"A ONE YEAR CROSS-SECTIONAL CLINICO-MYCOLOGICAL STUDY OF ONYCHOMYCOSIS IN KLES DR PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

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LIST OF ABBREVIATIONS USED

%	-	percentage
^{0}C	-	Degree celcius
C.	-	Candida
CO	-	Candidial onychomycosis
DMSO	-	Dimethyl sulfoxide
DLSO	-	Disto-lateral Subungal Onychomycosis
DTM	-	Dermatophyte test medium
E.	-	Epidermophyton
eg.	-	Example
EO	-	Endonyx onychomycosis
etc	-	Etcetera
HIV	-	Human immunodeficiency virus
i.e.	-	That is
IFN-γ	-	Interferon-γ
IgE	-	Immunoglobulin E
IgG	-	Immunoglobulin G
KOH	-	Potassium Hydroxide
LCB	-	Lactophenol Cotton Blue
M.	-	Microsporum
mg	-	Milligram
l	-	Litre
mm	-	Millimeter
n	-	Total number
NaOH	-	Sodium hydroxide

NDM	-	Non-dermatophytic moulds
OM	-	Onychomycosis
P	-	Probability
PAS	-	Periodic Acid Schiff
PCR	-	Polymerase chain reaction
PNF	-	Proximal Nail fold
PSO	-	Proximal Subungal Onychomycosis
SD	-	Standard deviation
SDA	-	Sabouraud's dextrose agar
Sp.	-	species
T.	-	Trichophyton
TDO	-	Total Dystrophic Onychomycosis
WSO	-	White Superficial Onychomycosis

ABSTRACT

Background:

Onychomycosis is no longer considered as a simple cosmetic problem, but it is an important and significant disease, which can generate many occupational, physical and psychological problems impairing patient's quality of life.

Objectives:

To study the clinical types, etiological agents, sex distribution, age, occupation, duration of infection, aggravating factors, associated fungal infections causing onychomycosis and to compare KOH and culture in diagnosis

Methodology:

The present one year cross-sectional study was done in KLES, Dr Prabhakar Kore Hospital and MRC, Belagavi from January 2015 to December 2015. A total of 50 patients presenting with clinical features of onychomycosis were subjected to KOH examination and culture for fungi.

Results

Commonest age group was 51-60 years (22%) with male preponderance (52%). The maximum number of patients were housewives (34%) and most of them presented with in 1 year (56%). Hyperhidrosis and warmth were common aggravating factors. Discolouration (62%) of nails was the most common presenting complaint. Subungual hypertrophy (80%) and onycholysis (40%) were the most common clinical changes observed. Toe nails (44%) were most commonly involved. DLSO (66%) was the most common morphological pattern observed. Tinea corporis (6%) and diabetes mellitus

(8%) were the most common associated conditions. Isolation rate of fungus was 92% (46 out of 50). The higher incidence of culture positivity (92%) than KOH positivity (50%) was observed. Out of 46 culture positive specimens, the maximum were NDM (63%). The most common NDM isolated was Aspergillus niger (13). The most common dermatophyte isolated was Trichophyton mentagrophyte (8). Yeasts like Candida kruzei (C.kru), Candida tropicalis (C.trop), Candida lusitanea (C.lusi) were isolated. DLSO, PSO and TDO were most commonly caused by NDM.

Conclusion

The present study highlights the necessity of microbiological confirmation in case of onychomycosis and also stresses that diagnosis of onychomycosis solely based on clinical features is often misleading. Culture was considered the gold standard for the diagnosis of onychomycosis. It is cheaper and routinely available test.

Keywords:

Culture; Etiological agents; Nail changes; Non-dermatophytes; Onychomycosis.

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF PHOTOGRAPHS

INTRODUCTION

Fungi appear most common in nature that no part of the world is free from them.[1] Diseases caused by fungi are called as "mycoses.[2] Most of the cutaneous infections are caused by homogeneous group of keratinophilic fungi known as dermatophytes.

Based on site mycoses are classified as: [3]

1. Superficial and cutaneous
2. Subcutaneous
3. Systemic (deep) infections.

Superficial and cutaneous fungal infections are defined as infections in which the pathogen is restricted to stratum corneum, with or without tissue reaction.

- Superficial mycoses include: White piedra (trichosporon beigelii), black piedra (piedraia hortae), tinea nigra (phaeoannellomyces werneckii) and pityriasis versicolor (malassezia furfur).
- Cutaneous mycoses may be classified as dermatophytoses or dermatomycoses. Dermatophytoses are caused by the agents of the genera trichophyton, epidermophyton, and microsporum which cause onychomycosis, tinea capitis, tinea corporis, tinea barbae, tinea pedis. Dermatomycoses are cutaneous infections due to other fungi, the most common are candida sp. which cause candidiasis of skin, mucosa, and nails.[3, 4]

Dermatophytosis of epidermis is called epidermomycosis, dermatophytosis of hair is called trichomycosis, and dermatophytosis of the nail apparatus is called onychomycosis.[5] Onychomycosis are commonest nail disorders caused by dermatophytes, non-dermatophyte moulds or yeasts.[6] Onychomycosis affects approximately 5% of the population worldwide and represents about 30% of mycotic cutaneous infections and 20-40% of onychopathies.[7]

Compared to other superficial and cutaneous mycosis, onychomycosis is intractable, persistent and poses serious concern to the clinicians as it represents a chronic course of recurrent superficial fungal skin infections, besides causing considerable disfigurement.[8] It is a well-established fact that geographical distribution of fungi may change from time to time.[8]

Treatment of onychomycosis is becoming a challenging task as the nail fungus usually do not live on the nail surface but within the nail bed where there is a rich blood supply, which encourages growth of the fungus. Nail bed uses the nail as a protective shield, so that many topical medicines do not reach the affected area. In addition, most of the systemic drugs have poor affinity to the nail bed tissues. Even with currently available treatments, rate of complete elimination of the disease is not satisfactory.[9]

The present study is undertaken to study the clinical patterns of onychomycosis and various mycological species causing it by KOH and culture, so that an exact antifungal agent could be used for treatment.

The present work refers to clinico-mycological study of onychomycosis in patients attending KLES Dr Prabhakar Kore Hospital and MRC, Belgaum.

AIMS AND OBJECTIVES:

Primary objective:

1. To study the clinical types of onychomycosis
2. To study the etiological agents causing them by KOH and fungal culture.

Secondary objective:

1. To study the sex distribution, age, occupation, duration of infection, aggravating factors, associated fungal infections causing onychomycosis.
2. To compare KOH and culture in diagnosis

REVIEW OF LITERATURE

Historical review:

Fungi are an ancient group which have a fossil evidence of 3.5 billion years old.[10] Ancient ayurvedic literature of India which are written during 800 BC to 100 AD described "Dadru" a skin condition which clinically resembles the ring worm infection but nothing was mentioned about the causative agent.[11]

"Onyx" which means nail, "mykes" which means fungus and "osis" which means condition i.e., fungal infection of the nail. The name onychomycosis comes from the Greek words.[11] Descriptions of mycotic infections of the nail had appeared first in 1828 by a Perisian emperor Mahon.[13]

The real founder of dermatomycology was David Gruby based on his discoveries during 1841 to 1844, his publications and his communications to the French academy of science during this period. [14] Trichophyton genus was first identified by Malmsten in 1845 with the discovery of species T. tonsurans. In 1843, Gruby identified microsporum with the discovery of M. audouinii.[15]

Credit of discovery of dermatophytes in nail substance goes to George Meissner in 1853, who observed hyphae in a potassium hydroxide preparation of a "thick finger nail".

