1, 3- β-D-glucan Assay for Diagnosis of Candidiasis Versus Candida Colonization Index in ICU Patients

Thesis

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Abstract

Background: The incidence of candidiasis has been increasing over the past 2 decades. In addition, Candida species accounts for the fourth most common cause of nosocomial bloodstream infections after Coagulase-negative staphylococci, Staphylococcus aureus and Enterococcus. Candida species are ubiquitous and constitute part of the normal human flora. Only a small percentage of the identified species cause disease in humans. Invasive candidiasis is a life-threatening infection associated with high mortality rates. Candida species is responsible for an extremely large spectrum of diseases**.** Measurement of the serum (1, 3) - β -D-glucan (BG) is a non-invasive testing for circulating fungal cell wall components, that allows the systemic screening and prompt identification of fungal infections, using one serum sample by activation of serine proteases that cleaves p-nitroaniline from the peptide substrate found in Limulus amebocyte lysate reagent found in the Kit, free p-nitroaniline is then measured, with a sensitivity of 100%, specificity of 90%.

Objectives: The aim of the work is to evaluate the serum (1,3)- β -glucan assay as an effective marker to aid in the early screening and diagnosis of candidiasis in comparison with candida colonization index.

Conclusion: The findings of this study suggest that the BG assay is a useful screening tool with high sensitivity and specificity compared to candida colonization index for discriminating between patients with and without Invasive candidiasis to start antifungal therapy in early and appropriate time, In clinical practice, BG assay results should be evaluated together with clinical and microbiological findings. Certain issues regarding the optimal utilization of BG testing require further evaluation, especially the optimal sampling strategy for patients who are at high risk, also, blood cultures should be re-evaluated as a standard diagnosis for invasive candidiasis.

Keywords: 1, 3- β-D-glucan, Candidiasis, candida colonization index, BG assay

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Introduction

The incidence of candidiasis has been increasing over the past 2 decades. In addition, Candida species accounts for the fourth most common cause of nosocomial bloodstream infections after Coagulase-negative staphylococci, Staphylococcus aureus and Enterococcus **(Wisplinghoff et al., 2004).**

Candida species are ubiquitous and constitute part of the normal human flora. Only a small percentage of the identified species cause disease in humans. Invasive candidiasis is a life-threatening infection associated with high mortality rates. Candida species is responsible for an extremely large spectrum of diseases **(Moran et al., 2010).**

Invasive Candida infections include candidemia with or without endopthalmitis; disseminated hematogeneous infections; involvement of a single deep organ site (e.g. peritonitis, other abdominal infections, meningitis and infective endocarditis) and chronic hepatosplenic candidiasis mostly in hematological patients, Source of Candida infection can be endogenous (gastro-intestinal flora or mucocutaneous colonisation) and exogenous

(hands of healthworkers, contaminated infusates) even leading to local outbreaks **(Tragiannidis et al., 2013).**

Early identification of ICU patients at high risk of Invasive Candida is challenging due to the complexity of the patients' underlying conditions and the clinical presentation of invasive disease is nonspecific and cultures are only positive in only half of affected patients **(Ellepola and Morrison, 2005).**

Delaying the appropriate antifungal treatment of Invasive Candida infection while awaiting cultures results has been associated with increased hospital mortality **(Morrell et al., 2005).**

Only clinical prediction rules of the risk of Invasive Candida or simpler scoring systems that differentiate patients with Candida colonization from those with ongoing but still occult Candida infection are available **(León et al., 2006).**

The Candida colonization index has been developed to help identify colonized patients who are likely to develop invasive disease. The index is the ratio of body sites colonized with genotypically identical strains of Candida over the total number of body sites investigated; a

value of ≥0.5 indicates invasive candidiasis considered to be over 90% specific for invasive candidiasis, with a very high negative predictive value **(Luzzati et al., 2000).**

One of the developed biomarkers is (1,3)-β- D-Glucan (BG), which is a major cell wall component of almost all fungi except for Zygomycetes and Cryptococcus species **(Douglas, 2001).**

Measurement of the serum (1, 3) - β -D-glucan (BG) is a non-invasive testing for circulating fungal cell wall components, that allows the systemic screening and prompt identification of fungal infections, using one serum sample by activation of serine proteases that cleaves p-nitroaniline from the peptide substrate found in Limulus amebocyte lysate reagent found in the Kit, free p-nitroaniline is then mesured, with a sensitivity of 100%, specificity of 90% **(Mennink-Kersten and Verweij, 2006).**

Aim of the Work

The aim of the work is to evaluate the serum (1,3)- β -glucan assay as an effective marker to aid in the early screening and diagnosis of candidiasis in comparison with candida colonization index.

