Arch Pediatr Infect Dis. 2017 July; 5(3):e41317. doi: 10.5812/pedinfect.41317.

Published online 2017 January 16. **Research Article**

Incidence of Fungal Infections in Pediatric Patients with Hematologic Neoplasms

Parisa Badiee,[1] Soheila Zareifar,[2] Pedram Haddadi,[1,*] and Hadis Jafarian[1]

[1]Prof. Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran
[2]Hematology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran

[*]*Corresponding author*: Pedram Haddadi, Prof. Alborzi Clinical Microbiology Research Center, Namazee Hospital Zand Ave., Shiraz, IR Iran. 7193711351. Tel: +98-7136474292, Fax: +98-7136474303, E-mail: badieep@sums.ac.ir

Received 2016 August 09; **Revised** 2016 October 13; **Accepted** 2016 October 29.

Abstract

Background: Fungal infections are one of the most important causes of morbidity and mortality in patients with hematological disorders. The frequency of these infections has increased during the past decades.
Objectives: The rate of fungal infections was investigated in pediatric patients with hematological disorders, using traditional and real-time PCR methods, in order to establish proper management of these patients.
Methods: Over a 13-month period, 86 patients with hematological disorders were admitted and were kept under observation for the development of fungal infections. Fungal colonization was determined and clinical samples were examined by direct microscopic examination and culture. Blood specimens were cultured by bedside inoculation into a BACTEC medium. The results of the pathology smear were collected from the patients' records. Real-time PCR was performed on all patients' sera to diagnose invasive candidiasis and aspergillosis.
Results: *Candida* colonization was seen in 42 (48.8%) and oral candidiasis was diagnosed in 7 (8%) patients. The incidence of invasive fungal infections was 16.3% (14/86) with a mortality rate of 50% in pediatric patients. The etiologic agents were *Candida albicans* in 5 cases, *Aspergillus flavus* in 3, and *Aspergillus fumigatus*, *Fusarium*, *Alterneria* and *Mucor*, each in 1 case. In 2 cases with liver abscess, only *Candida* PCR was positive. Fungemia was observed in 4 patients.
Conclusions: The rate of invasive fungal infections in our study was high. Early and accurate detection of these infections could result in a better outcome. In critical ill cases where only blood samples are available, molecular methods such as PCR could be more effective than culture for the detection of etiologic agents.

Keywords: Candidiasis, Aspergillosis, Hematologic Neoplasm

1. Background

Invasive fungal infections (IFIs) are one of the most important causes of morbidity and mortality in patients with hematological malignancies. Factors such as neutropenia, damaged mucosa, receiving high-dose chemotherapy, undergoing invasive medical procedures, and using broad-spectrum antibacterial drugs, constitute the risk factors for IFIs (1, 2). The frequency of candidemia has increased during the past few decades. The mortality rate in non-immunocompromised children hospitalized in the pediatric intensive care unit was reported to be 32% (2), whereas children with a hematologic disease displayed a mortality rate of approximately 60% (3). The mortality rate related to invasive fungal infections in children with hematologic disorders was found to be 30% in a study by Baytan et al. (4). "In children, the mortality rate for invasive aspergillosis is 2.5 to 3.5 higher than that for invasive candidiasis (respectively 70% vs. 20% and 30%)" (5).

Clinical symptoms and radiological patterns are not specific and late diagnosis may lead to delayed therapy, which is associated with a poor outcome (6). Examination commonly includes a direct smear with potassium hydroxide, culturing, and histopathological analysis, however, in some cases, this can be challenging since sampling requires invasive procedures, which are not possible in critically ill patients. Hence, the use of a non-invasive method, such as real-time PCR, for the detection of fungal infections in clinical samples could be a suitable approach in aiding early diagnosis in high-risk infected patients (7).

2. Objectives

Since the role of PCR in diagnosing fungal infections is not yet well established (8), the aim of this study was to investigate the rate of IFIs in pediatric patients with hematological disorders, using traditional (direct smear and culture) and real-time PCR methods.