Rudolf Virchow had first coined the term "Onychomycosis" in 1856.[12] The epidermophyton genus was firstly established by Sabouraud in 1910 after the invention of Epidermophyton rubrum species.[16]

In 1910, a book "Les teignes" published by Raymond Sabouraud, which described classification of dermatophytes into three genera: (1)Trichophyton (2)Epidermophyton (3)Microsporum.[16] The first case of onychomycosis caused by Aspergillus species was reported by Emile-Weil and Gaudin in 1919.[18] In 1941 Bereston and Keil isolated Aspergillus flavus for the first time.[19] Papers discussing the potential uses of lasers in clinical practice started in the 1980s, focusing on the high powered carbon dioxide (CO_2) systems available at that time.[20]

Elewski (1998) stated that subsequent 20th century developments including the modern health and the associated use of the locker rooms, occlusive footwear and migration of people due to invention of jumbo jet, promoted an increased incidence of athletes foot and onychomycosis.[1] In July 2014, tavaborole topical solution is approved by the FDA as a novel, boron based pharmaceutical, for the treatment of onychomycosis caused by T. rubrum and T. mentagrophytes.[21]

Clinically used terms to describe dermatophytic infections: Tinea or "ringworm" - a term derived from the appearance of the characteristic lesions over the skin in dermatophytosis which affects the nail (tinea unguium), feet (tinea pedis), scalp (tinea capitis), beard (tinea barbae), glabrous skin (tinea corporis), hand (tinea manuum), groin (tinea cruris).[22]

BASIC ANATOMY OF NAIL UNIT:

It is important to focus on basic structure and science of nails in the present topic which is referred to as fungal infection of nail.

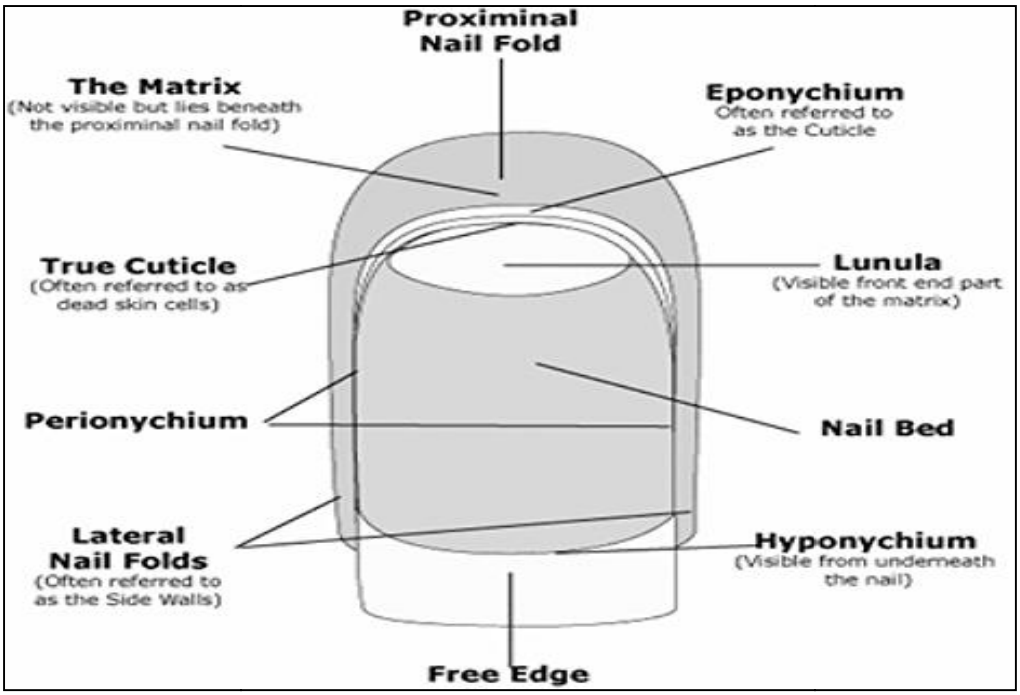


Figure 1. Dorsal View Of Nail Unit

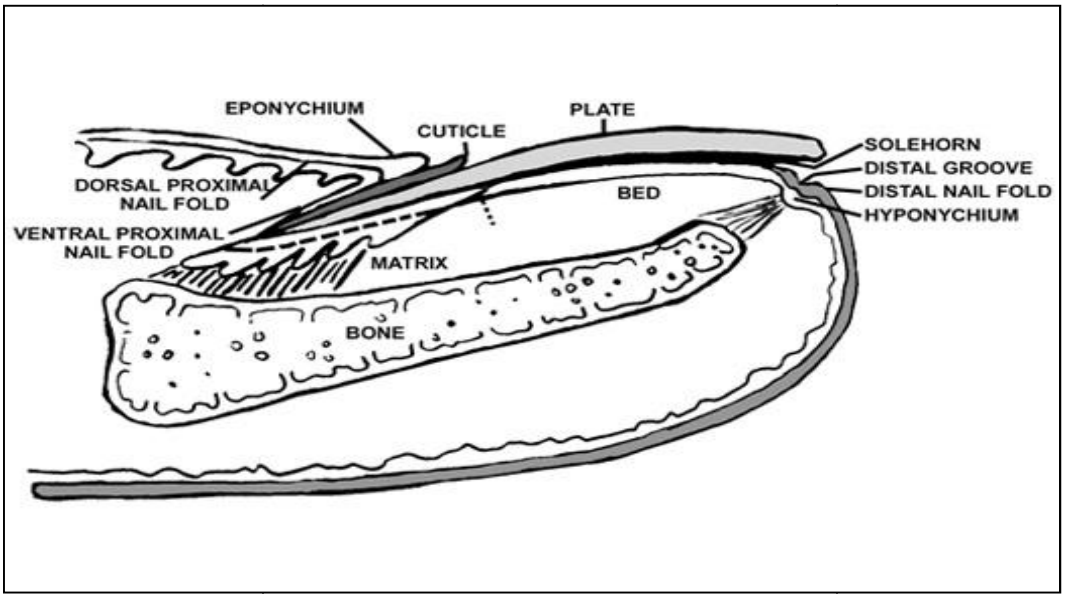


Figure 2. Saggital View Of Nail Unit

Embryology:

The nail apparatus appears in the fingers of embryo by 9th week in-utero. The earliest matrix was found in the area of the lunula by 13 weeks. The nail plate grows over the nail bed by 20 weeks.[23]

Structure:

Nail organ consists of four components: The matrix, nail bed, nail plate, nail folds.

Nail matrix:

The matrix forms the nail plate and it begins its development in the ninth week from the nail anlage. It is a convex band of highly proliferative epithelium. About 80% of the nail plate is produced by the proximal half of the matrix.[24]

Nail bed:

The nail bed is the continuation of distal matrix. It does not produce nail plate substance so it is called as ‘‘sterile matrix’’. It begins where the lunula ends.[25]

Nail plate:

It is a chemically and mechanically resistant sheet of compacted keratinized cells. It is analogous to stratum corneum of the epidermis.

Nail folds:

There are two lateral nail folds and one proximal which direct the nail growth in the correct position. The PNF is a crescentic sheet of tissue and it has a sharp angle at its free margin and it is attached to the dorsal surface of the nail plate. The lateral nail folds overlap the nail plate at the sides.[26]

Lunula:

The lunula or half-moon can be seen through the nail emerging from beneath the PNF as a pale convex structure.

Cuticle:

The stratum corneum of both dorsal and ventral side of the PNF forms a desquamating tissue that seals the nail cul-de-sac.

Onychodermal band:

The onychocorneal band represents the first barrier against the penetration of materials to beneath the nail plate. Disruption of this barrier by a disease or trauma precipitates further events which affects the nail bed.

Eponychium:

Refers to the upper portion of the ventral aspect of the proximal nail fold and adheres closely to the nail for a short distance.

Hyponychium:

It is the cutaneous margin underlying free nail, bordered distally by the distal groove.

Distal groove:

A cutaneous ridge demarcating the border between the finger pulp and the subungual structures.[27]

Blood supply:

The nail organ has a rich blood supply. Two digital arteries, the bigger volar arteries and the small dorsal artery bring oxygenated blood to each side of the nail. Arteriovenous anstomoses form the glomus bodies that function in thermoregulation.

The blood is collected into a superficial system and a deep that drains into few larger veins that run subcutaneously on the dorsal and volar aspect of the digits.[28]

Nerve supply:

The periungual soft tissues are innervated by dorsal branches of digital nerves which supplies the digital tip. The main branch passes under the nail bed and innervates both nail bed and matrix.[29]

Nail growth:

The rate of finger nail growth varies between 1.9 and 4.4 mm per month and toenails grow around 1 mm per month.

Why nails grow flat instead of vertically or heaped up keratinous masses is, it is hypothesized that the restraint of the PNF, the adherence of the nail plate to the nail bed, containment by the lateral nail folds, the influence of the underlying phalanx and the direction of differentiation of cells contribute to the flat growth of the nail.[30]

ONYCHOMYCOSIS

The terms onychomycosis and tinea ungium are confusing.

