INTRODUCTION

The prevalence of invasive fungal infections (IFI) has increased in recent years due to an increasing population of immunocompromised patients, intensive immunosuppressive chemotherapy, increasing awareness of these infections, and the widespread use of broad-spectrum antibiotics and central venous catheters ***(Sheevani et al., 2013)***.

Among immunocompromised children, the impact of IFI can be devastating, with a high rate of mortality and morbidity despite the development of more active, less toxic antifungal agents and the use of antifungal prophylaxis ***(Lehrnbecher et al., 2010)***. Thus, timely diagnosis and initiation of appropriate antifungal therapy is imperative for improving outcomes ***(Lass-Florl, 2010)***.

Candida (C.) albicans and *Aspergillus (A.) fumigatus* species are the most commonly encountered organisms ***(Lass-Florl, 2010)***. However, since *non-albicans C.* species *and non-fumigatus A.* species are increasing in importance, new diagnostic approaches covering a large number of fungal species are required ***(Bille et al., 2005; and Dornbusch et al., 2009)***.

Although conventional diagnostic tests such as histology, microscopy, and culture remain the cornerstone of proving fungal disease, their yield is low. Therefore, their impact on

clinical decisions to treat patients is limited. Furthermore, cultures become positive at a late stage of infection and delayed therapy is associated with a poor outcome. Moreover, rapid diagnostic strategies for fungal infections including detection of antibody or antigen is limited due to unpredictable humoral responses ***(Dornbusch et al., 2009)*.**

Polymerase chain reaction (PCR) for the detection of fungal nucleic acids may be the optimal diagnostic approach because it offers the potential of being more sensitive than current culture-based methods, encompassing multiple fungal genera, and being applied to a variety of specimen types ***(Cuenca, 2011)*.**

Both conventional and Real Time (RT) PCR can be used for the detection of fungal nucleic acid although the introduction of RT-PCR has increased the reliability of the results compared to the conventional PCR. Moreover, Real-Time PCR can give the results in less than 2 hours, which is an important requirement for the clinical decision making ***(Landlinger et al., 2010)***.

AIM OF THE WORK

The aim of the present work is to evaluate the role of real time PCR, using a pan-fungal marker, as a rapid test for the early diagnosis of invasive fungal infection in immunocompromised patients compared to conventional culture techniques.

FUNGAL INFECTION

The word Fungus was derived from the Greek word 'Sphongirs' or Sponge. The term Mycosis was derived from the Greek word 'Mykes' or Mushroom. The earliest known record of mycotic infection was in Atharva Veda in India (about 2000-1000 BC) and was that of mycetoma of the foot which was described under name of 'foot anthill' ***(Topley and Wilson, 2005).***

In the seventeenth century, the microscopist Hook of England presented the first illustration of microfungi such as Mucor. Followed by the Dutchman Vanleeuwenhock to be credited with being the first to have observed the yeast microscopically in 1680. Since its discovery, more than 100,000 species of fungi have been described ***(Barnett, 2003).***

Fungi form a separate group of eukaryotic organisms. A fungus is different from bacteria in different aspects as described in table (1) ***(Wang et al., 2012)***.

Table (1): Difference between fungi and bacteria.

	Fungi	Bacteria
Kingdom	Eukaryotes [organisms whose cells contain a nucleus and other structures (organelles) enclosed within membranes].	Prokaryotes [organisms whose cells lack a membrane-bound nucleus].
Cell wall	-Mainly polysaccharides [glucose, mannose polymers and glycoproteins). -Chitin (rigid cell wall].	-Formed of peptidoglycan [mucopeptide or murein] formed of N-acetylglucoseamine and N-acetylmuramic acid. -Cellulose.
Nucleus	Membrane-bound nuclei 1 - 3 µm in diameter, with double number of chromosomes that contain DNA. The nuclei are haploid and divide by meiosis or mitosis and have a nuclear membrane or nucleoli.	Single nuclear body. The Bacterial DNA is not enclosed inside of a membrane-bound nucleus but instead resides inside the bacterial cytoplasm. They lack nuclear membrane or nucleoli.
Organelles	Membrane-bound organelles i.e. mitochondria (they have a double bilayer membrane and contain complex internal membranes).Cytoplasm that contains plasmid,endoplasmic reticulum (smooth and rough), golgi apparatus, ribosomes and vacuoles.	Bacteria do not usually have membrane-bound organelles in their cytoplasm, and thus contain few large intracellular structures. They lack mitochondria, endoplasmic reticulum, chloroplasts and the other organelles present in eukaryotic cells.
Cytoplasmic membrane	Contain sterol	No sterol except in mycoplasma.
Cellularity	Multicellular (molds) or Unicellular (yeasts)	Unicellular
Reproduction	Sexual or asexual processes	Asexually only by simple binary fission.
Nutrition	Heterotrophic, they lack chlorophyll and require performed organic carbon and nitrogen compounds.	Exhibit different modes of nutrition, they are either autotrophic (synthesize their own organic food from inorganic substances) or heterotrophic (depends on other organisms for their nutrition).