3. Methods

During a 13-month period (January 2014 to February 2015), 86 pediatric patients with hematological disorders were admitted to Amir Hospital, Shiraz University of Medical Sciences, in Iran and were kept under observation for the development of IFIs. Patients with hematological disorders and clinical signs including fever, ascites, epistaxis, febrile neutropenia, chest pain, dyspnea, and dry cough as well as patients with a suspicious fungal rash were included in this study. Pediatric patients admitted due to malignancies, with the exception of hematological malignancies, were excluded from the study. In order to determine fungal colonization, clinical samples such as urine, nasal, mouth, and rectal swabs were obtained at time of admission. The samples were cultured on saboaroud dextrose agar (Merck, Germany) and incubated at 24°C for 10 days. Colonization was defined as the presence of *Candida* spp. in any of the body sites without any local or systemic symptoms or signs of infection. During the hospital stay, samples (e.g., plural and abdominal tap, cerebrospinal fluid, blood, urine, oropharyngeal, biopsy, and broncho-alveolar lavage samples) from patients with clinical signs of infection were examined by culturing on sabouraud dextrose agar with chloramphenicol, and direct microscopic examination. In patients suspicious to fungal infections according to clinical signs and symptoms, blood specimens were collected for PCR and cultured by bedside inoculation into BACTEC medium (Becton- Dickinson, Sparks, Md., USA), twice weekly. The clinician and an expert radiologist analyzed the imaging modalities in order to detect IFIs. The isolated molds were diagnosed by colony morphologic and microscopic characterization of species with the use of lactophenol cotton blue, whereas yeasts were identified using the API 20 C AUX system (bioMerieux, France), according to the manufacturer's instructions. The demographic characteristics of the patients were extracted from their medical files. The results of the pathology smear were collected from the patient's records.

Fungal DNA was extracted from the patients' sera by using the QIAmp DNA minikit (Qiagen,Hilden, Germany), according to the manufacturer's recommendations. To perform PCR, the suggestions of Shin et al. and Kami et al. (9, 10) were taken into consideration. The primers and a TaqMan probe (Metabion Martinsried, Germany) were used, and thermal cycling conditions for the Aspergillus and *Candida* real-time PCR assay were performed, as described previously (9, 10). Thermal cycling conditions (11) were carried out using the ABI 7500 FAST instrument (Applied Biosystem, Foster City, CA, USA).

Invasive fungal infections were defined according to the European Organization on research and treatment in cancer and the mycoses study group (8). To be defined as a proven infection, fungal elements in tissue (by histopathology) or isolates from etiologic agents were required to be found in cultures of samples from a normally sterile site, such as blood, CSF, and tissue in immunocompromised patients. Combination of a susceptible host, clinical signs compatible with fungal infection and mycological evidence or indirect (non-culture/non-histopathologic evidence) from non-sterile clinical samples like sputum and bronchoalveolar lavage were defined as probable IFIs.

The ethics committee of Shiraz University of Medical Sciences reviewed and approved the study, which was carried out in accordance with the 1975 declaration of Helsinki, as revised in 1983. Written consents of the patients were obtained before participating in the study.

Data were analyzed by descriptive statistical methods using the SPSS statistical package (SPSS, Chicago, IL, USA).

4. Results

The female-to-male ratio was 38:48, and the mean age of the patients was 6 years (standard deviation: 3.9). The most common hematological disorders in these patients were acute lymphoblastic leukemia (ALL) in 50 patients (58%), acute myeloid leukemia (AML) in 21 patients (24%), followed by Hodgkin's lymphoma, Burkit lymphoma, megaloblastic anemia, and aplastic anemia. The mean white blood cell count was 3522 (standard deviation: 3300).

Candida colonization was seen in 42 patients (48.8%) and the most frequent sites of colonization were oral cavity 53.3%, rectum 33.3%, nose 6.7% and urine 6.7%. The etiologic agents were found to be *C. albicans* in 73.3% of the cases, followed by *C. krusei*, *C. tropicalis*, *C. dubliniensis* and *C. famata*. Oral candidiasis was diagnosed in 7 (8%) patients (*C. albicans* was grown in 5 cases, *C. tropicalis* in 1 and *C. krusei* in 1 case).

A total of 225 clinical specimens consisting of blood, urine, cerebrospinal fluid, pleural and abdominal tap, bronchoalveolar lavage, abscess secretion, and sputum were examined for fungal infection. Candidemia was detected in 4 patients. According to clinical, radiological and mycological data, proven and probable IFIs were detected in 14 of the 86 patients (16.3%), using pathological, conventional and molecular diagnostic methods. Demographic and clinical characteristics of the pediatric patients are presented in Table 1. The etiologic agents based on the culture results from clinical specimens consisted of *C. albicans* in 5 cases, *A. flavus* in 3, and *A. fumigatus*, *fusarium*, *Alterneria* and *Mucor*, each in 1 case. In 2 cases with liver abscess, only the *Candida* PCR was positive. Blood culture results in these

2 cases were negative and sampling from the abscess was not done. Lungs and sinuses were the most infected sites (Table 1). The detection limit of real-time PCR was 10 copy numbers per ml. Real-time PCR was positive in 10 patients (5 of 8 proven, 5 of 6 probable). Despite appropriate antifungal therapy, 50% (7/14) of the patients unfortunately passed away.