Definition:

"Onychomycosis" was traditionally referred to as non-dermatophytic infection of nail, but now it is used as a general term to denote any fungal nail infection. Tinea ungium is a term which specifically describes a dermatophytic infection of the nail plate.[6]

Epidemiology:

Dermatophytes account for almost 90% cases of onychomycosis of the toenails and around 50% of fingernail infections.[31] Both dermatophytes and nondermatophytes cause onychomycosis.

Nondermatophyte moulds cause 1.5-6% of onychomycosis. Onychomycosis caused by nondermatophytes is most commonly seen in immunocompromised patients, elderly patients and in patients with other skin diseases which can also infect the nails. It is more frequent in toenails than in fingernails as the moulds are present in soil.[32]

The prevalence rates of onychomycosis are determined by age, social class, predisposing factor, climate, occupation, living environment and frequency of travel.[33] The prevalence is higher in patients with HIV.[34] Prevalence rates of onychomycosis among children are variable. A recent review of different studies in several countres shows the prevalence rates varying from 0% (Finland, wales and United States) to 2.6% (Guatemala). A decrease in prevalence of onychomycosis

among children in contrast to adults may include faster nail growth, reduced exposure to fungus, lower prevalence of tinea pedis and smaller nail surface for invasion.[35]

Etiology:

Onychomycosis can be caused by dermatophytes, non-dermatophyte moulds or yeasts.[36, 37]

Dermatophytes:

These are a group of fungi that have the capacity to invade keratinized tissue i.e skin, hair, and nails of humans and other animals. The infection is restricted to the nonliving layers because of the inability of the fungi to penetrate the deeper organs or tissues of immunocompetent hosts.[38]

Dermatophytes are classified into 3 genera:

1. Trichophyton
2. Epidermophyton
3. Microsporum

According to recent data available, there are two species in epidermophyton, 18 species in microsporum and 25 species in trichophyton.[39]

Species of dermatophytes: [7]

Trichophyton:

T. Concentricum

T. Equinum

T. Gourvilii

T. Kanei

T. Megninii

T. Mentagrophytes

T. Raubitschekii

T. Rubrum

T. Schoenleinii

T. Simii

T. Soudanense

T. Tonsurans

T. Verrucosum

T. Violaceum

T. Yaoundei

Epidermophyton:

E. floccosum

E. stockdaleae

Microsporum:

M. Audouinii

M. Canis

M. Equinum

M. Ferrugineum

M. Fulvum

M. Gallinae

M. Gypseum

M. Nanum

M. Persicolor

M. Praecox

M. Racemosum

M. Vanbreuseghemii

Trichophyton species can infect skin, hair and nails. Microsporum species infect skin and hair. Epidermophyton species infect skin and nails.[3]

Only a few species in trichophyton and epidermophyton can cause onychomycosis. For identification of the species, microconidia or macroconidia and hyphae should be observed on fungal culture.

Causative agents of onychomycosis: [37]

Dermatophytes	**Yeasts**	**Moulds**
T. mentagrophytes	C. albicans	Acremonium species
T. rubrum	C. tropicalis	Alternaria species
E. floccosum		Aspergillus species
		Onycochola canadensis
		Botryodiplodia Theobrome
		Fusarium species
		Scopulariopsis species
		Scytalidium species

Dermatophytes:

A. Trichophyton species:

1. Trichophyton rubrum:

Trichophyton rubrum is anthropophilic and is the most common cause of dermatophyte infections in humans.[40]

Microscopy:

Isolates of T. rubrum produce only few conidia. They are called as "tear drop" micro conidia which are produced lateral to the hyphae. "Pine tree" arrangement of clusters of conidia are seen. Macrconidia are usually absent or rarely present. If macro conidia are present they are narrow, long, fusiform and multicelled.

Colony characteristics:

The culture primarily shows cottony and pure white surface, but later converts into rose purple or reddish with velvety surface on the back of the colony.[41, 42]

2. Trichophyton Mentagrophytes:

It is so variable in its morphology. It is a zoophilic species.

Microscopy:

T. mentagrophytes produces grape like clusters of globose microconidia. Macroconidia are smooth, thin walled and the shapes are variable. "Rat tail appearance" is seen.

Culture characteristics:

Primary cultures are powdery and tan in colour. Some strains produce pink to red pigment in Saubaouraud's glucose agar. T. rubrum can be differentiated from T. mentagrophytes by cultivation on corn meal agar in which it produces red pigment.

B. Epidermophyton:

This genus has only two known species, only E. floccosum is known to be pathogenic.

Epidermophyton floccosum:

Macroconidia are abundant, single or in clusters, has one to nine septa, and are broadly clavate and typically smooth, thin to moderately thick walls. Microconidia are absent.

Culture characteristics:

Primary growth shows "khakhi" surface, yellowish brown or olive green with pleomorphic tuft and radial folding. Yellow brown colour is seen on the reverse side. [6, 17]

Non- dermatophytes:

These are subdivided into 2 major groups of fungi:

1. Yeasts
2. Moulds

Non-dermatophyte moulds and yeasts (especially Candida albicans) and may affect the nail plate directly especially in previously traumatized nails.[43, 44, 45] In some cases they act as primary pathogens also.[46]

It is important to diagnose opportunistic molds to ensure adequate treatment because the therapeutic management of onychomycosis by non-dermatophytes is complex and unsatisfactory and more difficult than tinea unguium.[47]

1. Yeasts :

Candida are budding yeasts like fungus. They produce both pseudomycelium and blastospores. The incidence of yeasts causing onychomycosis is not well known, because they are colonising organisms in oral cavity or intestinal mucosa rather than pathogens.[48]

The main etiological agent in humans is Candida albicans. Other Candida species which can cause a variety of infections includes C. tropicalis, C. dubliniensis, C. krusei, C. guillermondii, C. parapsilosis, C. kefyer and C.glabrata.[49]

Microscopy:

On KOH examination, the fungal elements like budding yeasts, hyphae or pseudo hyphae can be demonstrated. But the species identification requires isolation and biochemical or physiological characterization.[50]

Culture characteristics:

On SDA, Candida produces pasty, creamy, smooth and convex colonies which may become wrinkled on further incubation. Colonies appear within 1-3 days on the laboratory media at 25-30^{o}c.

Followed by culture on SDA medium, seeding on differential media like CHROM agar Candida and incubation at 30ºC for 48 hour is done which aids in identification of the strains. C. albicans or C. dubliniensis produce green colonies, C. tropicalis produce metallic blue colonies, C. krusei shows pink colonies and the other species show white to mauve coloured colonies.[51] A rapid method for identifying C. albicans and C. dubliniensis is by its ability to produce germ tubes

which are short, slender, tube like structures, when it is incubated in human and non-human sera.[52]

2. Moulds :

Moulds are non-dermatophytic, filamentous, saprophytic fungi. The five most common species of NDM worldwide are: Scopulariopsis brevicaulis, Aspergillus sp., Onychocola canadiense, Fusarium sp., Acremonium sp. and Scytalidium sp. Aspergillus species are frequently found saprophytes when culturing nails. Asp. fumigatus, Asp. niger, Asp. flavus, Asp. nitidus are some subspecies isolated from nails.[53]

Many of these fungi are highly sensitive to cycloheximide. They may be missed if the specimen is not inoculated on a cycloheximide free medium also, such as Sabouraud glucose agar, Potato dextrose agar or Littman's Oxgall agar. This is because cyclohexamide inhibits growth of many saprophytes.[54] NDM may be present as a contaminants in glabrous skin, hair, and nails.

Clinical clues that a NDM is the causative pathogen may include the absence of tinea pedis, a history of trauma, a history of nonresponsiveness to systemic antifungals and association with periungual inflammation.[55] A NDM growth on culture, from at least 5 out of 20 nail fragment inoculums with no dermatophyte growth is considered to be clinically significant.[42]

Direct microscopy in case of a NDM involvement of the nail usually shows irregular, vesiculated, tortuous or pigmented hyphae.[56]

Culture on potato dextrose agar (PDA):[57]

Asp. niger shows typical white colonies with typical black spores. Penicillium species show dark green colonies. Acremonium species show yellowish orange colonies. Chaetomonium species show golden brown colonies. Fusarium species show magenta coloured colonies.

PATHOGENESIS:

Fungal invasion of nail apparatus is less known aspect. Chemical and mechanical factors play a role.

