

Invasive Fungal Infections: A Comprehensive Review

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Abstract Fungi are a versatile group of microorganisms which can be freely present in the environment, be a part of the normal flora of human and animals and have the ability to cause mild superficial infections to severe life threatening invasive infections. Invasive fungal infections (IFI's) are those infections where fungi have invaded in to the deep tissues and have established themselves resulting in prolonged illness. IFI's usually are seen in debilitated and immunosuppressed individuals. There are many reports of IFI's even in immunocompetent individuals thus making IFI's a potential threat in the present century. Fungi are saprophytic microorganisms which have evolved mechanisms to survive in the mammalian hosts. Most of the fungal infections have been accidental and systemic fungal infections are a rarity that may result in high mortality. In systemic fungal infections the outcome of the disease depends more on the host factors rather than the fungal virulence. Immune response to fungal infections is a complex subject where in fungi invading goes unrecognised by the immune system and that invasive fungal infections can result in severe inflammatory reactions resulting in morbidity and mortality. From being uncommon during the earlier part of the 20th century when the world was plagued with bacterial epidemics, fungi have evolved as a major global health problem.

Keywords: Invasive Fungal Infections (IFI's), immunocompromised individuals, opportunistic pathogens, predisposing factors for IFI's

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1. Introduction

The presence of fungal elements either as mould or yeast in deep tissues of biopsy or needle aspirates that is confirmed on culture and histo-pathological examination can be described as an Invasive Fungal infection (IFI). This definition was proposed by Invasive Fungal Infections Cooperative group (IFICG) of European Organization for Research and Treatment for Cancer (EORTC) and Mycology Study group (MSG) of National Institute of Allergy and Infectious Diseases (NIAID) [1]. Clinical features including febrile illness that continues even after prolonged broad spectrum chemoprophylaxis, radiological evidence of lesions with culture confirmation for the presence of fungi also establish the invasive fungal infection. Invasive fungal infections can involve any part of the body and are common in immunocompromised patients which usually result in high mortality [2]. So by definition IFI's are established by the host factors, clinical features and cultural confirmation. Among the fungi that have potential to cause IFI's include Yeasts (*Candida spp*, *Cryptococcus spp*) and moulds (*Aspergillus spp*, *Fusarium spp*, *Scedosporium prolificans*, *Mucor*, *Rhizopus* and

Rhizomucor Absidia) [3-8]. IFI's are also caused by dimorphic fungi including *Histoplasma capsulatum*, *coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides spp*, *Sporothrix spp* and *Penicillium marneffii*) [9,10,11]. Scanty reports of IFI's are also reported in literature with rare yeasts like *Saccharomyces spp*, *Trichosporon spp*, *Malassezia spp*, *Geotrichum candidum*, *Hansenula anomala*, *Rhodotorula spp* and *Picchia spp*. [12,13,14]. Among these fungi *Candida spp*, *Cryptococcus spp*, *Aspergillus spp*, *Mucor* and *Rhizopus* are either saprophytes in soil and environment or can be present as commensally in human as well as animals. Fungi are saprophytic microorganisms which have evolved mechanisms to survive in the mammalian hosts. Most of the fungal infections have been accidental and systemic fungal infections are a rarity that may result in high mortality (Figure 1). In systemic fungal infections the outcome of the disease depends more on the host factors rather than the fungal virulence. From being uncommon during the earlier part of the 20th century when the world was plagued with bacterial epidemics, fungi have evolved as a major global health problem. This could be attributed to the immunosuppression either because of infections (AIDS) or cancer and metabolic disorders like diabetes.

