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Prevalence and description of invasive fungal infection in adults with hematological neoplasms

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Abstract

Background: Fungi have emerged as important causes of human infection, due primarily to the increased numbers of patients subjected to severe immunosuppression; therefore, the demand for information on the pathogenic role of these microorganisms and the diseases they cause is growing. This study aimed to evaluate invasive fungal infection (IFI) in adult patients with hematological neoplasms to identify common site of infection, type of causative fungi, the percentage of patients developing fungemia and determine high risk patients required early intervention.
Methods: A prospective study was conducted in South Egypt Cancer Institute, Assiut University. Diagnosis of fungal infection was made by conventional culture media (Sabaroud′s agar), radiological finding and fungal DNA-PCR that performed on serum samples of patients to detect fungemia.
Results: Of 960 hematologic malignancy patients with high risk for infection, rate of fungal infection as documented both clinically and microbiologically was 8.3% (80 cases). Acute leukemia was the majority of the underlying hematological disease with fungal infection (58.8%). AML patients represented 33.8%, which was the highest percentage of cases followed by patients with ALL 25%, then NHL 23.8%. The most encountered hematological finding was Neutropenia which recorded in 71/80 (88.75%) patients, 35 out of 80 patients (43.75%) suffer from severe neutropenia. Lower respiratory tract infection (LRTI) was the most common presentation of fungal infection in patients (n 39, 48.75%), followed by fungemia and fungal oral mucositis grade III or IV (n 30, 37.5%) for each. Isolated pathogens were yeasts in 25 patients (31.25%), molds in 19 patients (23.75%), mixed yeast and mold in 4 patients (5%) and polymicrobial pathogens (fungus and bacteria) in 32 patients (40%). Among isolated fungi, Candida species was the commonest, followed by Aspergillus species. .
Conclusion: Hematological malignancy especially acute leukemia patients were at high risk of invasive fungal infection. LRTI was the commonest detected clinical presentation specifically in patients with marked and prolonged neutropenia.

Background

Fungi have emerged as important causes of human infection, due primarily to the increased numbers of patients subjected to severe immunosuppression, Despite the development of more active, less toxic antifungal agents and the use of antifungal prophylaxis, mycoses (especially those invasive) continue to be a serious infective complication in several patients' outcomes, resulting in high mortality rates[1]. Various factors account for the increased frequency of fungal infections in cancer patients. Regimens that are more intensive are associated with more profound neutropenia and mucosal barrier damage [2]. In hematologic malignancy patients, Candida and Aspergillus species are the major invasive fungal pathogens [3]. Although historically invasive candidiasis (IC) was the most common fungal pathogen, invasive aspergillosis (IA) has a higher incidence than IC in this patient population [4].

Timely recognition and treatment of invasive fungal infection (IFI) in these patients are essential and decrease mortality. New diagnostic methods facilitate an early diagnosis of invasive disease and allow for utilization of a pre-emptive treatment approach, which may ultimately lead to improved treatment outcomes and reduced toxicity [5].

This study was performed in South Egypt Cancer Institute, Assiut University, to evaluate IFI in adult patients with hematological neoplasms to identify common site of infection, type of causative fungi, also percentage of fungemia among patient and identifying high-risk patients requiring early intervention.

Methods

This prospective study was performed at the South Egypt Cancer Institute (SECI), Assiut University, in a period from April 2010 to July 2013.

Patients.

Eighty hematologic malignancy patients with clinical and microbiological documented fungal infection were enrolled in the study.

Inclusion criteria:

Patients were included if they were 18 years of age or older, newly diagnosed or in their first relapse with clinical suspicion of fungal infection in addition they have one or more of these risk factors; 1- Febrile with or without neutropenia. 2- Presence of localized signs and symptoms of infection [oral mucositis grade III or IV, lower respiratory tract infection (LRTI)] [6]. 3- Receiving induction chemotherapy or steroid therapy for more than 10 days. 4-Intravenous intake of therapeutic antibiotic for more than 10 days [7].