The essential steps involved in the pathogenesis are:

1. Surface adhesion
2. Invasion into sublayers

The anatomy of nails aids in easy entry of pathogens. The immunological aspects of nail are different from skin. Nail has relatively low expression of MHC (Major histocompatibility) Class1a antigens, dysfunction of antigen presenting cells (APC) and inhibition of natural Killer (NK) cell activity.[58]

With respect to their capability of antigen presentation, the macrophages and langerhans cells in the nail matrix are functionally impaired.[59] So basically because of lack of effective cell-mediated immune mechanism in the nail matrix associated with exposure to predisposing factors aids in susceptibility of nail apparatus to various fungal organisms.

Different groups of fungi causing onychomycosis have varied clinical behavior.

Dermatophytes:

They have keratinolytic, proteolytic and lipolytic activities.[60] The invasion into the tissues is facilitated by hydrolysis of keratin. They are strongly keratophilic organisms as they have ability to form perforating organs which quickly digest keratin.[61] Trichophyton mentagrophytes is more active destroyer than Trichophyton

rubrum. This active pathogenicity for the nail could be a result of mechanical or enzymatic processes.[62]

Most of the dermatophytes affect the ventral and middle layers of the nail plate because the keratin is in close proximity to the underlying living cells and it is comparatively soft. The junctions between cells on the ventral surface are more flexible than the tight junctions in the dorsal part. The ventral surface has an irregular topography with parallel grooves and ridges which provides channels for hyphae to penetrate the nail plate.[63] The intermediate layer is involved less commonly and the hardest, dorsal nail plate is rarely involved as in white superficial onychomycosis.[64]

Yeasts:

Nail has strong innate immunity as it has a local increased expression of antimicrobial peptide (human cathelicidin LL.37) which is induced upon exposure to infection. As a result there is inflammation due to increased activity of these antimicrobial peptides in case of candida infections as they are soluble antimicrobials.[65] Candidal OM is seen in most of the patients who are engaged in domestic activities which involve wet work that predispose to repeated trauma.[48] Invasion of nail by Candida albicans is usually secondary to abnormal nail bed and nail folds or chronic paronychia. It invades the nails in both yeast and mycelial forms.

Moulds:

Moulds reside on the unkeratinized intercellular cement of nail as they are non-keratinolytic fungi. Unlike dermatophytes, moulds are situated in the outer layer of the nail, away from the living tissue. These moulds grow in between the layers of hard keratin by digesting the intercellular cement through their frond like mycelium.[42]

CLINICAL FEATURES:

The different patterns and sites of invasion by different fungi, leads to different clinical types of onychomycosis.

The clinical types of onychomycosis can be divided into: [48, 66, 67]

a) Disto-lateral subungual onychomycosis (DSLO)
b) Proximal subungual onychomycosis (PSO)
c) White superficial onychomycosis (WSO)
d) Endonyx onychomycosis (EO)
e) Candidial onychomycosis (CO)
f) Total dystrophic onychomycosis (TDO)

a) Disto-lateral subungual onychomycosis (DSLO):

This is the most common clinical type of OM. The keratin of the hyponychium is infected first which progresses to involve the nail bed and then, the nail plate.[68] This is also described as "nail bed dermatophytosis." The fungus migrates proximally i.e., against the growth of the nail plate. Mild inflammation with subungual hyperkeratosis, focal parakeratosis which leads to onycholysis and subungual thickening. This subungual space can serve as a reservoir for superinfecting molds and bacteria which gives the nail plate yellowish brown appearance.[69] DSLO is usually caused by dermatophytes, especially T. rubrum and less commonly T. mentagrophytes, T. tonsurans, and E. floccosum. Finger nails are affected less commonly than toenails. Co-infections of tinea pedis and tinea mannum may be present.[70]

b) Proximal subungual onychomycosis (PSO):

The fungus first invades the stratum corneum of proximal nail fold and then penetrates to the matrix and undersurface of the nail plate and migrate distally.[71] It is a relatively uncommon subtype. PSO can present as a rapidly developing disease which involves several nails within few days, especially in HIV associated immunosuppression. It is considered an early clinical marker of HIV Infection.[72] A hypothetic role of lymphatic spread, auto-reinfection or endogenous reactivation from a deeper site is proposed, than any new external infection being acquired through the proximal nail fold.[73]

c) White superficial onychomycosis (WSO):

It is a rare variant. The dorsal part of the nail plate directly is the initial site of invasion in which the fungus cause superficial small white patches which coalesce and cover the entire nail plate. WSO is mostly limited to toenails. The most common isolated organism is T.mentagrophytes.[69] WSO has been documented usually in fingernails and is most commonly caused by T. rubrum in HIV patients.[74] It can be recognized by appearance of opaque "white islands" over the external nail plate and the nail becomes soft, rough, and crumbly. Inflammation is minimal in WSO as viable tissues are not involved.[69]

d) Endonyx onychomycosis (EO):

There is an exclusive attack of nail plate with the fungus growing in between the nail plate lamellae. EO is specifically caused by T. soudanense (also by T. violaceum). Clinically, EO presents with diffuse milky white discoloration, pits and lamellar splits. There is absence of nailbed hyperkeratosis and onycholysis.

Presence of fungal filaments within the tunnels of nail plate causes alteration in diffraction of light and causes milky white discolouration.[75] Nail plate surface and nail thickness, both are normal. A large number of fungal hyphae are present in the nail plate but are absent in the nail bed and hyponychium.[76]

e) Candidial onychomycosis (CO):

Candida spp. may cause other syndromes, including onycholysis and paronychia. CO occur in patients with chronic mucocutaneous candidiasis. C. albicans is the causative agent in 70% of cases of CO caused by the yeast. Other species which can cause CO are: C. parapsilosis, C. tropicalis and C. krusei.[77]

It has three subtypes:

- *Candida paronychia*: It is also called a whitlow. This is the most common type of paronychia which presents as marked swelling and erythema of the proximal and lateral nail folds. Both toenails and fingernails may be involved.
- *Candida granuloma*: It is an uncommon type and is characterized by direct invasion of the nail plate and associated paronychia. This condition is seen mostly in immuno-compromised patients. Presents as a pseudo clubbing or chicken drumstick appearance of the digits.
- *Candida onycholysis*: The nail plate separates from the nail bed. This can be seen as a yellowish grey mass lifting up the nail plate. This type resembles DSLO.[78]

f) Total dystrophic onychomycosis (TDO):

TDO is the end stage nail disease. Some clinicians consider it to be a distinct subtype of onychomycosis. It presents as total destruction of the entire nail apparatus including whole thickness of the nail plate, nail bed and matrix. The involved nail

becomes crumbled and dystrophic. It could be primary as in cases of chronic mucocutaneous candidiasis, or secondary as an end result of any of the patterns of onychomycosis.[68]

DIFFERENTIAL DIAGNOSIS:

The nail changes of onychomycosis mimics many other dermatological and systemic conditions. So signs and symptoms of onychomycosis should be correctly differentiated from other diseases.

About 50% of dystrophic or discolored nails have a confirmed fungal infection on culture. Other causes include:

- Trauma due to tight shoes or nail biting.
- Eczemas (allergic or irritant contact dermatitis).
- Subungual melanomas
- Psoriatic nail disease
- Lichen planus
- Bacterial paronychia – e.g.: Pseudomonas spp. infection.
- Systemic disease – e.g.: peripheral arterial disease, thyroid disease, diabetes.
- Rare systemic disorders – e.g.: yellow nail syndrome, pachyonychia congenita, nail patella syndrome, keratosis follicularis.
- Drug reactions (tetracyclines, quinolones and psoralens)

Onycholysis:

When psoriasis affects the nails, it can produce onycholysis resembling that of DLSO. Also seen in conditions like pachyonychia, drugs (photo onycholysis secondary to tetracyclines), trauma, contact dermatitis, Darier's disease, erythroderma, ichthyosis, sezary's syndrome etc.[79]

Onychorrhexis: Central part of the nail is raised and the lateral part is depressed. Seen in lichen planus, ichthyosis, psoriasis, onychomycosis, syphilis, pemphigus, variola.[80]

Subungual hyperkeratoses: Thickening below the preformed nail plate. Also seen in psoriasis, icthyosis, Darier's disease and repeated trauma.

Onychogryphosis: (Hypertrophic and distorted nails) seen in syphilis, pemphigus, variola, icthyosis and psoriasis and onychomycosis caused by moulds.