Fungi though have established themselves as plant pathogens and have been a major threat to the agriculture, in course of time have evolved as both primary and opportunistic pathogens which have the ability to cause infection both in immunocompetent and immunocompromised individuals respectively [1]. There are about 100,000 fungal species of which only 300 species are known to cause animal or human infection [15]. Few fungal species can inhabit human and animals (*Candida spp*); some fungi like dermatophytes inhabit skin, hair and nail of human and animals and are highly transmissible. Most of the fungal infections in human are undiagnosed and under reported. The dermatophytic fungi are the common fungal infections in human. Criteria for establishing an IFI include isolation of fungi from blood culture/ sterile site, isolation of the same fungus from the source wound, radiological evidence, no evidence of other infections and improvement in the clinical condition of the patient after initiation of antifungal therapy.

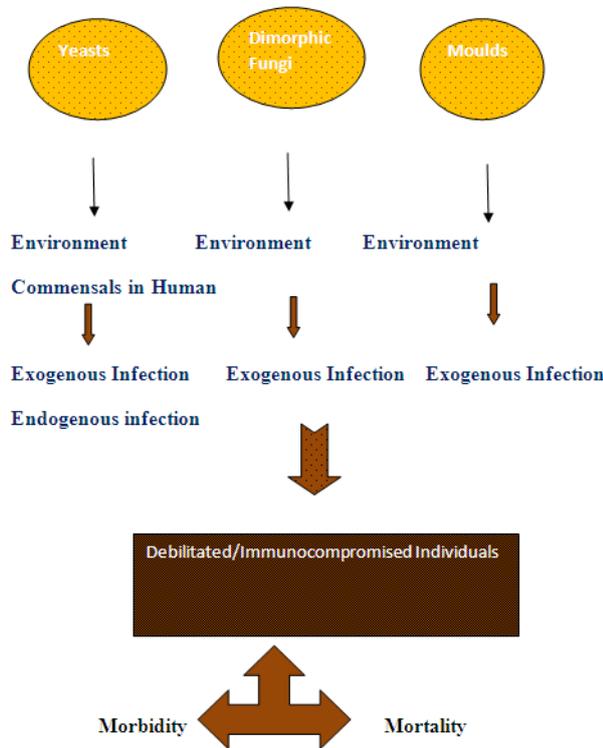


Figure 1. Flow chart representing source and consequence for IFI's

2. Pathogenicity and Virulence

Depending on the virulence and pathogenicity there are broadly two types of fungi: true pathogenic fungi and opportunistic fungi [16]. Fungal virulence can be attributed to many factors. Amongst them, the ability of the fungi to grow at 37°C and adapt to the environment inside the host tissues (especially dimorphic fungi) helps them to establish and cause infection. Factors responsible for fungal pathogenicity are specific to the fungus. Enzymes, toxins and by products of various fungi play an important role in their virulence and pathogenicity. *Aspergillus spp* produces proteases (elastin-serine protease and elastin-metalloprotease) and aspartic acid proteinase. Gliotoxin and restrictocin are the toxins produced by *Aspergillus spp*. Phenoloxidase/melanin synthesis along

with the presence of capsule make *Cryptococcus* an opportunistic pathogen [17]. *Candida spp* is an opportunistic fungi, that being a normal flora of human can be responsible for significant infections from superficial skin and nail infections to urinary tract infections and candidaemia. *Candida spp* have been able to form biofilms: described as microbial adherence to either biotic or abiotic surfaces forming a polymer matrix which becomes a substrate on which microbes grow [18]. This ability makes *Candida spp* a potential cause of nosocomial infections. Few fungi like the *Cryptococcus neoformans*, *Candida albicans* and *Aspergillus fumigatus* have been reported to be producing a sterol regulatory element binding proteins (SREBPs) that help the fungus to tolerate the hypoxic environments in host tissues as well as demonstrate antifungal resistance [19]. Fungal pathogenesis is complex and it has been reported that pathogenic fungi of plants have certain proteins including non ribosomal peptide synthetases (NRPS's) and polyketide synthases (PKS's) that were not found or present only in minute quantities in saprophytes [20]. Predisposing factors for IFI's include neutropenia, organ transplant recipients (bone marrow, stem cells or solid organs). HIV seropositive with marked immunosuppression (AIDS), preterm neonates, patients on long term immune suppressive therapy, those undergoing intensive care treatments. Long term broad spectrum antimicrobial therapy, cancer chemotherapy, presence of indwelling catheter or other medical devices, extremes of age and other biological factors also predispose to individuals to IFI's [21,22]. Genetic predisposition to IFI's due to impaired NADPH oxidase activity, abnormal synthesis of tumour necrosis factor α , Interleukin 10 and other cytokines has been reported recently [23,24] (Table 1).

