Prevalence and risk factors of fungal infections in leukemia patients treated with chemotherapy

Dr . Suzan Samra [*]
Dr . Samer Akel**
Dalia Suliman [***]

(Received 19 / 1 / 2023. Accepted 7 / 3 / 2023)

□ ABSTRACT □

Fungal diseases are serious complications in the treatment of leukemia patients and are linked to a high morbidity and mortality rate. Therefore, a correct definition of the risk level of fungal infections helps to improve strategies for the use of an antifungal prophylactic regimen. In the current study; we compared the prevalence of fungal infections pre and during chemotherapy in patients newly diagnosed with leukemia at Tishreen University Hospital in Lattakia, Syria. We investigated the risk factors for fungal infections in these patients during chemotherapy. Fungal infections diagnosis relied on both clinical symptoms and culture results on selective media (sabouraud dextrose agar and potato dextrose agar). We included fifty leukemia patients in this study (19 ALL, 14 AML, 12 CLL, and 5 CML). Fungal infections appeared in 11 patients (22%) before starting chemotherapy, all of these infections were of the genus Candida. While these infections appeared in 30 patients (60%) during chemotherapy, 48% were Candida, 10% were Rhizopus, and 2% were Aspergillus. Our results showed that the difference was significant in the percentage of fungal infections between pre-chemotherapy and during chemotherapy (p-value = 0.0001). While using the chi-square test or Fisher exact test, we found statistically significant differences between the two groups classified according to the incidence of fungal infections during chemotherapy in terms of smoking (p = 0.01), corticosteroid therapy (p = 0.0001), and the combination of the two previous risk factors (p = 0.0001), but there were no statistically significant differences between the two groups in terms of gender (p = 0.2), age (p = 0.1), and type of leukemia (p = 0.073).

Keywords: fungal infections, leukemia, antifungal prophylaxis, chemotherapy, Candida, Rhizopus, Aspergillus, risk factors.

[*]**Assistant Professor, Department of Biochemistry and Microbiology at the Faculty of Pharmacy-Tishreen University, Lattakia, Syria.**
****Assistant Professor, Department of Oncology and Hematology, Faculty of Medicine, Tishreen University, Lattakia, Syria ..**
*****Postgraduate Student, Department of Biochemistry and Microbiology at the Faculty of Pharmacy-Tishreen University, Lattakia, Syria dalia.solaiman@tishreen.edu.sy**

انتشار وعوامل خطورة الإنتانات الفطرية عند مرضى الابيضاضات الخاضعين للعلاج الكيميائي

د. سوزان سمرة*

د. سامر عاقل**

داليا سليمان***

(تاريخ الإيداع 19 / 1 / 2023. قبل للنشر في 7 / 3 / 2023)

□ ملخّص □

تعدّ الإنتانات الفطرية من المضاعفات الخطيرة لعلاج مرضى الابيضاضات كما وترتبط مع ارتفاع معدل الإمراضية والوفيات لديهم. يساعد التعريف الصحيح لمستوى مخاطر الإنتانات الفطرية في تحسين استراتيجيات استخدام العلاج الوقائي المضاد للفطريات. تمت في هذه الدراسة مقارنة انتشار الإنتانات الفطرية بين مرحلة ما قبل العلاج الكيميائي وأثناء العلاج الكيميائي لدى مرضى الابيضاضات المشخصين حديثاً في مستشفى تشرين الجامعي في اللاذقية، سوريا. تم تحديد عوامل خطورة الإنتانات الفطرية لدى هؤلاء المرضى أثناء العلاج الكيميائي. اعتمد تشخيص الإنتانات الفطرية على كل من الأعراض السريرية ونتائج الزرع على أوساط انتقائية (وسط سابورو دكستروز آغار و بطاطا دكستروز آغار). تضمنت الدراسة 50 مريضاً من مرضى الابيضاضات (19 من نمط الابيضاض اللمفاوي الحاد، 14 من نمط الابيضاض النقوي الحاد، 12 من نمط الابيضاض اللمفاوي المزمن و 5 من نمط الابيضاض النقوي المزمن). ظهرت الإنتانات الفطرية لدى 11 مريضاً (22%) قبل البدء بالعلاج الكيميائي، كانت جميعها من جنس المبيضات. بينما ظهرت هذه الإنتانات لدى 30 مريضاً (60%) أثناء العلاج الكيميائي، 48% منها من جنس المبيضات، 10% من جنس الـ Rhizopus و 2% من جنس الرشاشيات. أظهرت نتائجنا وجود فرق ذو دلالة إحصائية في نسبة الإنتانات الفطرية بين مرحلة ما قبل العلاج الكيميائي وأثناء العلاج الكيميائي (p-value=0.0001). تبين من خلال تطبيق اختبار كاي مربع أو فيشر وجود فروق ذات دلالة إحصائية بين المجموعتين المصنفتين تبعاً لحدوث الإنتانات الفطرية أثناء العلاج الكيميائي من حيث التدخين ($p = 0.01$)، العلاج بالكورتيزونات ($p = 0.0001$) واجتماع عاملي الخطورة السابقين معاً ($p = 0.0001$). بينما لم يكن هناك فروق ذات دلالة إحصائية بين المجموعتين من حيث الجنس ($p = 0.2$)، العمر ($p = 0.1$) ونوع الابيضاض ($p = 0.073$).