(Wang et al., 2012)

CLASSIFICATION OF FUNGI

A-Taxonomic Classification:

Fungi are usually classified to 4 classes according to biological taxonomy based upon the type of hyphae, spore, and reproduction. These are:

1-Class zygomycetes:

Zygomycetes are organisms with aseptate hyphae, reproduce asexually by spores produced inside sporangia, and sexually by production of zygospores. The productive cells formed by fusion of morphologically identical cells are called zygospores. Examples of this group are *Mucor, Rhizopus* and *Absidia as* shown in figure (1).

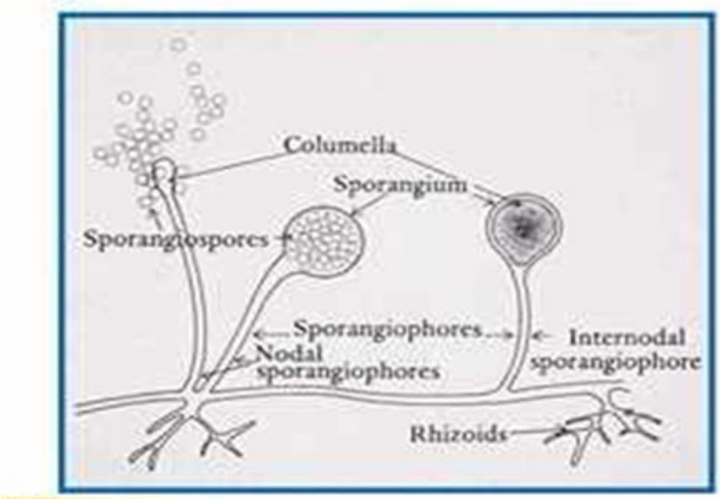

Figure (1): Zygomycetes structure showing spores inside the sporangia ***(Horst, 2013)***.

2- Class ascomycetes:

Characterized by the presence of sexually produced spores formed within a sac. Like most fungi, ascomycetes also reproduce asexually by the formation of nonsexual spores called conidia at the ends of filaments known as hyphae (septate hyphae). Example of this group is *A.* species (spp.) as shown in figure (2).

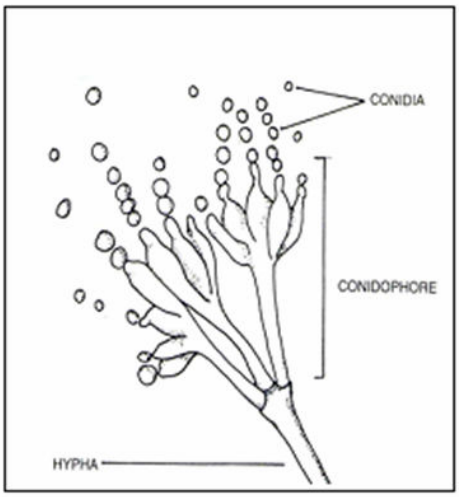

Figure (2): Ascomycetes structure showing conidia and sac like conidiophores ***(Tederso et al., 2009).***

3- Class basidiomycetes:

Organisms of this class are characterized by septate hyphae; reproduce asexually by conidia and sexually by basidiospores. Example of this group is *Cryptococcus* (basidium=club shaped) as shown in figure (3).

