

Laboratory Mycological Study of Visceral Fungal Infection in Tehran, Iran

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Background: Many comprehensive epidemiological studies have been done about fungal infections, which are one of the public health and therapeutic problems in different communities. Since prevalence rate of fungal diseases and their etiological agents are changing over time, the aim of this study was to provide additional information about visceral fungal infections (VFIs) in order to understand the ways of their dissemination, to prevent disease transmission, to eliminate contamination sources and predisposing factors, and to provide effective ways for their treatment.

Materials and Methods: Samples were taken from the lesion of patients referred to medical mycology laboratory of Tehran University of Medical Sciences from 2014 to 2015. After providing direct wet mount of Potassium hydroxide (KOH) smears from these samples and samples sent from other medical centers, culturing on Sabouraud Dextrose agar with chloramphenicol (SC) and brain heart infusion agar (BHI) media was performed. After growth, species were identified.

Results: From a total of 295 suspected samples, VFI was proved in 69 cases (23%). Aspergillosis was the most prevalent infection among VFIs. Candidiasis, cryptococcosis and mucormycosis were in the late orders, respectively. Two patients were also infected by *Trichosporon*. The predominant species in aspergillosis infections was *Aspergillus flavus*, and the predominant species in candidiasis infections was *Candida albicans*.

Conclusion: According to the obtained results on the prevalence rate and incidence of VFIs between male and female patients in different age groups and also by taking into account the prevalent type of fungi and infectious site of patients' bodies, it is possible to take appropriate action for the prevention and treatment of these kinds of diseases by using the important keys of these results to research and study etiological and underlying factors involved in these diseases.

Keyword: Visceral, Fungal, Infection, Invasive

1. Background

Visceral fungal infections (VFIs) have significantly increased due to developments in medical care, provided for population that are in risk of immune compromising (1-5). Approximately one third of the immune compromised patient demise because of visceral mycoses. The most susceptible populations are those patients afflicted with a disease of hematological malignancies, AIDS, and transplant recipients. The epidemiological studies provide reasonable hints for direct experimental antifungal treatment and chemoprophylaxis. However, only, a described analysis of clinical data cannot efficiently estimate the rate of invasive fungal infections, because most of them remain undetected in died patients due to difficulty in diagnostics. Essentially, the diagnosis of proven VFIs needs culture or histopathology (6-8). VFIs can be transferred by inhalation of the spores or conidia (aspergillosis, cryptococcosis) or their penetration into the mucosa by some commensal organisms such as *Candida albicans*. Infections may cause life-threatening systemic illness such as candidiasis (9), aspergillosis (10), mucormycosis (11), and cryptococcosis (12). The clinical symptoms of a disease caused by mentioned fungal agent can be highly variable and related to condition of host immunity system and its physiological condition (5). Many comprehensive and spacious epidemiological studies have been carried out about fungal infections, which are one of the health and therapeutic problems in different communities (13-16).

2. Objectives

Because prevalence rate of mycotic diseases and their etiological agents becoming different over time due to the geographical conditions (17), the aim of this study was to

obtaining additional useful information about VFIs to understanding the ways of their spreading, to prevent from transmission of disease, for removing of contamination sources and underlying factors, and to prepare efficient ways for their treatment.

3. Materials and Methods

This cross-sectional study was carried out from 2014 to 2015 in the medical mycology laboratory, faculty of public health, Tehran University of Medical Sciences, Iran. The subjects were those patients referred to the medical centers due to their illness. After visiting by specialist physicians, they or their samples were sent to this center for the evaluation of histopathological and mycological characteristics of their infections. Some of the samples were biopsy sample taken from hospitalized patients (Table 1). In medical mycology laboratory, direct smears of samples were prepared by 15% KOH and cultured on SC with 0.005% chloramphenicol and BHI agar media (E. Merck, Germany). Culturing and preparation of direct smears of some samples such as cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL), and urine were done with sedimentation after centrifuging. Also, CSF samples were stained with Indian ink. After putting in moistures environment for an hour, direct smears were examined with an optical microscope (Olympus, Germany). Culture media were surveyed after incubation at 30 and 37 °C for 48-72 hours. Culture media for those cases with no growth were maintained up to two weeks. To identifying positive cultures, slide culture, surveying macroscopic characteristic of colonies, API, and standard mycological procedures were performed. The data analysis was performed by SPSS software (V.18). The study was assessed by using standard Chi-squared and 95 % Confidence intervals (CI). Statistically, *P* value < .05 was considered as significant difference or correlation.