الكلمات المفتاحية: الإنتانات الفطرية، الابيضاضات، العلاج الكيميائي، العلاج الوقائي، مبيضات، رشاشيات، Rhizopus، عوامل خطورة.

* مدرسة ، قسم الكيمياء الحيوية والأحياء الدقيقة ، كلية الصيدلة ، جامعة تشرين، اللاذقية، سورية.

** مدرس، قسم الأورام وأمراض الدم ، كلية الطب ، جامعة تشرين، اللاذقية، سورية.

*** طالبة ماجستير، قسم الكيمياء الحيوية والأحياء الدقيقة ، كلية الصيدلة ، جامعة تشرين، اللاذقية، سورية.dalia.solaiman@tishreen.edu.sy

Introduction:

In recent years, the proportion of patients who develop fungal infections has increased significantly. The majority of these infections occur in patients with hematologic malignancies[1]. Patients with leukemia are most at risk for fungal infections during periods of severe neutropenia following chemotherapy. These patients are hospitalized during treatment and may be exposed to opportunistic fungal organisms found in building materials, soil, air conditioning ducts, hospital supplies, and other places[2]. The rate of fungal infections in these patients ranges from 2% to 49%, with mortality rates as high as 60%. Variation in incidence rates can be explained by patient population selection, chemotherapeutic regimens, varying patterns of systemic antifungal prophylactic medication, Treatment (cytotoxic regimens, antifungal prophylaxis), and host (older age, type and clinical status of hematologic malignancy, duration of neutropenia, candidal colonization) [3]. For these reasons, much effort has been devoted in recent years to optimizing the prevention of invasive fungal infections for patients in high-risk categories[4]. *Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans*, *Pneumocystis jirovecii*, endemic dimorphic fungi, and Mucormycetes continue to be the most common fungal pathogens worldwide, accounting for the majority of serious fungal diseases[5] .

The primary risk factor for all types of fungal infection is severe neutropenia cell count < 500/mL for more than 1 week. Other risk factors include: vascular and urinary catheters, chemotherapy disruption of the GI and oropharyngeal mucosa, use of broad-spectrum antibiotics, graft-versus-host disease (GVHD) treatment with high-dose corticosteroid therapy[2], renal failure, a prolonged stay in an intensive care unit, total parenteral nutrition, and a recent gastrointestinal surgical procedure[6] extremes of age, smoking, diabetes, Cushing's syndrome, immunosuppression, nutritional deficiencies[7], pancreatitis, sepsis, the severity of illness and Transplantation of solid organs and stem cells [8].

In this study, we aim to determine the prevalence and risk factors of fungal infections in leukemia patients undergoing chemotherapy in the patient population treated in Tishreen Hospital and compare the prevalence rate before and during chemotherapy in these patients.

Study design and methods:

1- Study population:

This study was conducted in the microbiology department/Laboratory wards of Tishreen University Hospital in Lattakia between June 2021 and July 2022. Fifty patients (37 adults and 13 children) newly diagnosed with leukemia and admitted to the oncology department at Tishreen University Hospital were included. None of them received antifungal prophylactic treatment. The first samples were collected at the time of the patient's first admission to the oncology department, and the following information was obtained from the patient's records: age, gender, personal habits (smoking), and type of leukemia. Then the second samples were collected after starting chemotherapy, and data related to this stage were obtained, such as treatment with corticosteroids, and antifungal prophylaxis.