5. Discussion

Acute leukemia (myeloid, lymphoid) was the most frequent underlying disease among the patients, as was similarly seen in other studies (12). *Candida* colonization in this study was reported in 48.8% of all patients and 100% of patients with invasive candidiasis. There was a significant correlation between invasive candidiasis and *Candida* colonization ($PV < 0.05$) in patients with hematologic malignancy. The rate of *Candida* colonization was reported to be 55.5% in two other studies, with 107 patients out of 197 in the first study and 35 patients out of 63 in the second study (13, 14). *Candida albicans* was the most prevalent etiologic agent in both this study and other earlier studies (13, 14). *Candida* colonization may be a pre-requisite for systemic candidiasis in patients with hematologic malignancy.

In the present study, the rates of IFIs among pediatric patients with hematological disorders were found to be 16.3%. The rate of proven IFIs in an autopsy study over a 15-year period (Chamilos et al.) revealed to be 31% in patients with with hematologic malignancies (15) and the incidence rate of invasive aspergillosis and systemic candidiasis in the pediatric patients was shown to be 27.4% and 12.9%, respectively (13, 16). Since many studies do not have the possibility to perform autopsies or molecular assays, lower incidence rates were found, including 13% (17), 6.9% (18), and 4.6% (19). Unfortunately, despite starting the antifungal agents, 50% (7/14) of the patients died. These patients were infected with *Mucor*, *Fusarium*, *Alternaria*, *A. flavus*, and *C. albicans*. The mortality rate of IFIs was reported to be 48.2% (18). In a review study on 29 episodes of IFIs, *Candida* spp. was the leading pathogen followed by the *Aspergillus* species (19). In a large epidemiological survey of fungal infections in patients with hematologic malignancies conducted by Ramirez et al. over half of the fungal infections were caused by molds, especially *Aspergillus* spp. followed by yeast infections including candidemia (20). The highest IFI-attributable mortality rates were associated with zygomycosis followed by fusariosis, aspergillosis, and candidemia (21).

The most common infected region presented in our proven and probable patients was the respiratory tract system, specifically 3 sinuses and 5 lungs (57.1%), followed by fungemia 4/14 (28.6%) and the liver 2/14 (14.3%). In a study by Kobayashi et al., the most common infected region in 334 patients with hematologic and malignant diseases were "lung in 14 patients, liver in 5, brain in 3, fungemia in 2, kidney in 1, and endophthalmitis in 1 patient" (18).

Culture of clinical specimens is the gold standard test for detection and identification of IFIs, however, the method is time consuming and sample collection through biopsy in some hematological patients requires invasive procedures. Blood cultures are rarely positive in infected patients with mold fungi. In this study, no positive blood culture for mold fungi was observed and 4 patients had a positive result for candidemia. If other samples such as tissue, bronchoalveolar fluid, sputum, needle aspiration and peritoneal fluid could be obtained from the patients, then, culture and direct smear (pathology smear) would serve as the best method for diagnosis of infections. Since such specimens are not available in some cases, it would be better to use non-invasive methods like PCR in sera. In this study, we selected real-time PCR as a facilitating and rapid screening test for medically important fungi in sera, and a feasible approach that enables culture-independent screening of fungal infections and helps identify infected patients at an early stage. The real-time PCR assay provides a high sensitivity and specificity for the detection of fungal DNA and rapidly identifies most of clinically relevant *Aspergillus* species (20). Many studies have reported the correlation between the presence of proven, probable, and possible criteria of IFIs according to the EORTC-MSG criteria and PCR results of the respective patients' sera (17, 21, 22). The results of the present study show 5/8 patients with proven and 5/6 patients with probable criteria. In proven patients, when the specific primers (*Aspergillus* and *Candida*) were used, PCR proved suitable for diagnosis. In probable patients, where clinical sampling is difficult or impossible (in patients with liver abscess), this method could help the clinicians with diagnosing the infection. As revealed in this study, aspergillosis was confirmed by PCR in patients with negative blood culture results, in accordance with the results by Schabereiter-Gurtner al. (23). The limitation of this study was the lack of using real-time PCR for the detection of fungal pathogens like *Fusarium*, *Alternaria*, and *Mucor* spp.

