INTRODUCTION

Thalassemias are a diverse group of genetic blood diseases characterized by absence or decreased production of either α or β- globulin protein chains, resulting in microcytic anemia of varying degrees and referred to as α or β-thalassemia ***(Hazza'a et al., 2010).***

Based on their clinical and genetic disorders, thalassemias are classified mainly into major (homozygous) and minor (heterozygous) types ***(Weatherall and Clegg, 2001).*** The ineffective erythropoiesis in β- thalassemias is due to defective hemoglobin synthesis; results in increased RBC turnover and severe anemia, which can be corrected by regular blood transfusions ***(Rund et al., 2013).***

Invasive fungal infections are the primary cause of morbidity and mortality in patients with different hematopoietic disorders ***(Buchheidt et al., 2003).*** The incidence of opportunistic fungal infections has increased dramatically in the past few decades, resulting from greater numbers of immunocompromised patients, the wide spread use of invasive medical devices and use of broad spectrum antibiotics ***(Chang et al., 2001).***

Candida species remain the most common cause of fungal infection in patients with hematological disorders ***(Donnelly, 2012).*** Identification of the species level of yeasts

cultured from various clinical specimen is increasingly necessary for clinical laboratories ***(Hazza'a et al., 2010).***

Generally yeast identification procedures start with a germ tube test in clinical laboratories. It is a rapid method to differentiate *Candida albicans* and Candida *dubliniensis* from other Candida *species*. Although it is a rapid test it may lead to false positive and false negative results ***(Peng et al., 2013).*** When the yeast cannot be identified using this method, cornmeal agar (CHROM agar) will be a good choice. This special medium yield microbial colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganisms ***(Murray et al., 2005).***

Isolated *Candida species* are less susceptible to traditional therapies and recovery of increasingly resistant isolates during antifungal therapy are growing problems, consequently, in vitro susceptibility tests should be performed to detect resistant strains ***(Sojakova et al., 2004).***

AIM OF THE WORK

Determination of the incidence of Candida infection among Thalassemia major patients, with detection of the degree of its resistance to antifungal agent.

Chapter (1)

CANDIDA

Definition & History

The genus *Candida* and species *C. albicans* were described by botanist Christine Marie Berkhout in her doctoral thesis at the University of Utrecht in 1923. Over the years, the classification of the genera and species has evolved. Obsolete names for this genus include *Mycotorula* and *Torulopsis*. The species has also been known in the past as *Monilia albicans* and *Oidium albicans* ***(Königstein, 2000).***

Many of *Candida species* are harmless commensals or endosymbionts of hosts including humans, but other species, or harmless species in the wrong location, can cause disease. *Candida albicans* can cause infections (candidiasis or thrush) in humans and other animals, especially in immunocompromised patients ***(Suh et al., 2008).***

Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts ***(Nguyen et al., 2007; Suh et al., 2008).***

Classification

1. Scientific Classification:

According to Berkhout, in 1923, it was classified as in table (1):

Table (1): Scientific Classification of Candida Spp.

Kingdom:	Fungi		
Phylum:	Ascomycota		
Subphylum:	Saccharomycotina		
Class:	Saccharomycetes		
Order:	Saccharomycetales		
Family:	Saccharomycetaceae		
Genus:	Candida		
Species:	C. albicans C. ascalaphidarum C. amphixiae C. antarctica C. argentea C. atlantica C. atmosphaerica C. blattae C. carpophila C. carvajalis C. cerambycidarum C. chauliodes C. corydali C. dosseyi C. dubliniensis C. ergatensis C. fructus	C. glabrata C. fermentati C. guilliermondii C. haemulonii C. insectamens C. insectorum C. intermedia C. jeffresii C. kefyr C. krusei C. lusitaniae C. lyxosophila C. maltosa C. marina C. membranifaciens C. milleri C. oleophila	C. quercitrusa C. rugosa C. sake C. shehatea C. temnochilae C. tenuis C. theae C. tropicalis C. tsuchiyae C. sinolaborantium C. sojae C. subhashii C. viswanathii C. utilis C. oregonensis C. parapsilosis