2- Study methods:

Oral swab samples were taken from the inside of the cheek and tongue using sterile cotton swabs. These samples were examined directly under a microscope with a 40x lens to detect the presence of fungal elements by dissolving them in a drop of physiological serum

on a glass slide, then they were covered with a clean glass coverslip and examined microscopically. The oral swabs were grown on selective media for fungi, namely Sapporo dextrose agar and potato dextrose agar, in Petri dishes, and then these dishes were incubated at 28 °C, during which the growth was monitored periodically every two days for a period of 15 days. Finally, the cultures were read, and the identity of the growing fungi was determined through macroscopic and microscopic examination of the growing colonies on the culture media.

Statistical analysis:

This is an observational, descriptive, cross-sectional study. Statistical analysis was performed using IBM SPSS Statistics (version 20). Descriptive statistics included measures of central tendency and measures of dispersion for quantitative variables, frequencies, and percentages for qualitative variables. The following statistical tests were used: the Kolmogorov-Smirnov test to find out the nature of the data distribution, prevalence rate, a Chi-Square or Fisher exact test to study the relationship between qualitative variables, and the results were considered statistically significant with the p-value < 0.05.

Results:

1- Patients characteristics:

We analyzed data from the oncology center at Tishreen University Hospital in 50 patients with newly diagnosed leukemia, 19 of whom were diagnosed with ALL, 14 with AML, 12 with CLL, and 5 with CML. Patients are evenly distributed according to gender. The median age of patients was 43 years (ranging from 3 to 75 years). 26 (52%) of the cohort were current smokers, and 30 (60%) of them were under treatment by taking corticosteroids during chemotherapy, as shown in Table (1).

Table (1): Baseline characteristics of 50 patients with newly diagnosed leukemia

Characteristics	Value (%)
Age	
<18	13(26%)
18-28	3(6%)
28-38	6(12%)
38-48	7(14%)
48-58	9(18%)
>58	12(24%)
Smoking	26 (52%)
Corticosteroids treatment	30 (60%)
Smoking and corticosteroids treatment	15(30%)
Sex	
Female	25 (50%) (6 of them were kids)
Male	25 (50%) (7 of them were kids)

Leukemia classification	
AML	14 (28%)
ALL	19 (38%)
CML	5 (10%)
CLL	12 (24%)

Eleven patients (22%) presented with a fungal infection before starting the chemotherapy, all of these infections were of the genus *Candida*. The number of fungal infection cases was increased to 30 (60%) during chemotherapy, and the highest percentage of these infections was from the genus *Candida*, followed by the genus *Rhizopus*, then the genus *Aspergillus*, as shown in table (2) and plotted in figures (1) and (2). By comparing percentages using the z-score, there were statistically significant differences in the percentage of fungal infections between pre-chemotherapy and during chemotherapy (p-value = 0.0001 < 0.05).

Table (2): Comparison of the prevalence of fungal infections between pre-chemotherapy and during chemotherapy in leukemia patients

	All cases (%)	Candida albicans(%)	Rhizopus(%)	Aspergillua flavus(%)
Pre-chemotherapy	11 (22%)	11 (22%)	No (0%)	No (0%)
During chemotherapy	30 (60%)	24 (48%)	5 (10%)	1 (2%)