Exclusion criteria:

Patients without microbiological documented fungal infection.

The study protocol was approved by the local ethical committee in SECI Assiut University. An informed consent was obtained from each patient enrolled in this study.

Primary diagnosis of hematologic neoplasms was determined by bone marrow aspirate, biopsy and flow cytometry immunophenotyping according to WHO 2008 criteria for classification of hematopoietic and lymphoid neoplasm. Proven, probable, or possible fungal infection were classified in accordance with criteria from the 2002 version of 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) outlined in table (1) [8]. Three criteria are used to define IFI: host factors, clinical factors and microbiological factors, which could help to assign a degree of certainty to the diagnosis. Three levels of probability are proposed: proven, probable and possible IFI. An analysis was restricted to infections classified as proven, probable or possible.

Definitions:

Febrile neutropenia defined as having an absolute neutrophil count (ANC) less than ٥٠0cells/µl, severe neutropenia was defined as a neutrophil count <100 cells /µl more than 10 days. Suspected fungal infection was defined as fever >38 ℃ persisting for more than 96 hour with intravenous antibiotics without positive blood culture. Proven IFI is defined by histological evidence with a concomitant positive culture result. Probable IFI is defined by a host factor plus a microbiological factor plus 1 major (or 2 minor) clinical factors. Possible IFI is defined by a host factor plus a microbiological factor or 1 major (or 2 minor) clinical factors.

Table 1. Criteria used to determine certainty of IFI. [9]

IFI infection criteria

Host factors

- Antibacterial refractory fever (for >96 h)
- Neutropenia (neutrophil count, <500 neutrophils/mm3 for >10 days)
- Fever of unknown origin
- Prolonged steroid use (>3 weeks)
- Graft-versus-host disease

Microbiological factors

- Positive result of culture of sputum.
- Positive microscopic analysis result.

Clinical factors

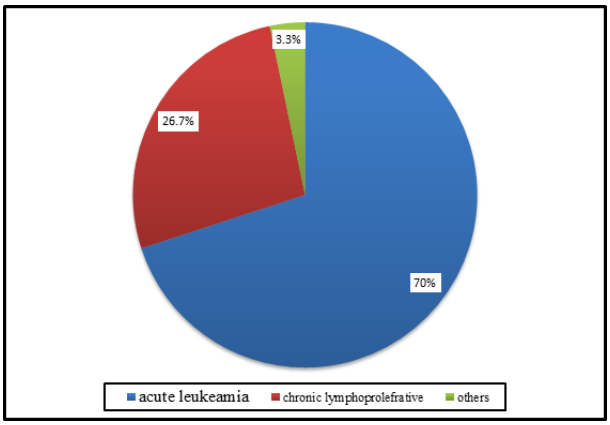
Major

- CT findings (halo, air crescent)

Minor

- New pleural infiltrate (on chest radiograph)
- Pleuritic chest pain
- Shortness of breath
- Cough
- Hemoptysis
- Pleural rub

NOTE: Proven IFI is defined by histological evidence with a concomitant positive culture result. Probable IFI is defined by a host factor plus a microbiological factor plus 1 major (or 2 minor) clinical factors. Possible IFI is defined by a host factor plus a microbiological factor or 1 major (or 2 minor) clinical factors.



Acute Leukemia= ALL+AML, chronic lymphoprolefrative =NHL+CLL, Others=MM+MPN

Figure (1): Distribution of fungeamia according to different patients' diagnosis

Methods:

Patients underwent the following investigations; 1- Full clinical history including localizing signs and symptoms for infection (fever, pain, cough, hemoptysis and dyspnea) and drug history.2- Radiological

examination chest CT scan. 3-Complete Blood Count (CBC). 4-Microbiological culture for morphological identification of fungi from oral swabs and sputum samples. 5- Pan- fungal DNA-PCR from serum samples for fungemia diagnosis.