Paronychia: Inflammation of the folds of tissue surrounding finger nails or toe nails. Seen in colonization with Candida albicans or bacteria, eczema, psoriasis, Reiter's syndrome, herpetic whitlow.[81] Candida albicans isolated from chronic paronychia is considered as a type of hand dermatitits rather than onychomycosis.[82]

Discolouration: [83]

- Greenish-black discoloration is seen in Pseudomonas aeruginosa and Aspergillus infections.
- Yellowish discolouration can be caused by Yellow nail syndrome, nail enamel and hardeners, Carotene, dermatophyte onychomycosis.
- Blue to grey pigmentation is seen in antimalarials, argyria, congenital pernicious anemia, minocycline.
- Black nails are seen in onychomycosis secondary to Trichophyton rubrum, malignant melanoma, silver nitrate.
- Brown nails are caused by pregnancy, thyroid disease, smoking and tar.
- Leuconychia: white nails can be seen in Psoriasis, leprosy, WSO.

DIAGNOSIS:

Although, diagnosis of onychomycosis can be made on clinical appearance, lab diagnosis is appropriate for the accurate diagnosis and initiation of treatment.[48]

Specimen collection:

A proper collection of specimen is required for appropriate diagnosis. As the site of invasion and location of infection differ in different types of onychomycosis, different approaches are necessary to obtain appropriate specimen.

Disto-lateral subungual onychomycosis:

In DLSO, the specimen should be collected from the nail bed, as the agents invade the nail bed rather than the nail plate, where concentration of the viable fungi is highest.[84]

With a no. 15 scalpel blade or a small curette, the specimen is collected from the infected edge most proximal to the cuticle. Care is to be taken to avoid bleeding by penetration of nail plate.[85]

Proximal subungual onychomycosis:

In PSO, using a scalpel blade, the healthy nail plate should be pared away. The scalpel blade or sharp curette is used to scrape material from the infected proximal nail bed as close to lunula as possible.[85]

White superficial onychomycosis:

In WSO, a no. 15 blade or a curette may be used to scrape the surface or the white area of the nail, and remove the infected debris.

Endonyx onychomycosis:

In EO, full thickness nail clippings or nail scrapings are used as specimens.

Candidial onychomycosis:

In CO, the material should be collected from proximal and lateral nail edges. In case of Candida onycholysis, scrapings of the lifted nail bed should be taken.[85']

Total dystrophic onychomycosis:

In TDO, scrapings or clippings from any abnormal area of the nail bed or plate can be used as specimen.

Specimen analysis:

The specimen is to be divided into two parts, one for direct microscopy (to visualize fungal elements) and the other for culture (to identify the species).

Direct microscopy:

This serves only as a screening test for the absence or presence of fungi but cannot specify the organism.

- **Potassium hydroxide (KOH) preparation:**

The specimen is placed in a drop of 10-30% KOH over a clean glass slide. As the nail material do not get softened easily, the slide can be placed in incubator at 37^0c for 1hour. For thick nail clippings 20% KOH and 36% dimethyl sulfoxide (DMSO) can be used. It helps in rapid penetration of KOH without the requirement of heating.[6] KOH digests the proteinaceous debris without damaging the fungus and making the fungal hyphae more prominent.[86]

Mount the preparation with a cover slip without air bubbles in it. Examine the preparation under microscope (10X and 40X objectives). Direct microscopy is usually time consuming as the nail debris is thick and hyphae are only sparsely present.

Some modifications of KOH preparation: [87]

- *Parker's ink method:* Parker's ink when added to potassium hydroxide, stains the fungal wall blue and so easy to recognise.
- *Eosin 1% method:* Eosin 1% when added to potassium hydroxide stains the keratin and gives a pinkish background, but the fungal filaments remain unstained.

Culture:

The purpose of culture is to identify both genus and species of the fungus because different species respond in a different way to a given antifungal agent. Bacterial and fungal contaminants may obscure the nail pathogen as the nails are non-sterile. So the culture results should be analysed cautiously.

Specimens should be subjected to culture on two different media:

- **Sabouraud's dextrose agar (SDA)** which is used for isolation of dermatophytes and also opportunistic pathogens. Sabouraud's agar is available in different commercial types which differ in pH, nitrogen source and dextrose concentration. Nail specimens are inoculated in SDA medium containing cycloheximide and chloramphenicol which inhibits the growth of saprophytic fungi and bacteria. But some saprophytic fungi have ability to invade nails and can start the disease process. So inoculation of the specimen in SDA with only chloramphenicol and

without cycloheximide should also be done which also allows the growth of saprophytes.[88]

- **Dermatophyte test medium** or Sabouraud peptone-glucose agar contains cycloheximide and phenol red indicator which turns red due to alkaline metabolites released by the dermatophytes. This is selective against most non-dermatophytic moulds and bacteria.[88]

Colony characters:

Note the colour of the surface, colony morphology, reverse of the colony, texture of the colony (wooly, cottony, granular, powdery or velvety).

Microscopic morphology:

Appearance of conidia and other structures are determined by slide culture or tease mount preparations made on Lacto phenol cotton blue (LCB). Tease mount on LCB is done by placing a fragment of fungal filament taken from a colony and placing it on a drop of LCB on a slide and then examined under microscope.

Interpretation of results:

a) Positive for dermatophytes, if either microscopy or culture is positive.
b) Positive for Candida spp., if both microscopy and culture are positive.
c) Positive for non-dermatophytic onychomycosis based on following criteria: [89]
    - ✓ Failure to isolate a dermatophyte on culture
    - ✓ Nail abnormalities showing clinical features of onychomycosis.
    - ✓ Presence of fungal hyphae on KOH in nail keratin.
    - ✓ If growth of more than five colonies of the same NDM in at least two consecutive specimens.

Other diagnostic modalities are:

- Nail plate biopsy by using periodic acid-Schiff (PAS): It is indicated if other routine diagnostic methods are negative but clinical suspicion is high.
- Serological tests like immunodiffusion techniques.
- Molecular identification techniques like PCR or restriction fragment length polymorphism from PCR products.[90]

TREATMENT

Selection of a most favorable antifungal drug whose spectrum of activity against infecting fungus can proceed only after accurate diagnosis.[1] It is necessary to avoid antimycotic agents in onychopathies which are not onychomycosis. It is logical to reduce the number of infected users by safe and effective treatment and thus reduce the disease prevalence.

Therapeutic options for onychomycosis range from no treatment, palliative care, chemical or mechanical debridement, topical and systemic antifungals to a combination of two or more of these.[92]

Topical therapy:

Indications for only topical therapy include: [93]

- Involvement limited to distal 50% of nail plate
- 3 or 4 nails involvement.
- No involvement of matrix.
- White superficial onychomycosis.
- In children with fast growing and thin nails
- As prophylaxis in patients who are at the risk of recurrence.
- In patients where oral therapy is not advised

Currently used topical antifungal agents include ciclopirox (8%) and amorolfine (5%) lacquers which have a broad action spectrum against dermatophytes, yeasts and NDM. These lacquers are specialized transungual drug delivery systems which produce evaporation of solvent and forms a non-water soluble film that remains in contact with the affected nail for long.[92]

Amorolfine once weekly application is feasible.[94] Ciclopirox is applied to the nail daily. The common side effects of lacquers are transient periungual erythema, burning, bluish or yellow brown discoloration that clears on discontinuing the treatment.[96]

Systemic therapy: Indication for systemic therapy: [93]

- Involvement of >50% of distal nail plate or involvement of multiple nails.
- Nail matrix involvement
- When topical drug penetration is suspected to be suboptimal.

Systemic antifungals to treat onychomycosis include griseofulvin, azoles like fluconazole, itraconazole, ketoconazole and terbinafine.[93]

Griseofulvin: Blocks the formation of mitotic spindle and is effective only against dermatophytes.[97] The dose is 500 mg to 1 g daily for adults. To be administered after a fatty meal. 4–9 months for finger nail onychomycosis and 10–18 months for toenails.[98]

Azoles: Acts by inhibiting ergosterol synthesis.

Ketoconazole is not used recently because severe hepatic side-effects.