Microbiology of Candida Infection

The genus Candida encompasses more than 350 species. They can be found among humans and other mammals, birds, insects, arthropods, fish, animal waste, plants, mushrooms, naturally occurring high-sugar substrates (e.g., honey, nectar, grapes) and fermentation products, dairy products, soil, freshwater, seawater, and on airborne particles **(Barnett et al., 1990 ; Nunn et al., 2007).**

Infection in humans was first described as oral thrush by Hippocrates in the fifth century BC. In 1853, Charles Robin microscopically observed budding cells and filaments in epithelial scrapings and he named the fungus Oidium albicans. Subsequently, more than 160 synonyms, including Monilia albicans, were used before Candida albicans became the accepted name for this species. It is capable of inhibiting not only intravascular fibrinolysis but also cell-associated proteolysis **(Tavanti et al., 2005).**

At least 13 Candida species have caused infection in humans. The most common of these are C. albicans, C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis. C. parapsilosis has been recognized as a heterogeneous

species, and it was proposed that it can be split into three morphologically and physiologically indistinguishable species: C. parapsilosis, C. metapsilosis, and C. orthopsilosis **(Sullivan et al., 2005).**

In 1995, Candida dubliniensis was described as a new species to accommodate a subset of isolates previously identified as C. albicans; C. dubliniensis is seen primarily among HIV-infected people **(Asmundsdóttir et al., 2009).**

Sequencing of the C. albicans genome has demonstrated that this species possesses 6354 genes contained in 8 chromosomes. These data will facilitate proteomic studies, which in turn could lead to better understanding of key biological characteristics of these species, including pathogen-host interactions, improved diagnostics, antifungal drug resistance, novel molecular targets for antifungal therapy, biofilm formation and dynamics, cell signaling, and responses to stress, nutrients, temperature, and pH **(Bennett and Johnson, 2003).**

Growth, morphology, and pleomorphism —the morphology of a given Candida species is fundamentally the same whether observed in vitro or in vivo. C. glabrata grows as small, elliptical, unicellular budding yeast at all times. Rarely, buds of C. glabrata can adhere to one another

in rudimentary short chains. In marked contrast, C. albicans, C. krusei, C. parapsilosis, and C. tropicalis form elliptical budding cells that typically are larger than those of C. glabrata; they can also form elaborate and well developed multicellular filaments, particularly when in contact with a solid substrate, such as human tissue or agar culture media (figure 1) **(Kniemeyer et al., 2011).**

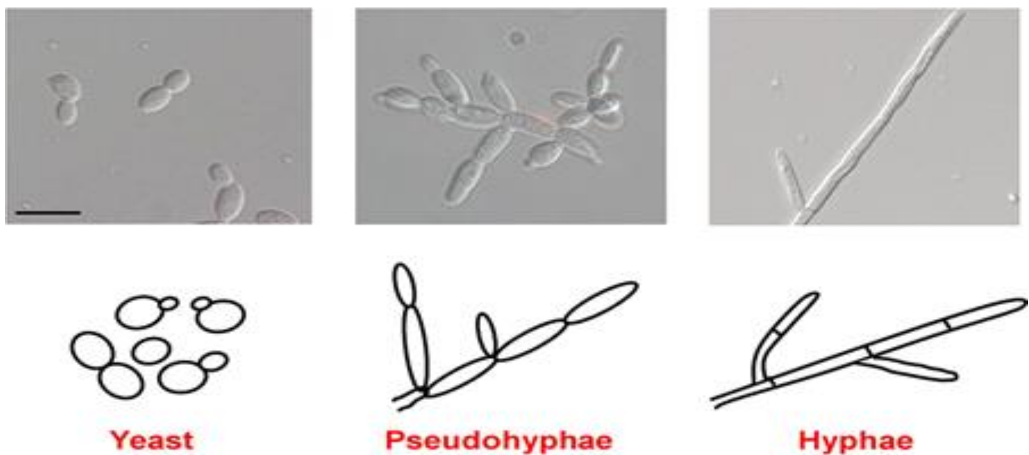

Fig. (1): Major morphologies of human fungal pathogens. (Top) Images of C. albicans cells as visualized by differential interference contrast (DIC) microscopy (bar 10 m). (Bottom) Schematic representation of each morphology **(Thompson et al., 2011).**

C. krusei and C. parapsilosis can be considered dimorphic because they exhibit budding and pseudohyphal forms. C. albicans and C. tropicalis can form true hyphae (in addition to buds and pseudohyphae) and thus can be considered pleomorphic. Each of these species is capable of

exhibiting budding and/or filamentous morphologies in infected tissue ,(Table 1) **(Kniemeyer et al., 2011).**

Pseudohyphae and hyphae — Pseudohyphae are morphologically and ontogenically distinct from hyphae. They are formed when buds remain attached to each other and subsequently elongate via differential rates of wall synthesis at various points along the cell wall. Elongation then stops and each terminal cell produce a new apical bud, which elongates in turn. This repeated process of budding and elongation can result in extensive filamentation **(Kniemeyer et al., 2011).**

Table (1): Morphologies of pathogenic *Candida* species (Thomson et al., 2011).