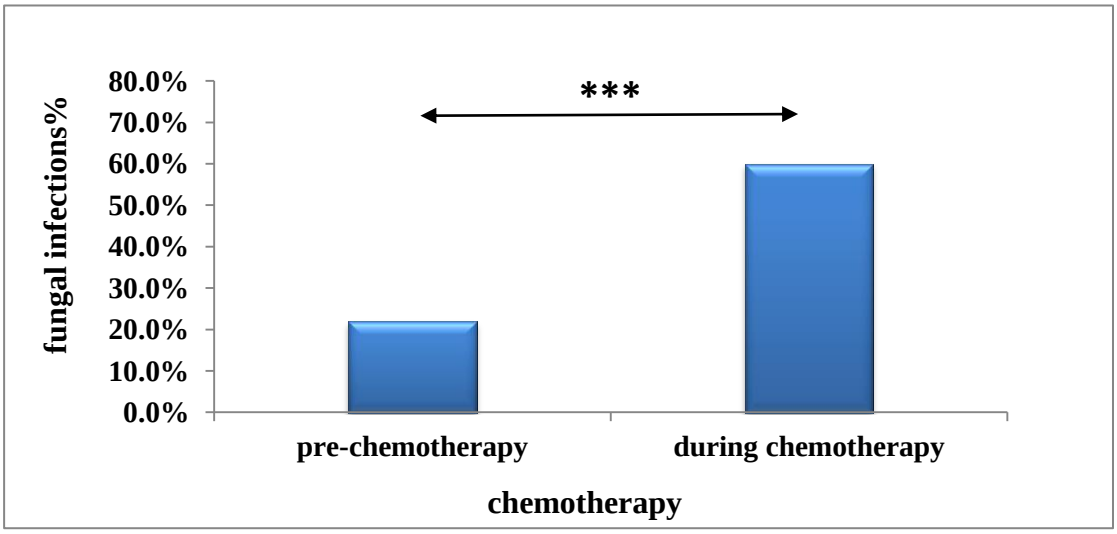

Figure (1): The difference in the percentage of all fungal infections in patients pre-chemotherapy and during chemotherapy (p-value = 0.0001 < 0.05)

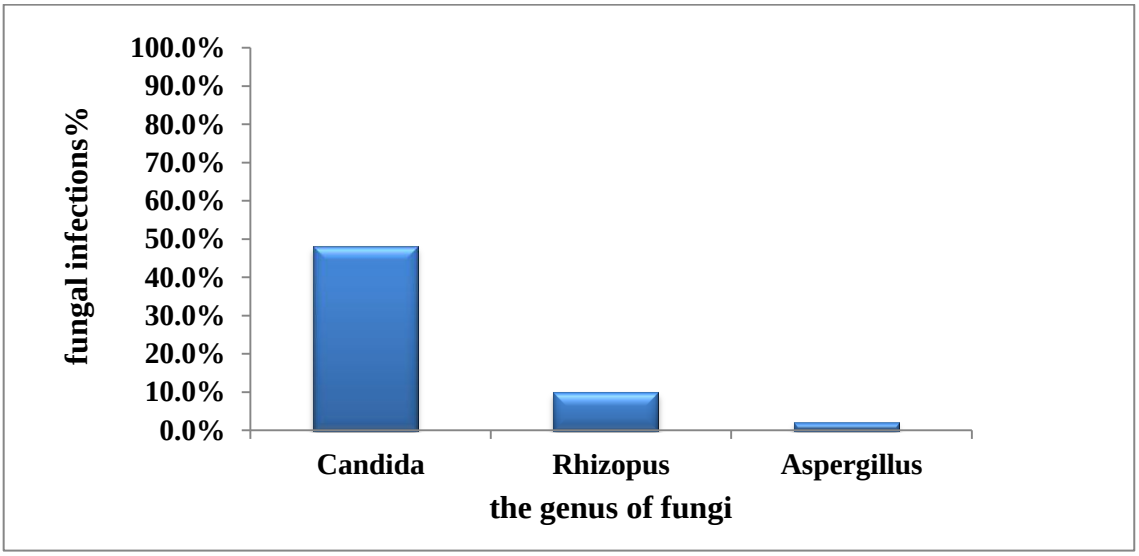

Figure (2): The prevalence of fungal infections during chemotherapy according to the genus of fungi

***

2- Evaluation of Risk factors associated with fungal infections:

Chemotherapy is the main risk factor for the increased incidence of fungal infections. We investigated the relationship between fungal infections and other risk factors such as age, gender, smoking, corticosteroid treatment, and type of leukemia. There were statistically significant differences between the two groups classified according to the incidence of fungal infections during chemotherapy in terms of smoking (p-value = 0.01), corticosteroid therapy (p-value = 0.0001), and the combination of the two previous risk factors together (p-value = 0.0001) as plotted in figure (3), but there were no statistically significant differences between the two groups in terms of age (p-value = 0.1), type of leukemia (p-value = 0.073), and gender (p-value = 0.2) as plotted in figure (4), figure (5) and figure (6).