Table 1. Predisposing Factors Associated with Invasive Fungal Infections

Infections
Human Immunodeficiency Virus (HIV)
Cytomegalo Virus (CMV)
Mycobacterium tuberculosis
Inadequate treatment of superficial fungal infection
Malignancy
Hematological Malignancy (Leukaemia, Multiple Myeloma)
Non-Hematological malignancy (Breast Carcinoma)
Debilitated patients
Critically ill on mechanical ventilation
Patients i ICU's and NICU's
Transplant patients
Stem cell transplants
Organ transplants (Kidney)
Non-Infectious causes
Severe trauma (Accident)
Major surgical procedure
Indwelling prosthetic devises
Fungal colonization in mucosal surfaces
Exposure to Building dust
Chronic airway obstruction (COPD)(Asthma)
Immunosuppressive therapy (Steroids)
Chronic broad spectrum antibacterial treatment
Extremes of age
Immunological Deficiency
Neutropenia
Autoimmune diseases
Genetic Predisposition
Impaired NADPH oxidase activity
Abnormal synthesis of tumour necrosis factor α (TNF- α)
Interleukin 10 and other cytokines

3. Laboratory Diagnosis

Laboratory methods for the diagnosis of IFI's include the conventional mycological techniques, biochemical (finding fungal products), Immunological and molecular methods. Wet microscopy of clinical samples using different concentrations of potassium hydroxide (KOH) reveal fungal elements. Gram's stain, Giemsa stain, india ink and fluorescent staining with calcofluor white increase the chances of finding fungal elements by microscopy. Immunohistochemistry and immunofluorescence increase the sensitivity and specificity of microscopy. Sabourauds Dextrose Agar (SDA), Chromogenic media, Czapek-Dox media, Corn Meal Agar (CMA), Muller Hinton agar (MHA) and RPMI 1640 are common culture media used for growth and identification. Antibodies (Specific Anti-*Candida*, Anti-*Aspergillus*) and Antigen of fungi in general (mannan, galactomannan) and specific fungal antigens (Cryptococcal Ag) are useful immunological methods for diagnosing IFI's [25]. Detection of fungal metabolites including enolase, arabinitol, creatinine and β -(1-3) D-glucan helps in species and genus specific diagnosis. PCR (conventional, nested-PCR, real-time PCR), Microarray, Nucleic acid sequence-based amplification (NASBA) and pyrosequencing are the molecular methods available for typing and confirmation of strains [26,27,28,29]. Rapid method (MALDI-TOF) for detection of common fungi responsible for IFI's has been reported recently [30].

4. Antifungal Therapy and Drug Resistance

When compared to antibacterial antibiotics, there are only limited numbers of available drugs against fungi. The antifungal antibiotics target various stages of metabolic pathways and are placed in different groups including azoles, polyenes, fluoropyrimidine analogs, echinocandins, morpholines, allylamines and thiocarbamates. 5-Fluorocytosine (5-FC) and 5-Fluorouracil are the two antifungals derived synthetically from DNA nucleotide cytosine that are used to treat human infections. 5-FC possesses a broad spectrum of activity not only against fungi (both yeasts and hyphal forms) but also protozoa (*Leishmania* and *Acanthamoeba* spp). 5-FC has been recommended against fungal infections always as a combination drug with other antifungal agent as fungi have ability to acquire resistance [31]. Pharmacological action of 5-FC is mainly due to its fungistatic activity, which inhibits protein synthesis, DNA replication and thereby inhibiting fungal growth. Side effects of 5-FC are minimal and confined to liver. Amphotericin-B (AmB), nystatin and natamycin are only the three polyene/macrolide group of antifungal drugs used to treat human infections which are synthesized from *Streptomyces* bacteria. Polyenes target the ergosterols the main component of fungal cell by destabilizing the plasma membrane and initiate cell lysis. Amphotericin-B drug toxicity is confined to liver and kidneys and this is minimised by using liposomal Amphotericin-B [32]. Advantage with AmB is that very rarely resistance has been observed in fungi. Azole group of antifungal drugs are pharmacologically divided in to two groups: the imidazoles that have two nitrogen atoms and the triazoles

