Fungal Biofilm in Onychomycosis

Thesis

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Mohamed Mustafa Reda Sayed

Contents

List of Abbreviations

AIDS	Acquired immunodeficiency diseases
Als1	Agglutinin-Like Sequence1
Als3	Agglutinin-Like Sequence3
Bcr1	Biofilm and Cell wall Regulator1
C.albicans	Candida albicans
CLSM	Confocal laser scanning microscopy
DLSO	Distal and lateral subungual onychomycosis
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
EPS	Extracellular polymeric substance
HIV	Human immunodeficiency virus
Hsg	Hyphae-specific genes
Hwp1	Hyphal Wall Protein1
Hyr1	HYphally Regulated1
IPL	Intense Pulse Light
KOH	Potassium hydroxide
MOPS	3-(N-morpholino) propanesulfonic acid
Nd:YAG	Neodymium-doped yttrium aluminum garnet
NDMs	Non-dermatophytes moulds
NIR	Near Infra Red
OD	Optical Density
PAS stain	Periodic acid Schiff stain
PBS	Phosphate Buffer Saline
PSO	Proximal Subungual Onychomycosis
RPMI	Roswell Park Memorial Institute
SAD medium	Sabouraud's dextrose agar medium
SAD medium +C	Sabouraud's dextrose agar medium + Chloramphenicol

List of Abbreviations (Cont.)

SAD medium+C+C	Sabouraud's dextrose agar medium + Chloramphenicol + cyclohexamide
SEM	Scanning electron microscopy
SWO	Superficial White Onychomycosis
T.mentagrophytes	Trichophyton mentagrophytes
T.rubrum	Trichophyton rubrum
TDO	Totall Dystrophic Onychomycosis
XTT	sodium 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium inner salt

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Introduction

Onychomycosis refers to fungal infection that involves components of nail unit including: nail matrix, nail bed and/or nail plate. It may cause nail disfigurement, produce physical and occupational limitations especially in patients who are providing food services or drinks to the public and may lead to psychosocial and emotional imbalance with a significant negative impacts on quality of life and self-image ***(Bristow and Spruce, 2009).***

Onychomycosis accounts for half of all nail disorders and its prevalence has been increasing. Almost, one third of patients with cutaneous fungal infections were found to have concomitant onychomycosis. Toenails are more likely to be infected than fingernails in male patients and vice versa ***(Carney et al., 2011).***

Treatment of onychomycosis with systemic antifungal agents must be continued for many months and some patients fail in compliance to full treatment course ***(Baran et al., 2008).***

Biofilms are differentiated masses of microbes whether bacterial or fungal. They were found to resist antimicrobial agents due to formation of special environment surrounded by a matrix of extracellular polymers ***(Peltroche et al., 2006).***

Dermatopyhtoma clinically presents as a longitudinal yellow band, spike or as a round yellow or white area in the nail plate. It is caused by dermatophytes which may resist systemic antifungal agents and tend to form biofilms

(Burkhart et al., 2002 and Bennett and Rubin, 2013). Other fungal species as C.albicans, Cryptococcus neoformans and filamentous moulds as aspergillus fumigatus had been shown to be implicated in biofilm-associated infections, too ***(Peltroche et al., 2006 and Ramage et al., 2009).***

In onychomycosis, several factors including firm adherence of fungi to the nail plate, presence of dormant fungal elements, and difficulty of fungal eradication despite long courses of systemic antifungal agents suggest that biofilms may be implicated in recalcitrant onychomycosis; yet evidence is lacking to advocate the existence of biofilms in onychomycosis infections and its role, as investigations to prove their presence in samples needs tedious steps, equipped labs and special microscopes ***(Aron et al., 2012).***

Aim of the Work

The aim of the current work is to study the incidence of different types of fungi in both fingernails and toenails onychomycosis and to investigate the ability of the isolates to produce biofilm.