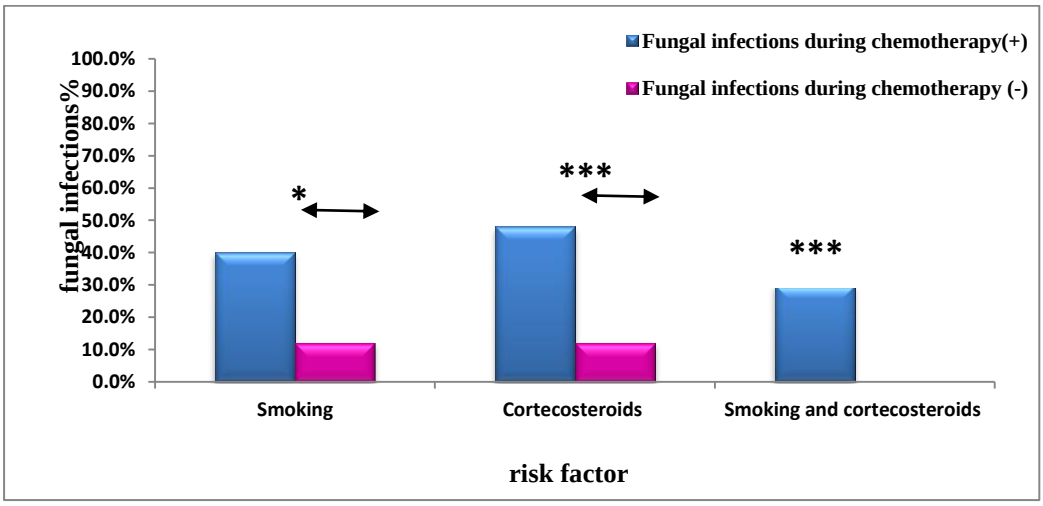

Figure (3): The effect of smoking and corticosteroid treatment on the increase of fungal infections during chemotherapy.

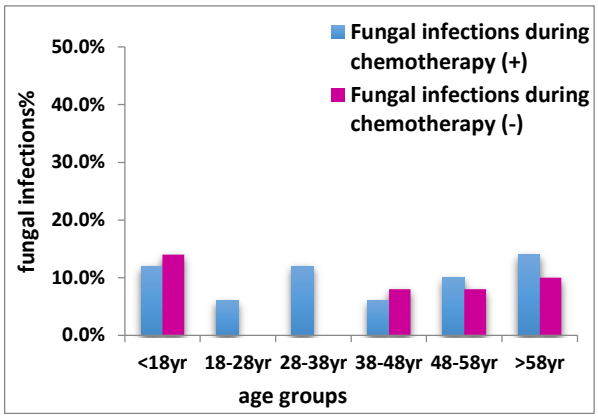

Figure (4): Prevalence of fungal infections during chemotherapy according to the age groups of the patients.

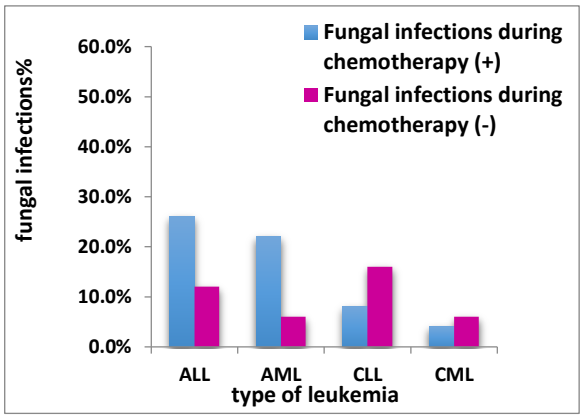

Figure (5): Prevalence of fungal infections during chemotherapy according to the type of leukemia.