Clinical samples

Oral or throat swabs and sputum samples were obtained from patients according to clinical presentation. Before initiation of therapeutic antifungals, under all aseptic conditions, 5 ml blood sample was obtained in sterile vacutainer tube for detection of fungal DNA in serum.

Microbial identification

Microbiological samples cultured on Sabouraud dextrose agar plates (Lab M, United Kingdom), blood agar and MacConky media and incubated at 37 ℃ to detect the possibility of presence of bacteria associated with fungal pathogen. Fungi that grew in culture were identified with the use of morphological and microscopic criteria [9] and Roth's flag technique [10]. Mixed IFI was defined as the presence of more than one fungal morphotype (e.g. yeast and moulds) by the growth of two or more fungal pathogens in cultures drawn from an infection site.

DNA extraction

Specimen manipulation and DNA extraction were performed in class II laminar flow cabinet, DNA extraction was conducted directly from serum by enzymatic method, which uses lyticase and proteinase K enzymatic digestion step and adapting a DNA extraction procedure from a commercial kit [QIAamp DNA Mini Kit (Qiagen, Germany)] [11,12]. DNA were stored in -20 until PCR is performed. To monitor contamination, each run was shadowed by a negative control containing molecular-biology-grade water

PCR amplification

PCR assay was performed utilizing the fungus-specific, universal primer pair ITS1 (′5TCCGTAGGTGAACCTGCGG3′) which hybridizes at the 3′ end of 18S rDNA and ITS4 (′5TCC TCC GCTTATGATAT GC3′) (Sigma, USA), which hybridizes at the 5′end of 28S rDNA [13]. After amplification, obtained product visualized using ethedium bromide under UV illumination. Molecular weight ladder were included in each run (Gen Ruler 100bp DNA ladder plus).

Statistical analysis

Descriptive results of continuous variables were expressed as mean (± SD) for parametric variables. Comparison of the demographic characteristics between cases and control was calculated using chi-square test for categorical data and independent sample t-test for numerical variables. Qualitative variable were expressed as number of positive cases (%). P value was considered significant at < 0.05 statistical calculation was performed with statistical package for social science (SPSS) Software (version 16.0: SPSS Inc, Chicago, IL).

Results

Demographic and clinical data of patients

During the 3-years period, 960 adult hematologic malignancy patients were cared for in SECI, Assiut University. Fungal infections were diagnosed clinically and by culture in 80 cases (8.3%). Patients' age ranged between 18 and 70 years with mean age 38.8 ± 14.3 years. Acute leukaemia were present in 47/80 (58.75 %) patients, which include 27/80 (33.8%) AML and 20/80 (25%) ALL. Chronic Lymphoprolefrative neoplasm presented in 26 / 80 (23.5%), which include: 19 / 80 (23.8%) NHL and 7/80 (8.8%) CLL. Multiple myeloma presented in 4/80 (5%) and myloprolefrative neoplasm in 3/80 (3.8 %). Table (2)

Regarding to the neutrophilic count; Neutropenia with neutrophils less than 500 / µl was present at the time of infection in 63/ 80 (79%). Sever neutropenia with neutrophil count less than 100 / µl was present in 35/80 (44%).

Diagnosis of fungal infection was built on consensus criteria of EORTC/ MSG which used to define IFI [9], patients were classified as probable infection in 59/80 (73.75%); and possible infection in 21/80 (26.25%).