Fluconazole: Highly effective against dermatophytes and candida. The cure rates were lower (31%) as compared to itraconazole (61%) and terbinafine (75%).[99] A daily and alternate day 100 mg /day regimen had also been reported to be successful (80%).[100] Higher weekly doses (300–450 mg) were recommended when the isolated agent is a NDM.[101] Side effects include skin rash, headache, insomnia, GI complaints and palpitations.[97]

Itraconazole: Has a broadest spectrum activity against dermatophytes, NDM and Candida. Dose of 200 mg daily, continuously for 12 weeks or 400 mg daily, 1 week per month. It is recommended that two of these weekly pulses, 21 days apart are given for fingernail infections and three pulses for toenail infections.[98] The common adverse effects are headache, gastrointestinal upset and hepatitis.[102]

Terbinafine: Inhibits squalene epoxidase which leads to accumulation of squalene, responsible for its fungicidal effect. Fungicidal against dermatophytes, NDM and C. parapsilosis and fungistatic against C. albicans. FDA approved treatment is 250 mg daily for 6 weeks for fingernail onychomycosis and 12 weeks for toenail onychomycosis.[98]

Treatment regimens for onychomycosis: [103]

	Itraconazole	**Terbinafine**	**Fluconazole**
Toe nails	200mg BD X 1week/month X 3pulses (or) 200mg OD X 3months.	250mg OD X 3months.	150mg once/week X 6months.
Finger nails	200mgBD, 1week/month X 2pulses (or) 200mg OD X 6weeks.	250mg OD X 6weeks.	150mg once/week X 3months.

Newer therapies:

Newer strategies which were developed to enhance penetration of topical drugs include iontophoresis, acid etching, physical modalities like manual and electrical nail abrasion, microporation, laser nail ablation, application of low-

frequency ultrasound, and application of chemicals that have ungual enhancing activity.[104]

The latest treatment for onychomycosis is laser therapy. The efficacy of Noveon dual-wavelength (using 870-nm, 930-nm light) near-infrared diode laser for the treatment of moderate to severe onychomycosis was reported by Landsman et al.[105] The effectiveness of 0.65 millisecond pulsed Nd:YAG 1064-nm laser, femtosecond infrared titanium sapphire lasers had also been reported.[106,107]

Photodynamic therapy has also shown promising results.[108, 109] Dai et al., had demonstrated the result of topical germicidal UVC radiation therapy in the treatment of onychomycosis.[110]

Among the systemic therapies, voriconazole is a new azole antifungal which was found to have low MIC against dermatophytes, NDM and Candida species but the current use of this novel drug is limited to immunocompromised patients, so it is not recommended as a first-line therapy in onychomycosis.[111] Efficacy of ravuconazole 200 mg/day in the treatment of onychomycosis has been reported with variable success.[112]

Combination regimens:

Combination regimes can be administered either as a parallel therapy (both topical and oral drugs are given simultaneously) for patients at risk of failure of therapy, or as sequential therapy (oral drug administration alone followed by topical).[113] Oral drugs take time to develop significant levels, but amorolfine shows its effect within few hours of treatment. Combination of topical and oral drugs may allow reduction in oral dosing which increases patient's compliance and tolerance

improving efficacy and reducing relapse. In Lecha M.'s open randomized trial, 83% and 41% of mycological cure rate was achieved in patients of toenail onychomycosis who received itraconazole and amorolfine lacquer together as compared to itraconazole alone for 12 weeks respectively.[114] Combination with surgical treatments like physical debridement may reduce the fungal load and increases drug penetration.[115]

Sequential therapy:

Sequential therapy combines the use of two or more oral antifungals which act on two different pathways. As a result, duration of the treatment and the cumulative drug exposure to each antifungal can be reduced.[116]

Supplementary therapy:

Microscopic examination and culture at 6 months following initiation of therapy was done. In patients who are found positive, extended administration of oral antifungal was given.[100]

Boosted oral antifungal treatment (BOAT): Following weekly pulse of itraconazole, a piece of SDA (Saboraud's dextrose agar) is placed on the affected nail plate for 48 hours.[116] This is to produce sensitive fungal hyphae which remain less refractory to antifungals. The dormant chlamydospores and arthroconidia are targeted within the nail plate.[100, 115]

Boosted antifungal topical treatment (BATT): This is designed to improve the efficacy of amorolfine nail lacquer.

These therapies carry the risk of over stimulation of the fungi and systemic spread that are non-susceptible to the antifungal agent and therefore not widely accepted.[117]

Surgical intervention**:**

Surgical methods can be used to remove a part (debridement) or a whole (avulsion) of nail plate. Partial removal is the treatment of choice for lateral nail plate involvement. Nails avulsion can be done using a carbon dioxide laser.[118]

Chemical nail avulsion includes application of keratolytic agents like 40% urea to the affected nail. Nail avulsion is usually not indicated in patients with multiple infected nails and the elderly. Apart from avulsion, nail abrasion mechanically by using sandpaper fraises or high speed hand piece before the treatment with antifungal nail lacquer reduces the fungal mass and aids in penetration of topical agent into the deepest nail layers.[100]

Treatment of non-dermatophytes: Prolonged duration of therapy as compared to dermatophytes. Treatment of NDM is often challenging and it may require combination of topical, oral and surgical methods.[119]

Some prognostic factors can determine the choice of therapeutic drug, length of the treatment, and duration of the follow-up and also may help in identifying cases which require extended treatment thereby reducing the chances of relapse/failure:

Poor prognostic factors: [120,121]

- Males
- Age >65 years
- Area of nail involvement >50%
- Poor nail growth
- Subungual hyperkeratosis >2 mm
- Significant lateral disease

- White / yellow / orange / brown streaks in the nail (includes dermatophytoma)
- Total dystrophic onychomycosis (with matrix involvement)
- Nonresponsive organisms (NDM like Scytalidium species)
- Immunosuppressed patients
- Reduced peripheral circulation
- Positive culture at 24 weeks

Strategies to improve cure rate in onychomycosis: [122, 123]

- Ensure proper diagnosis of onychomycosis.
- Choose appropriate antifungal agent
- Ensure compliance
- Monitor drug interactions
- Consider BATT and BOAT
- Consider supplemental therapy, if culture positive at 24 weeks
- Consider surgical avulsion in addition to oral or topical treatments if required.
- Consider combination or sequential therapy
- Educate regarding nail care
- Ensure treatment of contacts if they are also affected.
- Discard old pair of shoes

MATERIAL AND METHODS:

Study group comprised of those presenting with clinical features of onychomycosis to the Department of Dermatology Venereology and Leprosy, Jawaharlal Nehru Medical College, KLES, Dr Prabhakar Kore Hospital and MRC, Belgaum over a period of 1 year from January 2015 to December 2015.

INCLUSION CRITERIA:

All clinical types of onychomycosis irrespective of age and sex, immune status and those who give valid informed consent, presenting to the Department of dermatology, KLE hospital, Belgaum

EXCLUSION CRITERIA:

Patients who had used any topical application, taking systemic antifungal, medicated soap or powder in the past one month.

Study design: Cross sectional study

Sample size (n): 30

$n= Z\alpha^2 pq/d^2 PR$

where $Z\alpha^2$ = 1.96

p (sensitivity) = 62

q (100 - sensitivity) = 38

d = 8

PR (Prevalence) = 5

Ethical clearance:

Prior to the commencement, the ethical clearance was obtained from institutional ethics committee, Jawaharlal Nehru Medical College, Belgaum.

Method of collection of data:

Informed consent:

All the cases of clinical onychomycosis presenting to the Department of Dermatology were included in the study after obtaining informed consent. (Annexure I) For children whose age is less than 18 years, an informed assent from the parents was taken. (Annexure I)

History:

Detailed History with reference to age, sex, occupation, socioeconomic status, personal history, duration of nail involvement, any significant associated illnesses like diabetes mellitus and HIV were noted. Treatment history for onychomycosis, if any, were recorded in a predesigned proforma. (Annexure II)

Clinical examination:

General physical examination and cutaneous examination along with detailed examination of all finger and toe nails were done and documented on a predesigned proforma. (Annexure II)

Records were maintained and analysed statistically.

Specimen collection:

The affected nail was cleaned with 70% alcohol and nail clippings, nail scrapings, shavings and subungual debris were placed in a black sterile paper for easy visualization of the specimens and absorbs the moisture from the specimen which

reduces bacterial contamination. All collected samples were sent to the Department of Microbiology for mycological study and analyzed by direct microscopy and fungal culture.

