Evaluation of the Role of Fluorescence Microscope as a Potential Diagnostic Tool in the Diagnosis of Onychomycosis

Thesis

Submitted for Partial Fulfillment of Master Degree in

Dermatology, Venerology and Andrology

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Acknowledgment

First thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

I wish to express my deepest thanks, gratitude and appreciation to ***Prof.Dr.Hanan Mohammed Saleh,*** *Professor of Dermatology, Venerology and AndrologyFaculty of Medicine, Ain shams University for her meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to ***Prof .Dr. Mohamed Taha Elsayed,*** *Professor of Microbiology and Immunology Faculty of Veterinary Medicine, Zagazig University for his sincere efforts, fruitful encouragement.*

I am deeply thankful to ***Dr. Ghada Fathy Mohammed,*** *Assistant Professor of Dermatology, Venerology and Andrology Faculty of Medicine; Ain shams University for her great help, outstanding support, active participation and guidance.*

Mai Abdel-Naby Hamed

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List of Abbreviations

Abb.	Full term
A. niger	Aspergillus niger
A.flavus	Aspergillus flavus
ABSD	Autoimmune blistering skin diseases
AIDS	Acquired immunodeficiency diseases
AO	Acridine Orange
BCC	Basal cell carcinoma
BMZ	Basement membrane zone
BP	Bullous pemphigoid
C. albicans	Candida albicans
C. krusei	Candida krusei
C. parapsilosis	Candida parapsilosis
C. tropicalis	Candida tropicalis
C.glabrata	Candida glabrata
CMI	Cell-mediated immunity
Cyclic AMP	Cyclic adenosine monophosphate
CYP٤٥٠	Cytochrome P-٤٥٠
DDEB	Dominant dystrophic epidermolysis bullosa
DFA	Direct fluorescence assay
DIC	Differential interference contrast
DIF	Direct immunofluorescence
DLSO	Distal and lateral subungal onychomycosis
DMSO	Di-methyl sulfoxide
DNA	Deoxyribonucleic acid
DTM	Dermatophyte test medium
EB	Epidermolysis bullosa
EBA	Epidermolysis bullosa acquisita
ELISA	Enzyme-linked immunosorbent assay
FCM	Fluorescence confocal microscopy
FDA	Food and Drug Administration
FLTM	Fluorescence lifetime imaging
FRET	Fluorescence Resonance Energy Transfer
Fura-٢AM	Fura-٢-acetoxymethyl ester
H&E	Haematoxylin and Eosin

List of Abbreviations cont...

Abb.	Full term
HIV	Human immunodeficiency virus
HSP	Henoch-Schönlein purpura
HSV-١	Herpes simplex virus type ١
IF	Immunofluorescence
IFM	Immunofluorescence microscopy
IgA	Immunoglobulin A
IIF	Indirect immunofluorescence
KOH	Potassium hydroxide
KONCPA	Potassium hydroxide treated nail clipping with periodic acid-schiff
Laser	Light Amplification by Stimulated Emission of Radiation
LCV	Leukocytoclastic vasculitis
LE	Lupus erythematous
LP	Lichen planus
LPCB	Lacto phenol cotton blue
M. canis	Microsporum canis
M. cookei	Microsporum cookei
M. gypseum	Microsporum gypseum
M. nanum	Microsporum nanum
mAbs	Monoclonal antibodies
MPE	Multiphoton Excitation
MPM	Multiphoton microscopy
MPT	Multiphoton tomography
NDMs	Non dermatophyte molds
PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PSO	Proximal Subungual Onychomycosis
PV	Pemphigus vulgaris
RDEB	Recessive dystrophic epidermolysis bullosa
Rho-AKinase	Rho-associated protein kinase
RNA	Ribonucleic acid

List of Abbreviations cont...