that have three nitrogen atoms. These antifungals target the ergosterol biosynthesis pathway and trigger formation of toxic byproducts that are lethal to fungi. Clotrimazole, econazole, miconazole (imidazole), fluconazole, ketoconazole, itraconazole, voriconazole, posaconazole and ravuconazole (undertrial) (triazoles) are the azole group antifungal drugs. The imidazoles are used mostly as topical agents due to their severe toxicity when taken orally. Triazoles have been regularly modified and are now used to treat fungal infections caused by both yeasts and moulds. Echinocandins are the new antifungal agents which are synthetic derivatives of lipopeptides which are produced by various fungi (*Aspergillus rugulovalvus*, *Zalerion arboricola* and *Papularia sphaerosperma*) approved for treatment of invasive fungal infections (IFI's) including caspofungin B, micafungin, anidulafungin, pneumocandin b and papulacandin. Echinocandins act on the β (1-3)-glucan synthase, an enzyme that is critical for maintaining the cell wall integrity thereby resulting in cell lysis [33]. Advantages with echinocandins include less toxicity, broad spectrum activity and synergistic effect in combination therapy. Terbinafine, tolnaftate, amorolfine, ciclopirox and griseofulvin are all used as topical agents and are effective in treatment of superficial fungal infections including dermatophytic fungi.

Though rare, antifungal drug resistance has been a cause of concern in the treatment of IFI's since last decade. Resistance may be defined as the persistence of the microbe and progression of disease even after prolonged treatment. Resistance can be primary or innate drug resistance and acquired resistance. Mechanisms of resistance to antifungal agents differ in various groups of drugs. Reduced drug absorption and drug accumulation, decreased affinity of drug to its target, alteration in metabolic pathways to disturb the drug concentrations in cell are some of the reasons for antifungal drug resistance. Studies have also elaborated on the molecular mechanisms of drug resistance in fungi. CDR1 (*Candida* drug resistance 1), CDR2, CgCDR1 (*Candida glabrata* drug resistance 1), CgCDR2, CaMDR1 (*candida albicans* multidrug resistance 1), CgFLR1 (*Candida glabrata* fluconazole resistance1) and ERG11 (echinocandin resistance gene) are some of the genes identified in *Candida* spp that are responsible for drug resistance [17]. Biofilm formation has also been recognised as a potential factor for fungal drug resistance due to the production of an exopolymeric material that inhibits or restricts penetration of antifungal drugs [34,35,36].

5. Immune Response to Invasive Fungal Infections

As with any other microbial infection, an immunological response of a host plays an important role in the establishment and the resulting consequences of invasive fungal infections. Both innate and adaptive immune mechanisms play an important role during invasive fungal infections [37]. Fungi are a group of microorganisms that are better protected against the external stresses and immune defence due to their cell wall components made of hard, complex carbohydrate polymers including the mannans (chains of mannose molecules added to fungal proteins via N- or O-linkages),