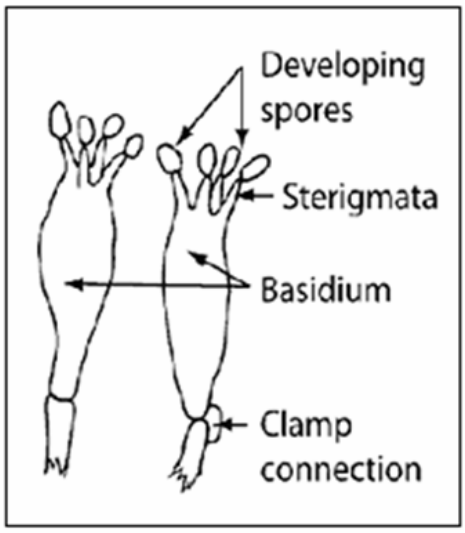

Figure (3): Basidiomycetes structure showing the spores and basidium ***(Horst, 2013)*.**

4- Class deuteromycetes:

Fungi imperfecti: a heterogeneous collection of fungi, this group of fungi produces their spores asexually. Most of the pathogens encountered in medical mycology belong to this class, including, *Penicillium*, and *Candida*.

Hibbett et al. (2007)

B- Morphologic Classification:

The most simple grouping, based on morphology, lumps fungi into either yeasts or molds as seen in figure (4) ***(Carris et al., 2012)***.

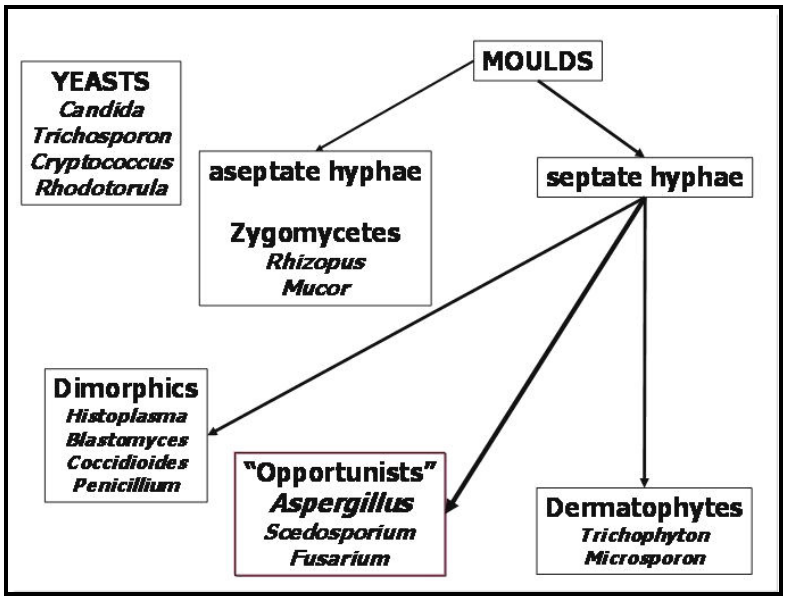

Figure (4): Morphological classification of fungi ***(Quoted from Bochud et al., 2008)***.

1-Yeasts:

A yeast (*C. species, Pichia, Rhodotorula, Trichosporon, and Saccharomyces* spp.) can be defined morphologically as a cell that reproduces by budding or by binary fission, where a progenitor or "mother" cell pinches off a portion of itself to produce a progeny or "daughter" cell. The daughter cells may elongate to form sausage like pseudohyphae. Yeasts are usually unicellular and this one cell is surrounded by a cell wall, followed by a space called the periplasmic space, a cell membrane and the cytoplasm. In the inside of the yeast there are many important organelles (golgi body, peroxisome, endoplasmic reticulum, mitochondrion vacuole and a nucleus) as shown in figure (5).

Yeasts produce round, pasty or mucoid colonies on the agar ***(Bowyereral, 2008)***.