Table (2): Characterization of the 80 studied patients

Variable	No.	%
Diagnosis		
- AML	27	33.8
- ALL	20	25.0
- NHL	19	23.8
- CLL	7	8.8
- MM	4	5.0
- MPN	3	3.8
Clinical documented infection		
- Mucositis	30	37.5
- LRTI	39	48.75
- Mixed sites of infection (oral mucositis and LRTI)	11	13.75
Radiology*		
- Positive CT chest finding	24/57	42.57
- Negative CT chest finding	33/57	57.89
Neutrophilic count		
- >500/µl	17	21
- 500-100/µl	28	35
- <100/µl	35	44

*57 patients underwent chest CT scan

MM: multiple myeloma, MPN: Myloprolefrative neoplasms, AML: acute myeloid leukaemia, ALL: acute lymphoplastic leukaemia, NHL: non Hodgkin lymphoma, CLL: chronic lymphocytic lymphoma, LRTI: lower respiratory tract infection

Qualitative data were expressed as (%).

CT: computerised tomography.

According to clinical site of infection (Clinical Documented Infection (CDI)), patients were distributed as follows: Patients with oral mucositis grade III or IV were 30 / 80 (37.5%). Lower respiratory tract infection (LRTI) were 39 / 80 (48.75%) and mixed site of infection (oral and lung) were 11/80 (13.75%).

Microbiological results showed that pure fungal growth was observed in 48 patients, whereas mixed bacterial and fungal growth was encountered in 32 patients. Candida species was the most encountered fungi. It was present in 42 specimen (including 4 specimens were mixed candida and mold pathogen) followed by Aspargillus in 38 specimen then penicillum in 4 specimen.

The relation between different infection sites (CDI) in our patients and their diagnosis showed a statistical significant difference (p <0.000) (Table 3). Another statistical significant relation was detected between patients diagnosis and occurrence of fungeamia with (p=0.000) (figure 1). The comparison between patients who had fungemia and those without fungemia regarding neutrophilic count and WBCs revealed significant decrease in neutrophilc and WBCs count in fungemic patients (Table 4).

Table (3): Relationship between Infection Site (CDI) and diagnosis in 80 studied patients.

Diagnosis	Fungal Infection Site			P-value*
	Oral mucositis (30)	LRTI (39)	Mixed infection sites (11)	
• Acute Leukaemia** (n=47)	18 (38.3%)	22 (46.8%)	7 (14.9%)	<0.001
• Chronic lymphoprolefrative*** (n=26)	11 (42.3%)	11 (42.3%)	4 (15.4%)	
• Others^{+} (n=7)	1 (14.3%)	6 (85.7%)	0 (0%)	

*Chi-square analysis was used to compare the difference in proportions
** Acute Leukaemia= ALL & AML, ***chronic lymphoprolefrative =NHL, CLL, +Others=MM,MPN

Table (4): Comparative Analysis between patients with and without fungeamia regarding peripheral hemogram findings.

variable	Fungal DNA-PCR + ve (no= 30)	Fungal DNA-PCR -ve (no= 50)	P- value
WBCs (× 10^3/ul)	1.38± 0.22	3.85± 0.31	< 0.000***
Neutrophilic count/ ul	0.20± 0.08	1.42 ± 0.16	< 0.000***
Platelet (× 10^3/ul)	78.47± 7.98	150.08 ± 8.89	< 0.000***
Hb (g/dl)	8.23± 0.21	8.56 ± 0.23	0.343

Quantitative variables are expressed as Mean Age ± standard error. No: number. WBC: White blood cells. Hb: haemoglobin. Mann-Whitney Test. Statistical significant difference (p <0.05).

Fungemia was detected in 30/80 (37.5%) patients. Distribution of fungemia in patients classified according to EORTC/MSG (2002) were as follows: 28/59 (47%) patients with probable IF, versus 2/21 (9.5%) patients with possible IFI. Also the highest percentage of fungemia found in patients with Acute leukemia 21/30 (70%), followed by patients with lymphoma 8/30 (26.7%), then patients with multiple myeloma 1/30 (3.3%).

The relation between site of infection (CDI) and presence of fungemia showed a statistical significant difference with (p<0.000). Patients with mixed infection site (oral and LRTI) were the most group associated with fungemia (72.7%), followed by patients with oral mucositis (36.6%) and lastly were patients with LRTI (28.2%).