Table 1: Frequency of different clinical visceral specimens based on the type of VFI and genus of patients.

| Fungal agents | Visceral Aspergillosis Nb. (%) | | Visceral candidiasis Nb. (%) | | Visceral Mucormycosis Nb. (%) | | Visceral Cryptococcosis Nb. (%) | | Visceral Trichosporonosis Nb. (%) | | Total of each genus Nb. (%) | | Total Nb. (%) |
|------------------------------|--------------------------------|-----------|------------------------------|-----------|-------------------------------|---------|---------------------------------|---|-----------------------------------|---------|-----------------------------|----------|---------------|
| | M | F | M | F | M | F | M | F | M | F | M | F | M&F |
| Lymph node biopsy | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Cervical bone biopsy | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Lumbar abscess | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 1 (1.4) |
| sinuses discharge | 9 | 9 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 11 (16) | 14 (20) | 25 (36) |
| Sputum | 1 | 1 | 2 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 4 (5.8) | 5 (7.2) | 9 (13) |
| Skull tumor biopsy | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Bronchoalveolar lavage (BAL) | 3 | 4 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 11 (16) | 8 (11.6) | 19 (27.6) |
| Brain microabscesses | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Stool | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 1 (1.4) | 2 (2.8) |
| Ventral buccal mucosa | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Wound discharge | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (2.9) | 0 | 2 (2.9) |
| Vaginal discharge | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (2.9) | 2 (2.9) |
| Urine | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 0 | 1 (1.4) |
| Cerebrospinal fluid(CSF) | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 (4.3) | 0 | 3 (4.3) |
| Total of each genus | 17 (24.7) | 15 (21.7) | 15 (21.7) | 10 (14.6) | 2 (2.9) | 5 (7.2) | 3 (4.3) | 0 | 1 (1.4) | 1 (1.4) | 38 (55) | 31 (45) | 69 (100) |
| Total(M&F) | 32 (46.4) | | 25 (36.3) | | 7 (10.1) | | 3 (4.3) | | 2 (2.9) | | 69 (100) | | 69 (100) |

M: Male, F: Female, Nb.: Number, (%): Percentage

4. Results

From a total of 295 suspected patients, VFI was proved in 69 cases (23%). The most prevalent infection was aspergillosis in VFIs. The late orders were belonged to candidiasis, cryptococcosis, and mucormycosis, respectively. About 32 patients (46%) were infected by aspergillosis with equal number of both male and female. The predominant species of *Aspergillus*, was *Aspergillus flavus*. The most patients infected by Aspergillosis were in the age group of 20-29 years. Also, 25 patients (36%) were infected by candidiasis, the number of males was more than females. *Candida albicans* was the predominant species in candidiasis. The most patients infected by candidiasis were in the age group of 50-59 years. Also, 7 cases were infected by mucormycosis, the number of females were more than males. Most cases of mucormycosis were in the age group of 20-29 and 50-59 years. The predominant fungus in mucormycosis was *Rhizopus*, and its most common underlying disease was diabetes. Three patients were also

infected by cryptococcosis, and all of them were males (one 51-year-old patient and two 71-year-old patients). Among patients, one 50-year-old woman and one 9-year-old boy were infected by *Trichosporon*. Significant difference was not observed between genus and age group of patients with the type of VFIs ($P > .05$). The most common organs involved in fungal infections were sinuses and respiratory tract (BAL), respectively (Table 1). There was a significant difference between organ involvement and the disease ($P < .05$). The most common underlying diseases of the patients identified with VFIs were diabetes, tuberculosis, and liver transplantation, types of cancer, sinusitis, heart surgery, kidney transplant, asthma, and bronchitis, respectively. Figure 1 shows the frequency of the patients with visceral aspergillosis based on age group and genus. Figure 2 shows the frequency of the patients with visceral candidiasis based on age group and genus. Table 1 shows the frequency of different clinical visceral specimens based on fungal agents and genus.

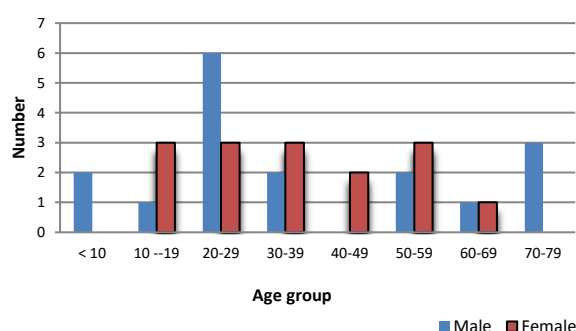


Figure 1. Frequency of patients with visceral aspergillosis based on age group and genus ($P>.05$).