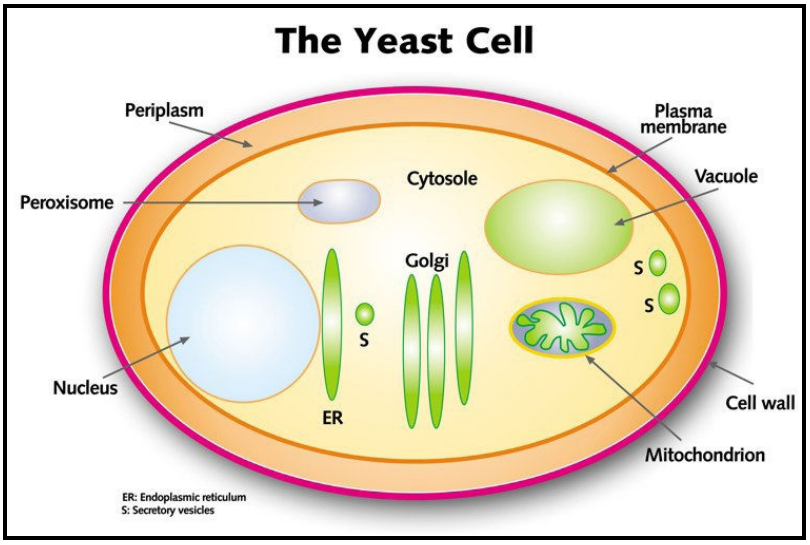

Figure (5): Cellular structure of yeast cell ***(Quoted from Bowyereral, 2008)***

2-Molds:

On the other hand, molds (*Acremonium, Alternaria, A., Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Stachybotrys and Trichoderma*) are multicellular organisms consisting of thread like tubular structures, called hyphae, that elongate at their tips by a process known as apical extension. Hyphae are either aseptate (hollow and multinucleate) or septate (divided by partitions or cross-walls). Molds are either dematiaceus with characteristic dark appearance of this group of fungi as it grows on agar or hyaline which are clear or colourless on agar. The hyphae form together

to produce a mat-like structure called a mycelium. The colonies formed by molds are often described as filamentous, hairy, or woolly ***(Carris et al., 2012).***

3-Dimorphic fungi:

Many fungi of medical importance change their growth form as part of the process of tissue invasion, these are called dimorphic fungi. They change from the multicellular mold form in the natural environment to a budding single-celled yeast form in tissues. These include *C. albicans, Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidiodes brasiliensis and Sporothrix schenckii* (***Chandler et al., 2008)***.

C-Clinical Classification:

Fungal infections may be classified according to the site of infection, route of acquisition, and type of virulence. When classified according to the site of infection, fungal infections are designated as superficial, cutaneous, subcutaneous, and deep ***(Anjali, 2010).***

1- Superficial and cutaneous mycoses

Superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation. Infection involves the integument and its appendages, including hair and nails ***(Malcolm et al., 2012).*** They include the following fungal infections and their etiological agent:

- Black piedra (*Piedraia hortae*): characterized by small, firm, black nodule involving the hair shaft (figure 6) ***(Renee, 2012).***

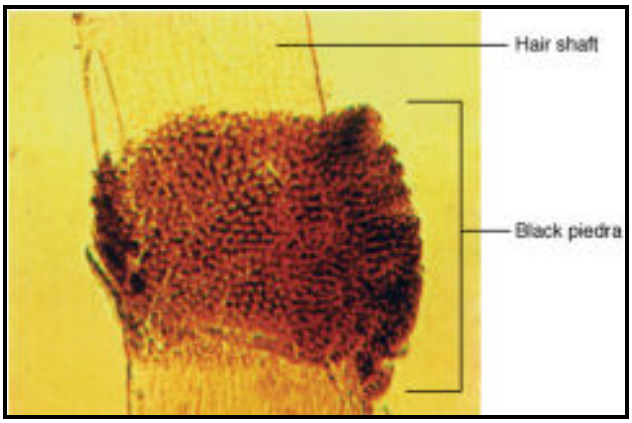

Figure (6): Microscopic image of black piedra on hair shaft ***(Renee, 2012).***

- White piedra *(Trichosporon beigelii*): characterized by a soft, friable, beige nodule of the distal ends of hair shafts.

- Pityriasis versicolor (*Malassezia furfur*): characterized by hypopigmentation or hyperpigmentation of skin of the neck, shoulders, chest, and back.