Species	Morphology
***C. glabrata*...**	Yeast, pseudohyphae
***C. lusitaniae*..**	Yeast, pseudohyphae
***C. guilliermondii*..................................**	Yeast, pseudohyphae
***C. parapsilosis*.....................................**	Yeast, pseudohyphae
***C. tropicalis*...**	Yeast, pseudohyphae, hyphae
***C. dubliniensis*.....................................**	Yeast, pseudohyphae, hyphae
***C. albicans*..**	Yeast, pseudohyphae, hyphae

In contrast, true hyphae elongate by a polarized growth process of apical synthesis that does not involve budding. Buds are not present at the hyphal tips; as a result, true hyphae do not exhibit the periodic constrictions that characterize pseudohyphae.

Septa — Septa form by centripetal synthesis from the interior of the cell wall inward toward the center of the cell and are present in pseudohyphae, hyphae, and between mature bud cells **(Sudbery et al., 2004).**

White-opaque switching — In addition to yeast, pseudo hyphal, and hyphal forms, C. albicans exhibits two additional phenotypes: white and opaque colonies (Figure 2), Although genetically identical, the two phases differ in virulence and gene expression, and switching between phenotypes occurs at a known frequency. The extent to which environmental (including host) stimuli control switching is not fully known. Opaque phase cells are prevalent as skin colonizers and are more readily killed by neutrophils, whereas white phase cells are more commonly associated with candidemia and are less susceptible to neutrophil defenses **(Chen et al., 2014).**

Genetic control — Signals that trigger each of the growth forms gradually are becoming better understood.

Signals include pH, temperature, carbon and nitrogen availability, oxygenation, serum, hormones, and density of the Candida cells within the infected host. Several pH-regulated genes involved in growth and morphology have been identified: PHR1 is expressed when the ambient pH is 5.5 or higher and correlates with filamentous growth, whereas PHR2 is expressed when the ambient pH is lower than 5.5 (as in the vagina) and correlates with yeast-like growth , a PHR2 null mutant was able to cause systemic infection **(Hnisz et al., 2009)**

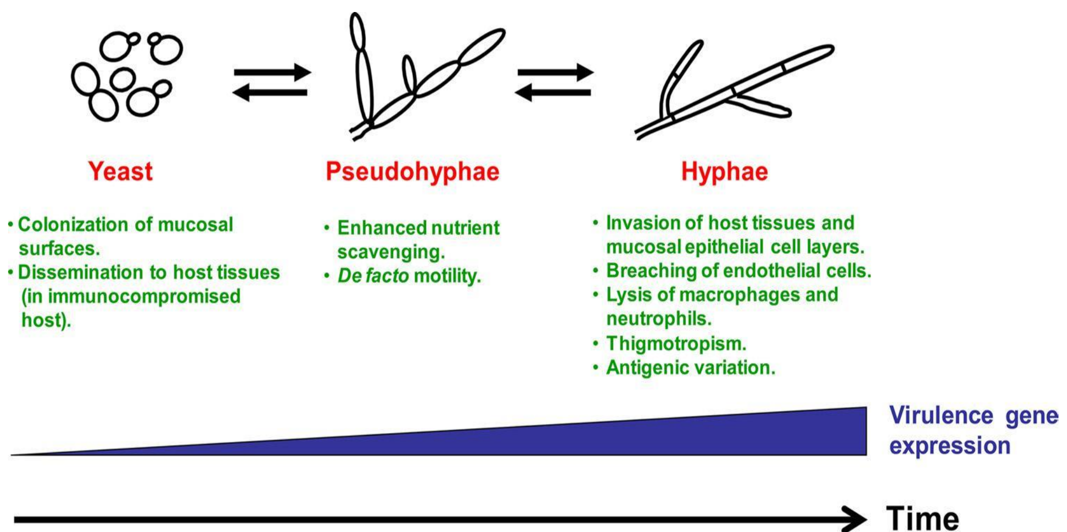

Fig. (2): Model for evolution of morphology and virulence in Candida species. In the mammalian host reservoir, the yeast form is believed to have adapted for colonization of mucosal cell surfaces in the oral cavity, gastrointestinal tract, and/or vagina. Stepwise evolution from yeast to pseudohyphae to hyphae is believed to be associated with increased virulence gene expression and the development of a variety of virulence properties (**Thompson et al., 2011**).

The gene EFG1 encodes a protein Efg1 that contains conserved regions with homology to the human Myc protein; Efg1 appears to activate pseudohyphal formation and downregulate hyphal development.

In one study of C. albicans, the gene CaRSR1, a RAS-related gene, was necessary for budding and also contributed to germ tube emergence and cell elongation, which is part of the process of hyphal formation **(De Bernardis et al., 1998).**

Both budding and filamentous forms presumably play a role in the progression of infection in humans, the ongoing formation and detachment of unicellular buds can facilitate hematogenous dissemination of the yeast following angioinvasion. In contrast, filamentation enhances the ability of Candida species to invade solid tissue via a burrowing process, Some investigators have suggested that the hyphae of C. albicans invade epithelial cells using thigmotropism (movement based upon touch), Others argue that chemotropism plays a more important role **(Yaar et al., 1997).**

Antigenic structure — The multilayered cell wall of C. albicans consists of glucans, mannans, mannoproteins, proteins, and chitin. With the exception of glucans and