The rate of invasive fungal infections in our study was high and *Aspergillus* and *Candida* were the most frequently encountered fungi. Some new non-culture based methods such as PCR would be useful for early and accurate detection of IFIs resulting in a better outcome for the infected pediatric patients.

Table 1. Characteristics of Pediatric Patients with Proven and Probable Invasive Fungal Infections

No	Background	Criteria	Culture Result	Serum PCR result	WB Ccount	Treatment	Site of Infection (Sample)	Outcome
1	ALL	Proven	*C. albicans*	A: negative C: positive	22000	Amphotericin	Blood	Survival
2	ALL	Proven	*C. albicans*	A: negative C: positive	1800	Amphotericin	Blood	Survival
3	ALL	Proven	*Fusarium*	A: negative C: negative	228	Amphotericin	Lung (tissue)	Death
4	ALL	Proven	*C. albicans*	A:negative C: positive	900	Amphotericin	Blood	Survival
5	ALL	Proven	*Alterneria*	A: negative C: negative	400	Amphotericin	Sinus (Tissue)	Death
6	ALL	Proven	*C. albicans*	A: negative C: positive	1400	Amphotericin	Sinus (Tissue)	Death
7	ALL	Proven	*C. albicans*	A:negative C: positive	22000	Amphotericin	Blood	Survival
8	ALL	Proven	*Aspergillus flavus*	A:negative C:negative	900	Voriconazole	Sinus (Tissue)	Death
9	ALL	Probable	*Mucor*	A:negative C:negative	6900	Posaconazole	Lung (sputum)	Death
10	AML	Probable	*Aspergillus flavus*	A: positive C:negative	1700	Voriconazole	Lung (sputum)	Death
11	AML	Probable	*Aspergillus flavus*	A: positive C:negative	18800	Voriconazole	Lung (sputum)	Survival
12	ALL	Probable	No sample	A:negative C: positive	500	Amphotericin	Liver Abscess	Survival
13	ALL	Probable	*Aspergillus fumigatus*	A: positive C:negative	13600	Amphotericin Voriconazole	Lung (sputum)	Survival
14	ALL	Probable	No sample	A: negative C: positive	1200	Amphotericin	Liver Abscess	Death

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; A, Aspergillus PCR in serum; C, *Candida* PCR in serum; EORTIC/MSG, European organization on research and treatment in cancer and the mycoses study group.

Acknowledgments

Our thanks are due to Hassan Khajehei for his help with language copy editing.

Footnotes

Authors' Contribution: Parisa Badiee developed the study concept and design, analysis and interpretation of data and drafting of the manuscript; Soheila Zareifar contributed to sample collection and development of the protocol and drafting of the manuscript. Pedram Haddadi contributed to sample collection, the development of the protocol, acquisition of data, drafting of the manuscript and study supervision; Hadis Jafarian contributed with technical support, acquisition of data and drafting of the manuscript.

Conflict of Interest: The authors declare that they have no

conflict of interest.

Funding/Support: The present article was extracted from the thesis of Pedram Haddadi and was financially supported by Shiraz University of Medical Sciences grant No. 89-01-0102328 and Professor Alborzi Clinical Microbiology research center, Shiraz University of Medical Sciences, Shiraz Iran.