Onychomycosis

Onychomycosis is defined as a fungal infection of any part of nail units such as: nail matrix, bed or plate with dermatophytes, yeasts or other non-dermatophytes moulds (NDMs), In Greek the term onychomycosis means "onyx", a nail and "mykes" a fungus ***(Kaur et al., 2008a).***

Although onychomycosis is not considered to be a life threatening condition, it does not self-heal and may be a source of widespread cutaneous fungal infections. Without treatment, fingernails become disfigured and look ugly. Negative emotional and psychosocial effects are common and may have respectable impacts on quality of person's life ***(Bristow and Spruce, 2009 and Papini et al., 2015).*** Moreover, it may cause many physical and occupational limitations. When handling objects and serve them to other people it may become cosmetically concerned. While, Thickened fingernails may interfere with fine finger movements, thickened toenails may press against the inside of shoes and cause pain ***(Hay, 2005, Seebacher et al., 2007 and Singal and Khanna, 2011).***

Prevalence

Onychomycosis is affecting about 8% of the general population. It accounts for 20-40% out of all onychopathies. It is found to be accompanying nearly 35% of superficial fungal infections ***(Achten and Wanet, 1978, Murray and Dawber, 2002, Carney et al., 2011 and Papini et al., 2015).*** The disease is twice more frequent among men than women and it seems to increase with advancing age. Higher prevalence is seen between 40 - 60 years old persons. Children have infection rates 30 times lower than elders. The

reason for such low rates among children may include: reduced exposure time to fungus, faster nail growth, smaller nail surface and lower prevalence of tinea pedis. Toenail onychomycosis is about 4 to7 times more frequent than fingernail infections. In about one third of patients with toenail onychomycosis, concomitant tinea pedis could be observed (***Garson et al., 2000 and Szepietowski, 2004).*** In some subgroups (e.g. immunocompromised individuals and those participating in sports) the incidence of onychomycosis may increase by 20% because the use of communal showers, tight fitting shoes and/or immune-suppression due to drugs or HIV infections. In addition, occupational exposure to repeated minor trauma and/or humid environments increases the incidence of onychomycosis, too ***(Gupta et al., 1997, Elewski, 1998, Kavanagh et al., 2002, Justin and Erin, 2007 and Papini et al., 2015).***

Aetiology

Fungal organisms implicated in onychomycosis are yeasts, non-dermatophytes moulds and dermatophyte species. Tinea unguium is referred to onychomycosis caused by dermatophytes that nearly participate in 85-90% of nail fungal infections ***(Richardson and Warnock, 2012).*** Prevalence rates of onychomycosis caused by non-dermatophyte moulds range between 1.45 -17.6 %. Toenail onychomycosis caused by yeasts infection is less common accounting for 1.7%. However, yeasts, precisely C.albicans, account for up to 30% of fingernail onychomycosis. Mixed fungal infections occur in approximately one-fifth (22%) of cases ***(Gupta et al., 2003 and Iorizzo et al., 2007).*** The prevalence of different pathogens in different area depends on several factors such as: temperature and geographic distribution ***(Gupta et al., 2000, Mugge et al., 2006 and Papini et al., 2015).***

Clinical patterns of onychomycosis

Different clinical patterns of onychomycosis could be observed, depending on the manner and extent by which fungi invade the nail and the fungal species type **(*Gupta et al., 2004 and Hay and Baran, 2011*).**

1. **Distal and lateral subungual onychomycosis (DLSO):**

   It affects distal nail bed, hyponychium and the ventral nail plate side. The main features are onycholysis with hyperkeratosis and varying forms of dyspigmentations (Fig.1) ***(Davies, 1968).***

2. **Superficial white onychomycosis (SWO):**

   In which the dorsal nail plate has well-circumscribed powdery sculpted surface with white patches (Fig.2) ***(Zaias, 1966).***

3. **Proximal subungual onychomycosis (PSO):**

   It represents as proximal nail plate encroachment. Clinically, subungual hyperkeratosis, leukonychia and proximal onycholysis which extend distally with nail growth are seen (Fig.3) ***(Dompmartin et al., 1990 and Tosti et al., 2000).***

4. **Endonyx onychomycosis:**

   The fungus directly infiltrates the nail plate keratin. The main feature is discolored white nail in the obscurity of onycholysis and subungual hyperkeratosis ***(Ameen et al., 2014).***

5. **Totall dystrophic onychomycosis (TDO):**

   It is defined as ‘’total destruction of the normal nail architecture’’ (Fig.4) ***(Baran and Badillet, 1982).***