2. Clinical Classification:

- Oral candidiasis (Thrush)
- Perlèche (Angular cheilitis)
- Candidal vulvovaginitis (vaginal yeast infection)

- Candidal intertrigo
- Diaper candidiasis
- Congenital cutaneous candidiasis
- Perianal candidiasis
- Candidal paronychia
- Erosio interdigitalis blastomycetica
- Chronic mucocutaneous candidiasis
- Systemic candidiasis
- Antibiotic candidiasis (Iatrogenic candidiasis)

(James et al., 2006)

Genetic Structure

1. Genome:

One of the most important features of the *C. albicans* genome is the occurrence of numeric and structural chromosomal rearrangements as means of generating genetic diversity, named chromosome length polymorphisms (contraction/expansion of repeats), reciprocal translocations, chromosome deletions and trisomy of individual chromosomes. These karyotypic alterations lead to changes in the phenotype, which is an adaptation strategy of this fungus. These mechanisms will be better understood with the complete analysis of the *C. albicans* genome. The *C. albicans* genome for strain SC5314 was sequenced at the Stanford DNA Sequencing and Technology Center ***(Jones et al., 2004; Braun et al., 2005).***

The sequencing of the *C. albicans* genome and subsequently of the genomes of several other medically relevant *Candida species* has profoundly and irreversibly changed the way *Candida species* are now investigated and understood. The *C. albicans* genome sequencing effort was launched in October 1996. Successive releases of the sequencing data and genome assemblies have occurred in the last 10 years, culminating in the release of the diploid assembly 19, which provided a haploid version of the genome along with data on allelic regions in the genome ***(Butler, 2009).***

A refined assembly 20 with the eight assembled *C. albicans* chromosomes was released in the summer of 2006. Importantly, the availability of sequencing data prior to the completion of the genome sequence has made it possible to start *C. albicans* post-genomics early on. In this regard, genome databases have been made available to the research community providing different forms of genome annotation. These have been merged in a community-based annotation hosted by the Candida Genome Database. The availability of the genome sequence has paved the way for the implementation of post-genomic approaches to the study of *C. albicans*: macroarrays and then microarrays have been developed and used to study the *C. albicans* transcriptome; proteomics has also been developed and complements transcriptional analyses; furthermore, systematic approaches are becoming available to study the contribution of

each *C. albicans* gene in different contexts. Other Candida genome sequences have been, or are being, determined: *C. glabrata, C. dubliniensis, C. parapsilosis, C. guilliermondii, C. lusitaniae, and C. tropicalis.* These species will soon enter the post-genomic era as well and provide interesting comparative data. The genome sequences obtained for the different *Candida species* along with those of non-pathogenic hemiascomycetes provide a wealth of knowledge on the evolutionary processes that shaped the hemiascomycete group, as well as those that may have contributed to the success of different *Candida species* as pathogens ***(Denfert and Hube, 2007).***

An unusual feature of the Candida genus is that in many of its species (including *C. albicans* and *C. tropicalis* but not, for instance, *C. glabrata*) the CUG codon, which normally specifies leucine, specifies serine in these species; this is an unusual example of a departure from the universal genetic code (most such departures are in start codons or, for eukaryotes, mitochondrial genetic codes) ***(Andrzej et al., 2010).***

The genome of *C. albicans* is highly dynamic, and this variability has been used advantageously for molecular epidemiological studies of *C. albicans* and population studies in this species. The genome sequence has allowed for identifying the presence of a parasexual cycle (no meiotic division) in *C. albicans* ***(Butler, 2009).*** This parasexual cycle is under the

control of mating-type loci and switching between white and opaque phenotypes. Investigating the role the mating process plays in the dynamics of the *C. albicans* population or in other aspects of *C. albicans* biology and pathogenicity will undoubtedly represent an important focus for future research ***(Denfert and Hube, 2007).***