Potassium hydroxide (KOH) preparation:

The specimen was placed on a clean sterilized glass slide, few drops of 20% KOH are added and then a cover slip was placed over it. The slide was left at room temperature for 24 hours. The specimen was examined under the microscope first under low power (10X) and then under high power (40X) for hyphae and arthrospores.

Culture:

All the samples irrespective of KOH results were subjected to culture on Sabouraud dextrose agar (SDA) with chloramphenicol, and Sabouraud dextrose agar with chloramphenicol + cycloheximide. The specimen was inoculated on to this media and it was incubated at room temperature. They were examined every week for growth of colonies and were discarded after 4 weeks if there was no growth. The colonies were identified on the base of micro and macroscopic characteristics and germ tube tests.

Macroscopic examination of colonies:

Colour, morphology, texture and rate of growth of the colonies were studied.

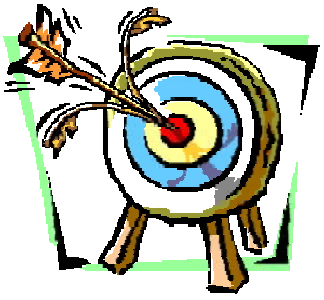
Microscopic examination of the colonies:

Tease mount: A fragment of the colony was teased on a glass slide and subjected to Lactophenol cotton blue staining and then observed under the microscope to identify the strains.

Statistical analysis:

The data obtained was coded onto Microsoft excel spreadsheet (Annexure III). The continuous data was expressed as mean ± standard deviation (SD). The categorical data was expressed as percentages and ratios. Sensitivity analysis method was used.

Introduction



Objectives

Review of Literature

Methodology

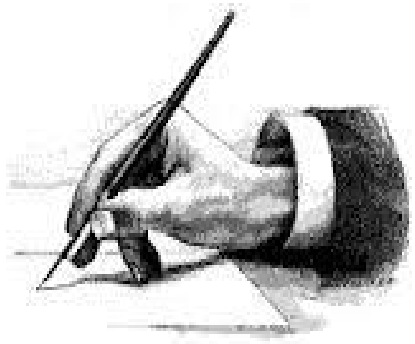
Results

Discussion

Conclusion

Summary

Bibliography



Annexure-I

Annexure-II

Annexure-III

Annexure-IV

Annexure-V

RESULTS

The present one year cross-sectional study was conducted in the Department of Dermatology, Venereology and Leprosy, KLES Dr.Prabhakar Kore Hospital and Medical Research Centre attached to Jawaharlal Nehru Medical College, Belgaum during the period of January 2015 to December 2015.

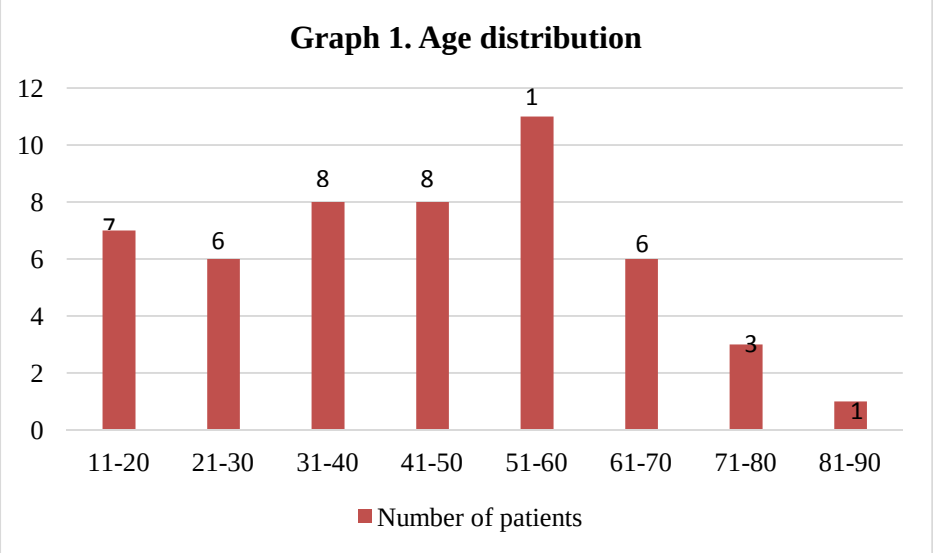
The study comprised of 50 patients presenting with onychomycosis. The data obtained was coded and entered into Microsoft excel spreadsheet and analysed. The results and observations were as below.

Age distribution:

In the present study of 50 patients, the youngest was 12 years old whereas the oldest was 85 years old.

Table 1. Age distribution:

Age group (years)	Number of patients	%
11-20	7	14
21-30	6	12
31-40	8	16
41-50	8	16
51-60	11	22
61-70	6	12
71-80	3	6
81-90	1	2



The above data suggests that out of 50 patients, 7 (14%) patients belonged to the age group of 11-20 years, 6(12%) to 21-30 years, 8(16%) to 31-40 years, 8(16%) to 41-50 years, 11(22%) to 51-60 years, (12%) to 61-70, 3(6%) to 71-80 years and 1(2%) to 81-90 years.

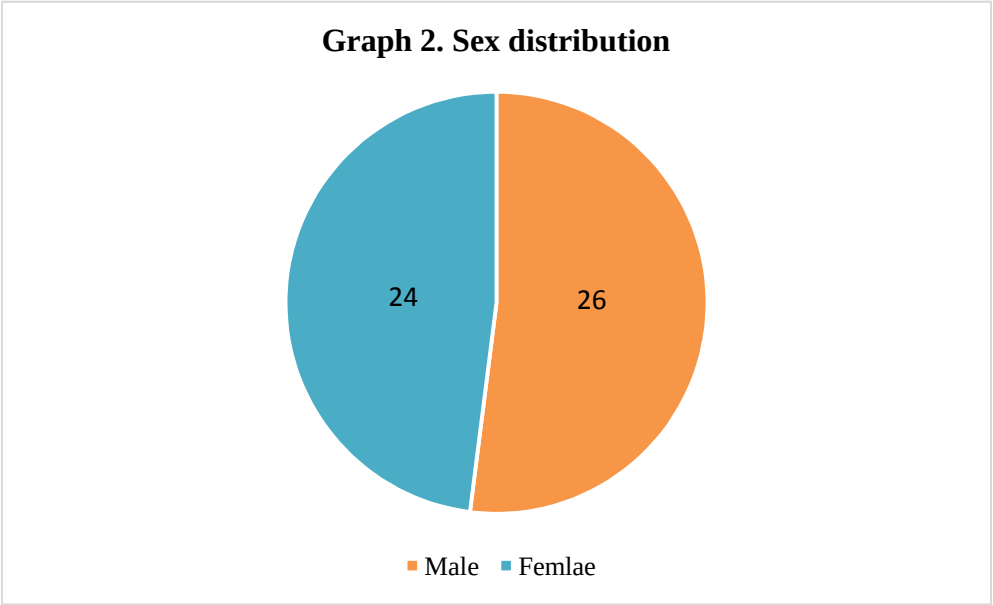
The highest incidence is seen in the age group of 51-60 years (22%) and the lowest incidence is seen in 81-90 years (2%).

Table 2.Sex distribution

In the present study, there were 26 males and 24 females.

Sex	Number of patients	%
Male	26	52
Female	24	48

Graph 2. Sex distribution

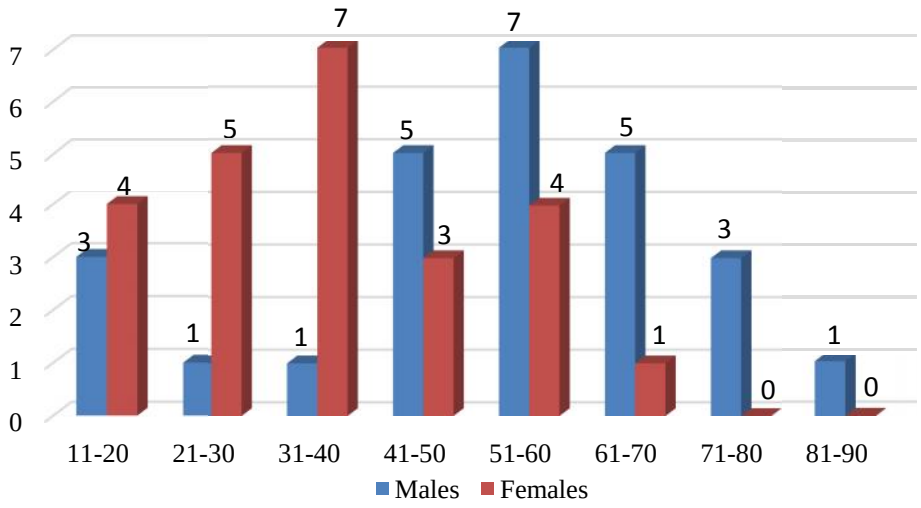


The above date suggests that incidence was higher in males (52%) than females (48%) with a ratio of 0.9230:1