chitin (polymers of N-acetylglucosamine) and glucans (polymers of glucose) [38]. The outcome of an invasive fungal infection depends on various factors including the current clinical condition of the patient (Underlying disease), the immunological status, pathogenicity and virulence determinants of the invading fungal species and the location of infected area. Recent studies have confirmed the interactions of fungal community (mycobiome) and the bacterial flora in the intestines have influence over immunosystem either resulting in protection or disease. A defect in the innate immune receptor Dectin-1 gene (CLEC7A) was found to be associated with reduced immune response to fungal colonization thereby resulting in severe ulcerative colitis. Dectin-1 receptor is a C-type lectin receptor that recognises β -glucans, the major fungal cell wall component. This study has also noted that there were more than 100 fungal species belonging to more than 50 genera that form a part of gut microbiota. *Candida tropicalis* was found to be the predominant fungal species followed by *Trichosporon* which are both opportunistic pathogens and *Saccharomyces* a non pathogen. Results of the study also indicate that a reduction in the numbers of non-pathogenic *Saccharomyces* and increase in *Candida* spp results in severe ulcerative colitis [39]. Fungal pattern-recognition receptors (PRR's) play an important role in sensing and recognition of fungal pathogen associated molecular patterns. Cells of the immune system that actively take part in the defence against the invading fungal pathogens include neutrophils, monocytes, macrophages and dendritic cells along with natural killer cells and other cytokines [40]. Studies have confirmed that Toll-like receptors (TLR's) and non-TLR's (TLR2, TLR4, TLR9, C-type lectin receptors; CLR's and galectin family proteins) on the macrophages have the ability to recognise the pathogen-associated molecular patterns (PAMP's) of the invading fungi and initiate an inflammatory response. This is dependent on the myeloid differentiation primary response gene-88 (MYD-88) initiated signalling pathway [41]. Other methods of sensing an invading fungi include the NLR's (NOD-like receptors), that get activated to stimulate the production of interleukins (IL-1 β and IL-18) [42]. Host response against invading fungi has not been completely understood yet but a recent study has demonstrated the role of chitinase (CHIT1 gene) activity in response to fungal infection, which demonstrates increased activity during an inflammatory reaction in the presence of various cytokines [43]. Studies have also demonstrated that single nucleotide polymorphisms (SNP's) at various genes (DECTIN1, TLR1, TLR4, TLR6, TLR9) coding for cytokines and the receptors is associated with either susceptibility or resistance to fungal infections [44,45].

6. Conclusion and Future Implications

IFI's have been on the ascending since the past three decades, due to the emergence of HIV, influenza virus, various other immunodeficient conditions including solid organ transplantations, cancers, genetic defects and autoimmune conditions. Global warming has also been attributed to be another cause of emergence of various fungal infections [46]. As evidenced from the available

literature it is clear that fungi, though are saprophytes and free living have evolved during the last century to be able to survive in the human tissues and produce disease. Invasive fungal infections (IFI) have become a cause of concern in debilitated population as more than 90% of the IFI's have been in immunosuppressed individuals [47,48,49,50]. Increasing reports of systemic fungal infections resulting in severe morbidity and mortality should be considered as an alarming bell. Elaborating and updating regularly the possible predisposing factors of IFI's and prompt diagnosis would certainly reduce the mortality arising from IFI's. Considering the fact that IFI's may result from the fungi that have already been colonized in the patients, screening for fungi in severely debilitated and immunosuppressed individuals will be effective in reducing the morbidity and mortality. The Paucity of the availability of newer antifungal chemotherapeutic agents and the drug toxicities make it difficult to manage IFI's. Evolving effective treatment strategies (to completely resolve IFI's and most other common fungal infections long term therapy is needed), regular antifungal susceptibility testing will be necessary to avoid treatment failure. IFI's should be considered as a potential threat in hospitals especially the critical care centres and effective infection control practices certainly reduce the incidences of nosocomial IFI's [51]. Unavailability of vaccines against fungal infections owing to their complex pathogenicity, immune adaptive and tolerance mechanisms should be considered as a major drawback in the control of IFI's. Studies on anti fungal vaccines though have proved their efficacy in lab, clinical utility is still unclear [52,53]. Environmental screening in the hospitals and effective disinfection strategies will be helping in reducing the incidences and thereby morbidity and mortality related to IFI's.

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