- Tinea nigra (*Phaeoannellomyces werneckii*): characterized by brown to black silver nitrate-like stain on the palm of the hand or sole of the foot, figure (7).

(Marc et al., 2012)

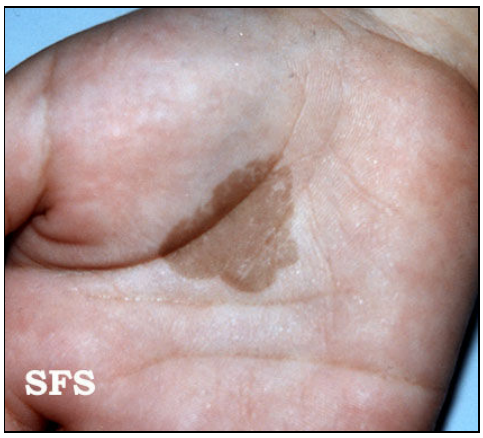

Figure (7): Tinea nigra appearing on the palm of the hand ***(Marc et al., 2012).***

2- Subcutaneous mycoses

There are three main general types of subcutaneous mycoses: chromoblastomycosis, mycetoma, and sporotrichosis. All appear to be caused by traumatic inoculation of the etiological fungi into the subcutaneous tissue ***(Davies et al., 2010).***

- Chromoblastomycosis (*Fonsecaea pedrosoi, Fonsecaea compacta*): characterized by verrucoid lesions of the skin; histological examination reveals muriform cells or so-called "copper pennies". It is generally limited to the subcutaneous tissue with no involvement of bone, tendon, or muscle ***(Murray et al., 2009).***
- Mycetoma (*Pseudallescheria boydii, Nocardia brasiliensis*): characterized by the presence of draining sinus tracts from which small but grossly visible pigmented grains or granules are extruded. It is a

suppurative and granulomatous subcutaneous mycosis, which is destructive of contiguous bone, tendon, and skeletal muscle ***(Kauffman, 2009).***

- Sporotrichosis (*Sporothrix schenckii*): it involves the subcutaneous tissue at the point of traumatic inoculation. The infection usually spreads along cutaneous lymphatic channels) of the extremity involved (lymphocutaneous sporotrichosis ***(La Hoz et al., 2012).***

3-Deep mycoses:

Deep mycoses involve the lungs, abdominal viscera, bones and or central nervous system. The most common portals of entry are the respiratory tract, gastrointestinal tract, and blood vessels. It is caused by either primary pathogenic or opportunistic fungal pathogens ***(Anjali, 2010).***

i- Primary mycoses:

- *Coccidiomycosis*: caused by inhalation of Coccidioides immitis arthroconidial spores, which then converts in the lungs to spherules. Most cases of coccidioidomycosis are clinically occult or mild infections. However, some patients have progressive pulmonary infection and also may suffer dissemination to the brain, bone, and other sites ***(Davies et al., 2010).***
- Histoplasmosis: is a primary pulmonary infection resulting from inhalation of conidia of Histoplasma

capsulatum which convert in vivo into the blastoconidial (budding yeast) form. Dissemination to the hilar and mediastinal lymph nodes, spleen, liver, bone marrow, and brain may be life-threatening in infants and other immunocompromised patients ***(Kauffman, 2009).***

- Blastomycosis: is also a primary pulmonary infection resulting from inhalation of conidia of Blastomyces dermatitidis which converts in vivo into blastoconidial form. The clinical pattern of pulmonary blastomycosis is one of chronic pneumonia. Dissemination occurs most commonly to the skin, bone, and, in males, prostate ***(Kenrad and Carolyn, 2014).***

ii- Opportunistic mycoses

- *Candidiasis (C. albicans and other species)* is the most common opportunistic fungal infection. It is classified as superficial or deep (visceral). Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx, esophagus, intestines, urinary bladder, and vagina. On the other hand the kidneys, liver, spleen, brain, eyes, heart, and other tissues are the major organ sites involved in deep candidiasis ***(Pettit et al., 2012).***

- *Aspergillosis*: *A. species (A. flavus, A. fumagatus and A. nigar)* are ubiquitous filamentous fungi, which sporulate abundantly and release conidia into the atmosphere