Abb.	Full term
RSM	Rapid sporulating medium
S. hyalinum	Scytalidium Hyalinum
SDA	Sabouraud dextrose agar
SDA+C	Sabouraud's dextrose agar medium with chloramphenicol
SDC+C+C	Sabouraud's dextrose agar with cyclohexamide and chloramphenicol
Spp	Species
SWO	Superficial white onychomycosis
T. equinum	Trichophyton equinum
T. erinacei	Trichophyton erinacei
T. mentagrophytes	Trichophyton mentagrophytes
T. rubrum	Trichophyton rubrum
T. verrucosum	Trichophyton verrucosum
TDO	Total Dystrophic Onychomycosis
TIRF	Total internal reflection fluorescence
UV light	Ultra violet light

ABSTRACT

Onychomycosis is a fungal infection of the toenails or fingernails that can cause serious complications…in the present study ٣٥ patints of clinically suspected onychomycosis of finger and/or toe nails subjected to examination by fluorescence microscope using acridine orange stain and H&E stain, mycological culture, KOH ٢٠٪ direct microscopic examination and H&E stained specimens' examinations under light microscopy were also done.

Key words: onychomycosis, fluorescence microscopy .H&E stain and Acridine orange stain.

INTRODUCTION

Onychomycosis is a fungal infection of the toenails or fingernails that may involve any component of the nail unit, including the matrix, bed, or plate. Onychomycosis can cause pain, discomfort, and disfigurement and may produce serious physical and occupational limitations, as well as reducing quality of life ***(Tosti et al., ٢٠٠٣).***

Accurate diagnosis is important since the treatment of onychomycosis can be long-standing, expensive and may be accompanied by severe adverse effects ***(Allevato, ٢٠١٠)*** currently; the diagnosis is made by clinical suspicion along with KOH examination followed by culture of the samples ***(Richert, ٢٠٠٩)***. The sensitivity and specificity of the potassium hydroxide examination and culture depend on the center where the study is conducted and can be as low as ١٥٪; when clinical suspicion of onychomycosis is high despite having a negative KOH examination and a negative culture, periodic acid-Schiff (PAS) staining of nail clippings can be used in the diagnosis of onychomycosis, but it is invasive and expensive compared to direct examination ***(Jesús-Silva et al., ٢٠١٥).***

Special illumination techniques such as ultraviolet (UV) light (Wood's lamp) have long been used in clinical dermatology for diagnostic purposes in dermatopathology, UV light is frequently used to confirm the presence of immune

deposits in skin ***(Caplan, ١٩٦٧).*** Utilization of ultraviolet light in the diagnosis of fungal infection ranges from *in vivo* diagnosis of dermatophytic infection to *in vitro* tissue diagnosis of fungal infection by immunofluorescent techniques ***(Rao et al., ٢٠٠٨).***

fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances ***(Spring and Davidson, ٢٠٠٨).***

Some prior studies have shown that pathogenic fungal species will fluoresce when examined under a fluorescent microscope, and this phenomenon has been advocated as a useful technique in the histopathologic diagnosis of fungal infections ***(Mann, ١٩٨٣; Margo and Bombardier, ١٩٨٥).*** Also fluorescence of pathogenic fungi has been previously shown when hematoxylin and eosin-stained sections are examined under a fluorescent microscope. There was a hypothesis about this phenomenon that it could aid in the evaluation of nail specimens for onychomycosis ***(Elston, ٢٠٠١).***

Most of these studies have focused on the study of systemic and deep fungal infections and most practicing dermatopathologists are unfamiliar with the use of fluorescent microscopy as a tool in the identification of fungal organism ***(Elston, ٢٠٠٢).***

Fluorescent substances have attracted a lot of attentions in the past few decades due to their unique optical properties and applications in imaging of various inorganics (metal and anions), organics (drugs) and cells ***(Kasibabu et al., ٢٠١٥).*** The relative ease and availability of fluorescence microscopy in the dermatopathology laboratory warrants the investigation of this modality as a possible diagnostic tool in onychomycosis ***(Idriss et al., ٢٠١٣).***