HIV positive patients is lower than that in AML patients [16]. Accordingly, the risk of aggressive candidiasis is higher in AML patients due to more prevalent oral Candida colonization [8].

Although *C. albicans* is the most prevalent species, the prevalence of non-albicans species has increased in recent years [2].

In this study, *C. glabrata* (27.5%) was the most prevalent species in AML patients, followed by *C. albicans* (13.7%). The possible reasons are the higher susceptibility of the host immune system to non-albicans species in cancer patients, epidemiologic differences in the distribution of Candida species, and long-term antifungal drug therapy as non-albicans species are more resistant to some types of antifungal drugs [8].

In this study, the frequency of *C. albicans* was lower in patients hospitalized in the oncology ward compared to healthy controls, which is similar to the results of previous studies [2,24].

Candida colonization with two or more Candida species was more prevalent in patients than in healthy controls. This result was similar to previous studies indicating that Candida colonization with two or more Candida species is more common in patients than in healthy individuals, although there is a greater difference between patients and controls in previous studies [8].

The difference in the prevalence of Candida species can be due to population characteristics, sampling protocols, and geographic regions [15,16]. Also, chemotherapy and radiotherapy are effective factors in the distribution of Candida species [2,25]. In the present study, the patients were examined before any cancer treatment (chemotherapy or radiotherapy); this might be one of the possible reasons for the difference in

Candida distribution in comparison with previous studies.

In the current study, the most prevalent Candida species in healthy controls was *C. albicans* (32.3%), which was similar to the findings of previous studies [8,26], although the prevalence of *C. albicans* was slightly lower in the present study.

CONCLUSION

In conclusion, AML patients are more susceptible to candidiasis as Candida colonization is more prevalent in these patients. The dominant Candida species in AML patients was *C. glabrata* followed by *C. albicans*.

Because of the increasing prevalence of non-*albicans* species and antifungal drug resistance of some Candida species, such as *C. glabrata*, antifungal drugs effective against dominant species are recommended, especially for high-risk patients.

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CONFLICT OF INTEREST STATEMENT

None declared.

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