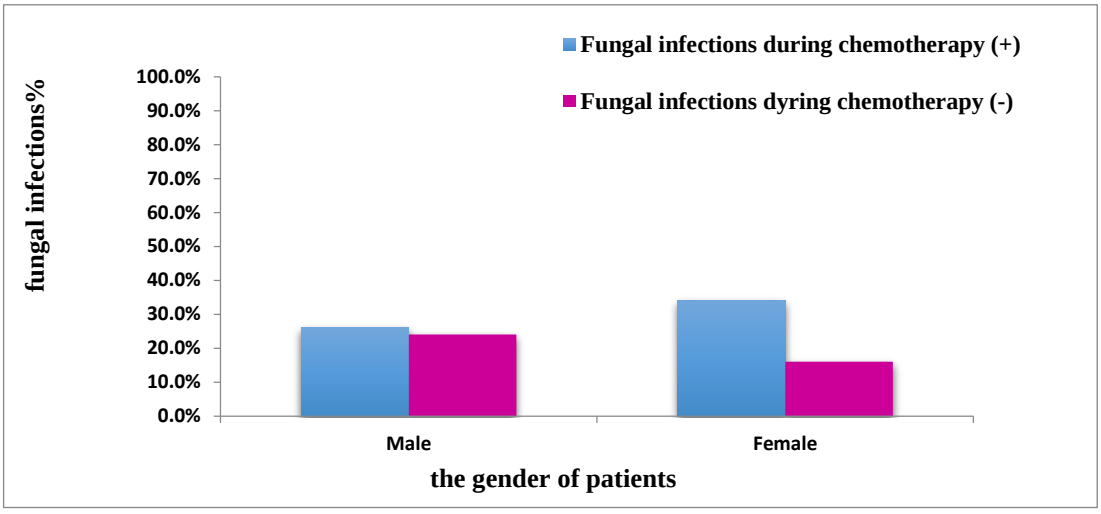

Figure (6): Prevalence of fungal infections during chemotherapy according to the sex of the patients.

Discussion:

The incidence of fungal infections is the highest among patients with leukemia during chemotherapy-induced neutropenia. We therefore, focused our analysis on a cohort of hematologic patients with newly diagnosed leukemia who received chemotherapy. Our results confirm a high incidence of fungal infections in leukemia patients undergoing chemotherapy. The percentage of fungal infections in leukemia patients increased during chemotherapy to 60%. In comparison with previous studies, the study conducted in China in 2015 showed that the prevalence of fungal infections during chemotherapy was confirmed in 34.6% of adult patients with acute myeloid leukemia who did not receive antifungal prophylaxis [9]. Another study conducted in Thailand in 2019 showed the prevalence of fungal infections during chemotherapy in 22.3% of acute myeloid leukemia patients who did not receive preventive treatment as well [10]. To be mentioned, the percentage of fungal infections varies between studies due to differences in study design, sample type, period of chemotherapy, and use of antifungal prophylactic treatment.

Candida was the most common pathogen, accounting for 48% of infections during chemotherapy. This result can be explained by the fact that candida is a part of the natural flora of humans and does not cause any pathogenicity in a healthy body, but with immunodeficiency and/or disruption of the balance of this flora, an opportunistic growth of these fungi occurs to turn into a pathogen [8]. In this study, chemotherapy was a major cause of immunodeficiency in leukemia patients, which made them susceptible to infection by opportunistic fungi such as Candida. On the other hand, the oral swabs taken in our study may have an effect on the high prevalence of Candida compared with the rest of the fungal genera, as oropharyngeal candidiasis is the most common form of candidiasis[11]. During chemotherapy, the second and third causes of fungal infections were *Rhizopus* and *Aspergillus*. These fungi are found everywhere (air, water, soil, organic residues), do not cause pathogenicity in a healthy body, but turn into a pathogen when human immunity is deficient [12, 13]. This result is consistent with the result of a previous study conducted in Taiwan in 2018 [14], which found *Candida* to be the leading cause of fungal infections in acute leukemia patients undergoing chemotherapy. This is in contrast to another study conducted in Japan in 2012 [15], where *Aspergillus* was the main cause of fungal infections in patients with malignant hematomas, followed by *Candida* and then Mucormycotes. This difference between the studies may be due to the different types of samples between them, as the samples in our study were oral swabs, while in these studies they were not completely specific, as they looked for fungal infections in the suspected sites, so the samples were skin, blood or respiratory samples.

In our study, smoking was associated with an increased risk of fungal infections during chemotherapy, which is consistent with the finding of a previous study conducted in Brazil in 2015 [16]. This can be explained by the fact that tobacco cigarettes contain *Aspergillus* spores; burning contaminated tobacco causes spore release, increasing immunocompromised patients' exposure. Furthermore, smoking has been shown to suppress the immune protection system of alveolar macrophages, dendritic cells, natural killer (NK) cells, and neutrophils due to immune cell damage and failure to activate intracellular signaling [17].