Discussion

Fungal infections affected 8.3% of all adult cases treated with chemotherapy at the SECI, Assiut University. Regarding patients diagnosis in this study, we found that acute leukemia represented the majority of the underlying hematological disease (58.8%). AML patients represented 33.8%, which was the highest percentage of cases followed by patients with ALL represented 25%, and then NHL patients recorded 23.8%. This finding is consistent with Egyptian study conducted by **EL-mahallawy et al [2]** who found that the most common underlying immunodeficiency associated with IFI was acute leukemia. Where 57.1% of studied patients were diagnosed with acute leukemia. Also **Racil et al [14]** study stated that acute leukemia was the commonest underlying hematological diseases with IFI in studied patients (58.5%). Therefore, patients with acute leukemia represented the typical population of hematological malignancy with the highest risk of IFI .This results may be due to the high-risk features of AML patients**,** related to a unique intrinsic functional defect or to relative reduction in the absolute numbers of neutrophils at the start of treatment [7,15]**.**

This study revealed that for all patients the commonest site of infections was LRTI (48.8%), followed by fungeamia and oral mucositis represented by the same incidence rate (37.5%). This result is agreed with **EL-mahallawy et al [2], Betts et al [16] and Racil et al [14].** All previous studies concluded that the lung is the commonest site of infection but with different recorded percentages 35.7%, 73% and 93.8% respectively. Our detected frequency is higher than that for the Egyptian study conducted by **EL-mahallawy et**

al [2], because the percentage of AML patients in our study is higher (33.8%) than that for EL-mahallawy study (25.7%). Moreover, the other two study [14, 16] have much higher percentage compared to ours because they selected AML patients only. AML patients were at high risk of invasive lung infection because the majority of these patients had active malignancies and multiple risk factors [17, 18]**.** The most frequent risk factor for invasive fungal infection in this study was neutropenia. Hence (88%) of patients were neutropenic <1000/µl in addition sever neutropenia was encountered in (44%)of patients .This recorded frequency is in harmony with **Racil et al [14]** study who stated that the most common classical risk factor identified in 61.4% of infection episodes was profound and prolonged neutropenia. **Li et al [19]** added that severe and prolonged neutropenia increases the incidence of IFI.

As regard the types of isolated pathogens this study revealed that pure fungal pathogens were detected in 60% of patients while polymicrobial infection presented in 40% of patients. Candida species. Were the most common encountered pathogen (52.5%) followed by aspargillus species (47.5%). This is in agreement with Gedik **et al [20]** study who stated that Candida species were predominated confirmed fungal infections. In addition, **EL-mahallawy et al [2]** study reported that yeast was isolated in (78.6%) of specimen and molds in (21.11%). Among yeasts, Candida was the commonest, while the most encountered molds were Aspergillus spp. The difference between our study and this study in percentage may related to difference in number of patients enrolled and different specimen types used by both of them. Reversely, some studies revealed opposite findings as those conducted by **Neofytos et al [21]** that report relatively low rates of invasive candidiasis and high rates of invasive mold infection. **Pagano et al [22]** study also found that patients with hematologic malignancies are currently at higher risk of IFI caused by molds 64.3% than by yeasts 35.7%. The majority of the mold infections (90%) were caused by Aspergillus spp., while candida spp. were still the predominant yeast pathogen. The Egyptian study conducted by **EL-Mahallawy et al [23]** found polymicrobial (mixed bacterial and fungal growth) was encountered in 62.5% of specimen, aspargillus spp. detected in 46.88 % while candida spp in 21.88% of specimen. The difference between those different studies than ours may related to different type of specimens or different used technique to obtain the specimens. In our study, we used oral swab and sputum samples only and we did not able to use bronchoalveolar lavage as most of our patients were thrombocytopenic and this procedure were risky to them.