A similar lack of meiosis was found in *Saccharomyces cerevisiae* altered to use the same genetic code as *C. albicans* ***(Silva et al., 2007).***

2. Dimorphism:

Although often referred to as "dimorphic", *C. albicans* is in fact polyphenic. When cultured in standard yeast laboratory medium *C. albicans* grows as ovoid "yeast" cells. However, mild environmental changes in temperature and pH can result in a morphological shift to pseudohyphal growth. Pseudohyphae share many similarities with yeast cells ***(Berman and Sudbery, 2002).***

3. Heterozygosity

The heterozygosity of the Candida genome exceeds that found in other genomes and is widespread among clinical isolates. Non-synonymous single-base polymorphisms result in two proteins that differ in one or several amino acids that may confer functional differences for each protein. This situation

considerably increases the number of different proteins encoded by the genome ***(Larriba and Calderone, 2008).***

Candidiasis

Candida yeasts are commonly present in humans, and their growth is normally limited by the human immune system and by other microorganisms, such as bacteria occupying the same locations (niches) in the human body ***(Mulley and Goroll, 2006).*** Treatment with antibiotics can lead to eliminating the yeast's natural competitors for resources, and increase the severity of the condition. Higher prevalence of colonization of *C. albicans* was reported in young individuals with tongue piercing, in comparison to non-tongue-pierced matched individuals. In the western hemisphere approximately 75% of females are affected at some time in their life ***(Zadik et al., 2010).***

Several species of the yeast genus Candida are capable of causing candidiasis which is the most common systemic mycosis and the most common agents are *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. guilliermondii* and *C. dubliniensis* ***(Geo et al., 2004).***

C. albicans was isolated from the vaginas of 19% of apparently healthy women, i.e., those that experienced few or no symptoms of infection. External use of detergents or

douches or internal disturbances (hormonal or physiological) can perturb the normal vaginal flora, consisting of lactic acid bacteria, such as lactobacilli, and result in an overgrowth of Candida cells causing symptoms of infection, such as local inflammation ***(Mardh et al., 2003).***

Almost 15% of people with weakened immune systems include AIDS, mononucleosis, cancer treatments, steroids, stress, and nutrient deficiency develop a systemic illness caused by *Candida species*. In extreme cases, the superficial infections of the skin or mucous membranes may enter into the bloodstream and cause systemic Candida infections ***(Choo et al., 2010).***

Clinical Finding

1. Cutaneous and Mucosal Candidiasis:

The risk factors associated with superficial candidiasis include: AIDS, pregnancy, Diabetes, extreme of age, birth control pills and trauma ***(Geo et al., 2004).***

a) Oral thrush: occur on the tongue, lips, gums or palate. It is a patchy to confluent, whitish pseudomembranous lesion composed of epithelal cells, yeasts and pseudohyphae (Figure 1) ***(Geo et al., 2004).***

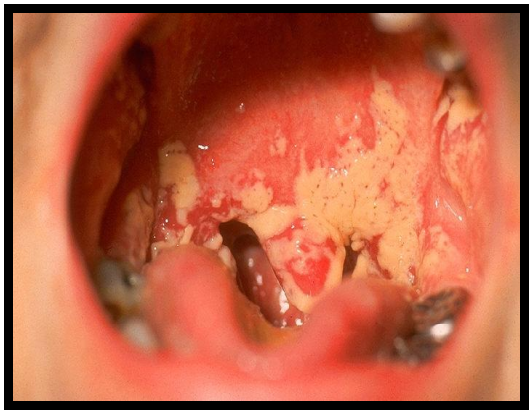

Fig. (1): Oral thrush.