We also confirmed an association between fungal infections and corticosteroid treatment. This result is similar to what was reported in a previous study conducted in Canada in 2012 [18]. Steroids reduce resistance to infection through various mechanisms: 1) reduction of the number of neutrophils migrating to the site of inflammation; 2)Stabilizing lysosomal membrane, which promotes the release of lysosomal catabolic enzymes; 3) Steroids may have a direct effect on the growth of both *Aspergillus* and *Candida*, reducing of the activity of the entire reticuloendothelial system, resulting in decreased anti-body synthesis, a property shared with other immunosuppressive drugs used to treat acute leukemia. As a result, there are numerous explanations for the higher incidence of fungal infections in the group where steroids were used as part of the chemotherapy regimen [19].

Age is a primary risk factor for developing infectious complications during chemotherapy [20]. The natural function of the immune system declines with age [21]. Furthermore, elderly patients are often frailer and have comorbidities that increase their susceptibility to infection [20]. In our study, we found that there was a trend for older patients (age > 58 years) to get fungal infections, but there was no significant association between fungal infections during chemotherapy and age; this may be due to the study design and alow number of patients in this subgroup.This result is similar to what was reported in a previous study conducted in China in 2018 [17].

In our study, there was no significant association between fungal infections during chemotherapy and sex, which is consistent with the finding of a previous study conducted in Italy in 2015 [4]. This may be due to the small size of our study sample or to the equal distribution of patients in terms of sex. In contrast, there was a difference with other previous reports [3, 22], where the male sex constituted a risk factor for fungal infections in leukemia patients. They explained this result by the difference in the underlying disease severity between the sexes, or it may be due to biological or environmental underlying reasons such as the exposure of males to inhaling fungal spores more than females because of their occupational risks.

Although acute leukemia patients have a higher risk of developing fungal infections [14, 23], in our study، there was no significant association between fungal infections during chemotherapy and the type of leukemia. This is in contrast to what was stated in a previous study conducted in Spain in 1994[24], and this may be due to the difference in the number of patients diagnosed with each type between the two studies.

Conclusion:

We show a high prevalence of fungal infections in leukemia patients during chemotherapy. Antifungal prophylaxis should be considered in leukemia patients undergoing induction chemotherapy. In our leukemia cohort, smoking status and corticosteroid treatment were associated with higher risk of fungal infections during chemotherapy. We recommend that fungal infections to be closely monitored and that antifungal drugs be used as soon as possible to reduce the risk of these infections. A prospective study is needed to determine the effect of antifungal prophylaxis given during leukemia remission-induction therapy.

References:

1.Pagano L., Caira M., Candoni A., Offidani M., Fianchi L., Martino B., Pastore D., Picardi M., Bonini A. ,Chierichini A. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study, Haematologica, 2006, 91(8), 1068-1075.

2.Mays S. R., Bogle M. A.,Bodey G. P. Cutaneous fungal infections in the oncology patient, American journal of clinical dermatology, 2006, 7(1), 31-43.

3.Neofytos D., Lu K., Hatfield-Seung A., Blackford A., Marr K. A., Treadway S., Ostrander D., Nussenblatt V. ,Karp J. Epidemiology, outcomes, and risk factors of invasive fungal infections in adult patients with acute myelogenous leukemia after induction chemotherapy, Diagnostic microbiology and infectious disease, 2013, 75(2), 144-149.

4.Caira M., Candoni A., Verga L., Busca A., Delia M., Nosari A., Caramatti C., Castagnola C., Cattaneo C. ,Fanci R. Pre-chemotherapy risk factors for invasive fungal diseases: prospective analysis of 1,192 patients with newly diagnosed acute myeloid leukemia (SEIFEM 2010-a multicenter study), haematologica, 2015, 100(2), 284.

5.Firacative C. Invasive fungal disease in humans: are we aware of the real impact?, Memórias do Instituto Oswaldo Cruz, 2020, 115(

6.Pfaller M. A., Pappas P. G.,Wingard J. R. Invasive fungal pathogens: current epidemiological trends, Clinical Infectious Diseases, 2006, 43(Supplement_1), S3-S14.

7.Akpan A. ,Morgan R. Oral candidiasis, Postgraduate medical journal, 2002, 78(922), 455-459.

8.Pappas P. G., Lionakis M. S., Arendrup M. C., Ostrosky-Zeichner L. ,Kullberg B. J. Invasive candidiasis, Nature Reviews Disease Primers, 2018, 4(1), 1-20.