References

1. Marr KA, Bowden RA. Fungal infections in patients undergoing blood and marrow transplantation. *Transpl Infect Dis.* 1999;**1**(4):237–46. [PubMed: 11428995].
2. Posfay-Barbe KM, Zerr DM, Pittet D. Infection control in paediatrics. *Lancet Infect Dis.* 2008;**8**(1):19–31. doi: 10.1016/S1473-3099(07)70310-9. [PubMed: 18156087].
3. Mor M, Gilad G, Kornreich L, Fisher S, Yaniv I, Levy I. Invasive fungal infections in pediatric oncology. *Pediatr Blood Cancer.* 2011;**56**(7):1092–7. doi: 10.1002/pbc.23005. [PubMed: 21319281].
4. Baytan B, Gunes AM, Celebi S, Gunay U. Invasive fungal diseases in children with hematologic disorders. *Turk J Haematol.* 2009;**26**(4):190–6. [PubMed: 27265631].
5. Brissaud O, Guichoux J, Harambat J, Tandonnet O, Zaoutis T. Invasive fungal disease in PICU: epidemiology and risk factors. *Ann Intensive Care.* 2012;**2**(1):6. doi: 10.1186/2110-5820-2-6. [PubMed: 22356683].
6. Hanson KE, Caligiuri P, Wiggins III RH, Quigley III EP, Kendall B. Radiologic Imaging Techniques for the Diagnosis and Management of Invasive Fungal Disease. *Curr Fungal Infect Rep.* 2015;**9**(3):180–9.
7. Alborzi A. Assessment of a real-time PCR method to detect human non-cryptococcal fungal meningitis. *Arch Iran Med.* 2011;**14**(6):381.
8. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. *Clin Infect Dis.* 2008;**46**(12):1813–21.
9. Shin JH, Nolte FS, Holloway BP, Morrison CJ. Rapid identification of up to three Candida species in a single reaction tube by a 5′ exonuclease assay using fluorescent DNA probes. *J Clin Microbiol.* 1999;**37**(1):165–70.
10. Kami M, Fukui T, Ogawa S, Kazuyama Y, Machida U, Tanaka Y, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis.* 2001;**33**(9):1504–12.
11. Maaroufi Y, De Bruyne JM, Duchateau V, Georgala A, Crokaert F. Early detection and identification of commonly encountered. *J Mol Diagn.* 2004;**6**(2):108–14. doi: 10.1016/S1525-1578(10)60498-9. [PubMed: 15096566].
12. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica.* 2006;**91**(8):1068–75. [PubMed: 16885047].
13. Badiee P, Kordbacheh P, Alborzi A, Zakernia M, Haddadi P. Early detection of systemic candidiasis in the whole blood of patients with hematologic malignancies. *Jpn J Infect Dis.* 2009;**62**(1):1–5. [PubMed: 19168950].
14. Mokaddas E, Burhamah MHA, Khan ZU, Ahmad S. Levels of $(1\rightarrow 3)$-β-D-glucan,. *BMC Infect Dis.* 2010;**10**(1):292.
15. Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). *Haematologica.* 2006;**91**(7):986–9.
16. Badiee P, Alborzi A, Karimi M, Pourabbas B, Haddadi P, Mardaneh J, et al. Diagnostic potential of nested PCR, galactomannan EIA, and beta-D-glucan for invasive aspergillosis in pediatric patients. *J Infect Dev Ctries.* 2012;**6**(4):352–7. [PubMed: 22505446].
17. Wiley JM, Smith N, Leventhal BG, Graham ML, Strauss LC, Hurwitz CA, et al. Invasive fungal disease in pediatric acute leukemia patients with fever and neutropenia during induction chemotherapy: a multivariate analysis of risk factors. *J Clin Oncol.* 1990;**8**(2):280–6. doi: 10.1200/jco.1990.8.2.280. [PubMed: 2299371].
18. Kobayashi R, Kaneda M, Sato T, Ichikawa M, Suzuki D, Ariga T. The clinical feature of invasive fungal infection in pediatric patients with hematologic and malignant diseases: a 10-year analysis at a single institution at Japan. *J Pediatr Hematol Oncol.* 2008;**30**(12):886–90. doi: 10.1097/MPH.0b013e3181864a80. [PubMed: 19131772].
19. Yeh TC, Liu HC, Wang LY, Chen SH, Liang DC. Invasive fungal infection in children undergoing chemotherapy for cancer. *Ann Trop Paediatr.* 2007;**27**(2):141–7. doi: 10.1179/146532807X192516. [PubMed: 17565811].
20. Ramirez M, Castro C, Palomares JC, Torres MJ, Aller AI, Ruiz M, et al. Molecular detection and identification of Aspergillus spp. from clinical samples using real-time PCR. *Mycoses.* 2009;**52**(2):129–34. doi: 10.1111/j.1439-0507.2008.01548.x. [PubMed: 18643921].
21. Landlinger C, Preuner S, Baskova L, van Grotel M, Hartwig NG, Dworzak M, et al. Diagnosis of invasive fungal infections by a real-time panfungal PCR assay in immunocompromised pediatric patients. *Leukemia.* 2010;**24**(12):2032–8. doi: 10.1038/leu.2010.209. [PubMed: 20882044].
22. Badiee P, Alborzi A, Joukar M. Molecular assay to detect nosocomial fungal infections in intensive care units. *Eur J Intern Med.* 2011;**22**(6):611–5. doi: 10.1016/j.ejim.2011.08.025. [PubMed: 22075290].
23. Schabereiter-Gurtner C, Selitsch B, Rotter ML, Hirschl AM, Willinger B. Development of novel real-time PCR assays for detection and differentiation of eleven medically important Aspergillus and. *J Clin Microbiol.* 2007;**45**(3):906–14. doi: 10.1128/JCM.01344-06. [PubMed: 17251398].