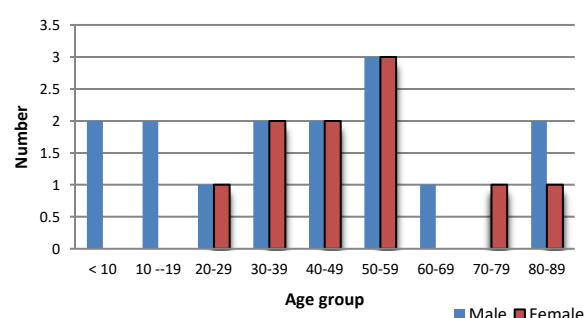


Figure 2. Frequency of patients with visceral candidiasis based on age group and genus ($P>.05$).

5. Discussion

In recent years, one of the most public health and therapeutic problems in different countries is fungal diseases, among which diseases causing mortality are more important than the other. Underlying diseases are considered as risk factors involved in incidence and prevalence rate of opportunistic fungal diseases (6-8,18-20). In this study, some underlying diseases such as diabetes, tuberculosis, liver transplantation, cancer, sinusitis, heart surgery, kidney transplant, asthma, and bronchitis were identified that certainly can be involved in VFIs. In current study, the most prevalent infection was aspergillosis in VFIs. The late orders were belonged to candidiasis, cryptococcosis, and mucormycosis, respectively. (Table1). These results are in accordance with Bassiri Jahromi's study conducted in Pasteur institute of Iran (21) and Sharifpour's study (22). However, by considering *Aspergillus* as the prevalent fungi of the environment and *Candida* as the common body's microflora, the high prevalence rate of these two genera of fungi in VFIs is justifiable. In Khodavaishi's study conducted in Sari and Babol (23), Badiie's study conducted in Shiraz in 2009 (24) and Pfaller's study conducted in the United States of America in 2010(25), candidiasis and aspergillosis were considered as the most prevalent infections, respectively.

In this study, the most common isolated species of aspergillosis was *Aspergillus flavus*, this finding is in accordance with Khodavaishi's (23), Badiie's (24), Zarrinfar's (26), and Hedayati's (27) studies conducted in Iran. However, It is not in accordance with some studies conducted in abroad such as Mantagna's (28) in Italy and Glare's in New Zealand (29), in which the predominant species was *Aspergillus fumigatus*. Non-compatibility of the current study results with some other results can be due to time and place conditions (geographical conditions).

In current study, the most common species isolated in candidiasis was *Candida albicans*, this finding is in accordance with Taghipour's (30) and Afsarian's (31) studies conducted in Iran, Omrani's study conducted in Saudi Arabia (32), Yang's study conducted in China (33), and Yapar's study conducted in Turkey (34). In this study, the prevalence rate of candidiasis in males was more than females, and in the age group of 50-59 years was the most prevalent (Figure2), these findings are also in accordance with the Khodavaishi's (23) and Bassiri Jahromi's (21) studies.

In this study, there was a significant difference between organ involvement and the disease ($P<.05$). The most common organs involved in fungal infections were sinuses and respiratory tract (BAL), respectively (Table 1). Fungal rhinosinusitis is considered as an uncommon disorder, and its frequency has been increasing in recent years. *Aspergillus* spp. is the most common species reported as a major cause of fungal sinusitis, but the most frequent fungal isolated from acute invasive form of fungal sinusitis belong to the Zygomycete order. However, etiological agents of these infections may vary according to the type of sinusitis and geographical epidemiology. In this study, fungal rhino sinusitis was the prevalent disease compared with others (36%). The predominant fungal agents were *Aspergillus* spp. and Zygomycete order, respectively. These results are in accordance with Nazeri's study conducted in Iran (35). Fungal respiratory tract infections were the second order diseases of VFIs in this study (27.6%). In the present study, *Candida* spp. were the predominant fungal agents, this finding is in accordance with Khodavaishi's study conducted in Iran (23) and Garnacho-Montero's and Sganga's studies conducted in other countries (36-37).

As Tehran is the capital of Iran, its hospitals are the references for many patients from all parts of the country. In Tehran, medical mycology laboratory of Tehran university of Medical Sciences is the reference laboratory for many specialist physicians and patients. Thus, the results of this study approximately can have more external validity in comparison with other local Iranian studies.

6. Conclusion

We analyzed the visceral mycoses and concluded that mycoses are still considered as an important problem for the clinician. According to the results that obtained on the prevalence rate of VFIs between males and females with different age groups and also by attention to the most common type of fungal agents and infectious body locations of patients, it is possible to prepare appropriate action for the prevention and treatment of these kind of infections by using the important results of this research. Also, it can be considered etiological and underlying factors involved in diseases of this study.

Conflict of Interests

The authors declare they have no conflict of interests.

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Authors' Contributions

All of authors contribute to this study.

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