9.Tang J.-L., Kung H.-C., Lei W.-C., Yao M., Wu U.-I., Hsu S.-C., Lin C.-T., Li C.-C., Wu S.-J. ,Hou H.-A. High incidences of invasive fungal infections in acute myeloid leukemia patients receiving induction chemotherapy without systemic antifungal prophylaxis: a prospective observational study in Taiwan, PloS one, 2015, 10(6), e0128410.
10.Nganthavee V., Phutthasakda W., Atipas K., Tanpong S., Pungprasert T., Dhirachaikulpanich D., Krithin S., Tanglitanon S., Jutidamronphang W. ,Owattanapanich W. High incidence of invasive fungal infection during acute myeloid leukemia treatment in a resource-limited country: clinical risk factors and treatment outcomes, Supportive Care in Cancer, 2019, 27(9), 3613-3622.
11.Degregorio M. W., Lee W. M.,Ries C. A. Candida infections in patients with acute leukemia: ineffectiveness of nystatin prophylaxis and relationship between oropharyngeal and systemic candidiasis, Cancer, 1982, 50(12), 2780-2784.
12.Sephton-Clark P. C., Muñoz J. F., Ballou E. R., Cuomo C. A. ,Voelz K. Pathways of pathogenicity: transcriptional stages of germination in the fatal fungal pathogen Rhizopus delemar, Msphere, 2018, 3(5), e00403-18.
13.Pattron D. D. Aspergillus, health implication & recommendations for public health food safety, Internet Journal of food safety, 2006, 8(19-23.
14.Lin G.-L., Chang H.-H., Lu C.-Y., Chen C.-M., Lu M.-Y., Lee P.-I., Jou S.-T., Yang Y.-L., Huang L.-M. ,Chang L.-Y. Clinical characteristics and outcome of invasive fungal infections in pediatric acute myeloid leukemia patients in a medical center in Taiwan, Journal of microbiology, immunology and infection, 2018, 51(2), 251-259.
15.Kurosawa M., Yonezumi M., Hashino S., Tanaka J., Nishio M., Kaneda M., Ota S., Koda K., Suzuki N. ,Yoshida M. Epidemiology and treatment outcome of invasive fungal infections in patients with hematological malignancies, International journal of hematology, 2012, 96(6), 748-757.
16.Garnica M., Oliveira da Cunha M., Portugal R., Maiolino A., Colombo A. L. ,Nucci M. Risk factors for invasive fusariosis in patients with acute myeloid leukemia and in hematopoietic cell transplant recipients, Clinical Infectious Diseases, 2015, 60(6), 875-880.
17.Lien M.-Y., Chou C.-H., Lin C.-C., Bai L.-Y., Chiu C.-F., Yeh S.-P. ,Ho M.-W. Epidemiology and risk factors for invasive fungal infections during induction chemotherapy for newly diagnosed acute myeloid leukemia: a retrospective cohort study, PloS one, 2018, 13(6), e0197851.
18.Dix D., Cellot S., Price V., Gillmeister B., Ethier M.-C., Johnston D. L., Lewis V., Michon B., Mitchell D. ,Stobart K. Association between corticosteroids and infection, sepsis, and infectious death in pediatric acute myeloid leukemia (AML): results from the Canadian infections in AML research group, Clinical infectious diseases, 2012, 55(12), 1608-1614.
19.Mirsky H. S. ,Cuttner J. Fungal infection in acute leukemia, Cancer, 1972, 30(2), 348-352.
20.Ossenkoppele G. ,Löwenberg B. How I treat the older patient with acute myeloid leukemia, Blood, The Journal of the American Society of Hematology, 2015, 125(5), 767-774.
21.Müller L., Benedetto S. D.,Pawelec G. The immune system and its dysregulation with aging, Biochemistry and Cell Biology of Ageing: Part II Clinical Science, 2019, 21-43.

22.Hammond S. P., Marty F. M., Bryar J. M., DeAngelo D. J. ,Baden L. R. Invasive fungal disease in patients treated for newly diagnosed acute leukemia, American journal of hematology, 2010, 85(9), 695-699.
23.Bhatt V. R., Viola G. M.,Ferrajoli A. Invasive fungal infections in acute leukemia, Therapeutic advances in hematology, 2011, 2(4), 231-247.
24.Somaia A. E., Hussein K.,Sohair I. Fungus infections in leukaemic patients under immunosuppressive therapy, EJMM-Egyptian Journal of Medical Microbiology, 1994, 3(1), 37-42.