b) Vulvovaginitis: Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation, and a whitish or whitish-gray cottage cheese-like discharge, often with a curd-like appearance. These symptoms are also present in the more common bacterial vaginosis ***(Terri-Warren, 2010).***

c) Cutaneous candidiasis: include invasion of the skin. This occur when the skin is weakened by trauma, burns or macerations as intertriginous infection occurs in moist warm parts of the body such as axillae, groin and intergluteal or inflammatory folds; it is most common in obese and diabetic individuals. The infected areas become red and moist and may develop vesicles, interdigital involvement between fingers follows repeated prolonged immersion in water; it is most common in homemakers, bartenders, cooks and vegetables or fish handlers ***(Geo et al., 2004).***

d) Onychomycosis: It is a candidail invasion of the nails and around the nail plate causes painful, erythematous swelling of the nail fold resembling a pyogenic paronychia, which may eventually destroy the nail (figure 2) ***(Geo et al., 2004).***

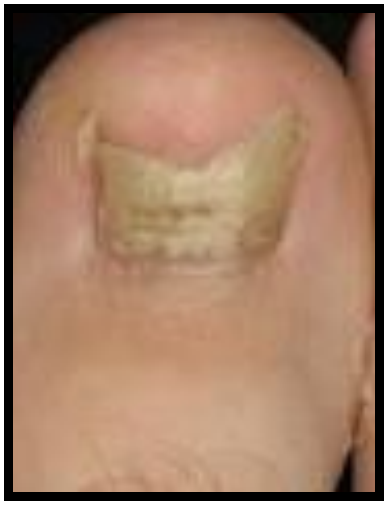

Fig. (2): Onychomycosis.

e) Infection of the male genitalia: include red patchy sores near the head of the penis or on the foreskin, severe itching, or a burning sensation. Candidiasis of the penis can also have a white discharge, although uncommon ***(Ferris et al., 2002).***

2. Systemic Candidiasis:

Candida represents the most common cause of invasive fungal disease ***(Pfaller and Diekema, 2007).*** Candidemia can be caused by indwelling catheters, surgery, intravenous drug abuse, aspiration or damage of the skin and gastrointestinal

tract. In most patients with normal host defense, the yeasts are eliminated and candidemia is transient. However patients compromised defenses may develop occult lesions anywhere, specially the kidney, skin, eye, heart and meninges. Systemic candidiasis is most often associated with chronic administration of corticosteroids or other immunosuppressive agents; with hematologic diseases such as leukemia, lymphomas and aplastic anaemia; or with chronic granulomatous disease. Candidial endocarditis is frequently associated with deposition and growth of the yeasts pseudohyphae on prosthetic heart valves or vegetations ***(Geo et al., 2004).***

3. Chronic Muococutaneous Candidiasis:

Most forms of this disease have onset in early childhood, are associated with cellular immunodeficiencies and endocraniopathies, and result in chronic superficial disfiguring infections of any or all areas of skin or mucosa ***(Geo et al., 2004).***

Pathogenesis

Infection with *C. albicans* varying in severity from asymptomatic colonization to superficial mucosal infections to life-threatening disseminated candidiasis. It is therefore not surprising that unravelling the mechanisms behind the pathogenesis and immunology is a complex process, with many apparent contradictions among the results obtained. A classic example is the controversy that has raged over whether or not antibodies have a

role to play in defence against this infection. This is an important issue, since if antibodies are protective, the technology is within our reach to engineer such antibodies and use them therapeutically. At first glance the evidence for and against the protective potential of antibodies is conflicting. But further examination of the nature of the animal models used in these assessments shows that where animals have been injected intravenously with the yeast, to produce a systemic infection, investigation often showed that immune sera were protective; however, those models in which mice were challenged orally usually demonstrated the importance of cell-mediated immunity. Clinical observation also lends support to the hypothesis that superficial candidiasis is highly dependent on cell-mediated immunity. The most obvious example is the predispostion of patients with AIDS to oral and oesophageal candidiasis and their relative resistance, except in the most advanced stages, to disseminated candidiasis in marked contrast to their susceptibility to other systemic fungal infections ***(Matthews and Bumie, 2008).***

Laboratory Diagnosis

Generally laboratory procedures in diagnostic mycology are directed mainly towards ***(Arunaloke et al., 2009):***

(1) Direct demonstration of the pathogenic fungi in clinical specimens (microscopy).

(2) Successful isolation of pathogenic fungi (culture).