The PCR used in the study detected the presence of fungal DNA in the serum to serve fungeamia diagnosis. It is expected to be positive only when the fungus invades the blood vessels. The percentage of fungemia in patients was (37.5%), the highest percentage were present in patient with mixed infection site (oral and lung) (72.7%) followed by, patients with oral mucositis (yeast infection) (36.6%) then patients with LRTI (mostly mold infection) (28.2%). This indicate that candida blood stream infection is more common than mold blood stream infection. Acute leukemia was the most likely diagnosis associated with fungeamia as 70% of fungeamia patients had acute leukemia (61.9% AML and 38% ALL). This previous result matched with the study of **Pagano et al [19]** who found that blood stream infection were detected in (32.5%) of patients *Candida* non–albicans species were responsible for over half the episodes of candidemia 83% percent of the cases of candidiemia occurred in Patients with acute leukemia (71% in AML versus 12% in ALL)

. Candida. albicans was isolated in 36% of patients with hematological disease in a surveillance study in cancer patients conducted by the European Organization for Research and Treatment of Cancer [24]**.**

Morace et al [25] found that 43% of patients had fungeamia. Also **EL-MahalLawy et al** [23] mentioned that 35% of the studied patients had fungeamia. That previously recorded studies in addition to ours may be due to the underlying compromised status of patients with IC. Patients with IC were more likely to be severely neutropenic. The majority of them received aggressive chemotherapy, which is associated with significant oral and lower gastrointestinal mucositis that lead to easy blood invasion. Fungemia associated with hematological malignancy most often, occur in acute leukemia patients in relapse, prior intensive chemotherapy, profound neutropenia, mucositis and steroid therapy [26]**.** On the other hand, some studies found that the percentage of fungemia in studied patients was lower than our results. As those conducted by **Neofytoes et al [21]** who found that, the percentage of fungemia was (11.3%) **and Badiee et al [27]** showed that 19.6% of patients in their study had fungemia. The difference between our study and these studies due to early diagnosis and start of powerful antifungal therapy in high risk individuals. Also may be due to different type of chemotherapy that cause less severe neutropenia or shorten its duration

Our study showed that the highest proportion of positive PCR tests were noted in patients classified as probable invasive infection according to EROTEC/MSG (2002) (47.5%) , while patients with possible infection had the lowest rate of positivity (9.5%). These results may supported that the PCR method was fungal specific and detect the presence of fungal DNA in serum in case of blood vessels invasion. Therefore serum fungal DNA-PCR expected to be positive more in patients with probable infection.

Conclusion

We concluded that patients with hematological neoplasms especially acute leukemia were at high risk of invasive fungal infection. LRTI was the commonest clinical presentation of invasive infection in those group of patients associated with marked and prolonged immune suppression. Sever neutropenia and multiple infection site in patients increase the risk for fungeamia development. Yeast pathogen were the commonest isolated fungi in our study. Ongoing clinical trials are expected to provide further insight into the management of these complex and serious infections.

Abbreviations

PCR:	Polymerase Chain Reaction
IFI:	Invasive Fungal Infection
LRTI:	Lower Respiratory Tract Infection
IA:	Invasive Aspergillosis
IC:	Invasive Candidiasis
SECI:	South Egypt Cancer Institute
EORTC/MSG:	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
WHO:	World Health Organization
ANC:	Absolute Neutrophil Count
CDI:	Clinically Documented Infection
CBC:	Complete Blood Count
CT:	Computerized Tomography
DNA:	Deoxy Neuclic Acid
ITS:	Internal Transcribed Spacer
bp:	base pair
SD:	Slandered Deviation
AML:	Acute Myeloid Leukemia
ALL:	Acute Lymphoblastic Leukemia
NHL:	Non Hodgkin Lymphoma
CLL:	Chronic Lymphocytic Leukemia
MM:	Multiple Myeloma
MPN:	Myloprolefrative Neoplasms
SPSS:	Statistical Package for Social Science

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