Table 3.Age and Sex distribution

Age in years	Males	Females
11-20	3	4
21-30	1	5
31-40	1	7
41-50	5	3
51-60	7	4
61-70	5	1
71-80	3	0
81-90	1	0

Graph 3. Age and sex distribution



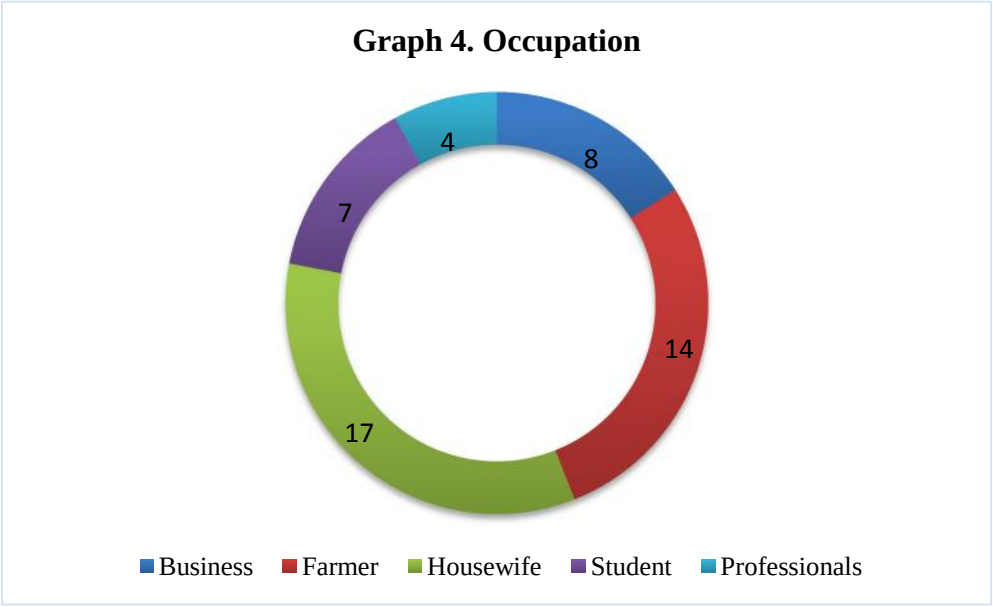
The above data suggests that 3 males and 4 females belong to the age group of (11-20), 1 male and 5 females belong to (21-30), 1 male and 5 females belong to (31-40), 5 males and 3 female belong to (41-50), 7 males and 4 females belong to (51-60), 5 males and 1 female to (61-70), 3 males belong to (71-80), 1 male to (81-90).

The mean age for females± standard deviation was 36.1±14.97 and for males was 52.4±19.

Table 4.Occupation

Occupation	Number of patients	%
Business	8	16
Farmer	14	28
Housewife	17	34
Student	7	14
Professionals	4	8

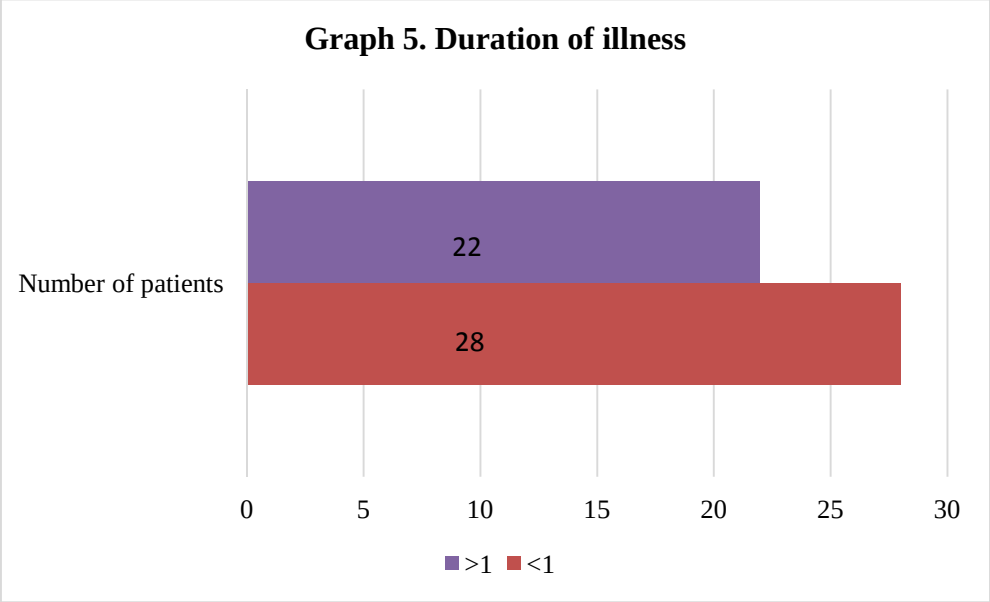
Graph 4. Occupation



Above data suggests that maximum number of patients were housewives 17(34%). Remaining were farmers 14(28%), businessman 8(16%), student 7(14%) and professionals 4(8%)

Table 5.Duration of illness

Duration in years	Number of patients	%
<1	28	56
>1	22	44

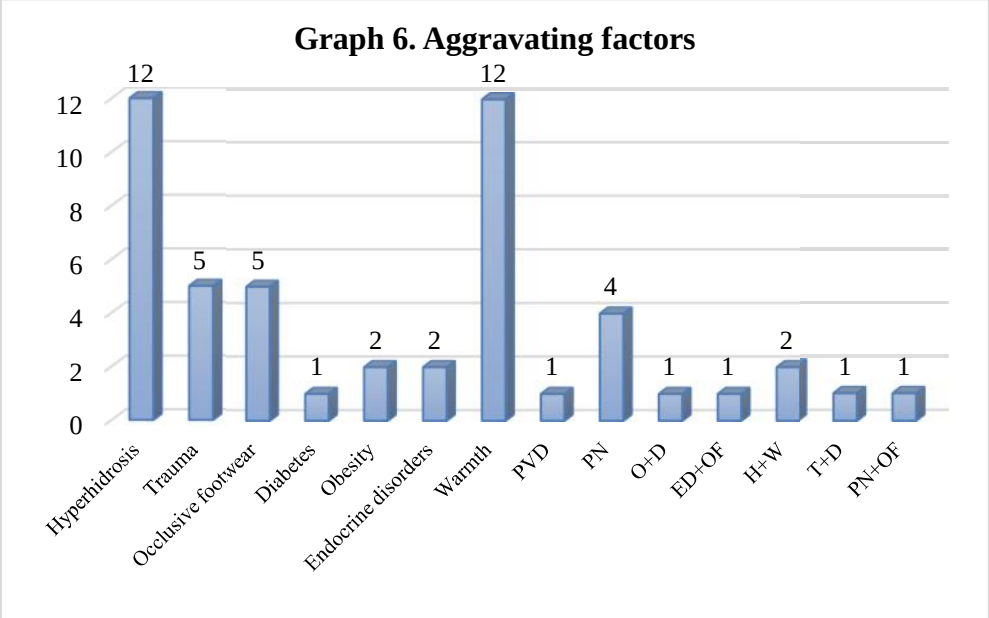


Above data shows that maximum number of cases 28(56%) had duration of <1 year, whereas 22(44%) had duration of >1 year.

Table 6.Aggravating factors

In the present study aggravating factors like hyperhidrosis (H), trauma (T), occlusive footwear (OF), diabetes(D), obesity (O), endocrine disorders (ED), warmth (W), peripheral vascular disease (PVD), (PN) and a combination of factors was observed.

Aggravating factors	Number of patients	%
Hyperhidrosis	12	24
Trauma	5	10
Occlusive footwear	5	10
Diabetes	1	2
Obesity	2	4
Endocrine disorders	2	4
Warmth	12	24
PVD	1	2
PN	4	8
O+D	1	2
ED+OF	1	2
H+W	2	4
T+D	1	2
PN+OF	1	2



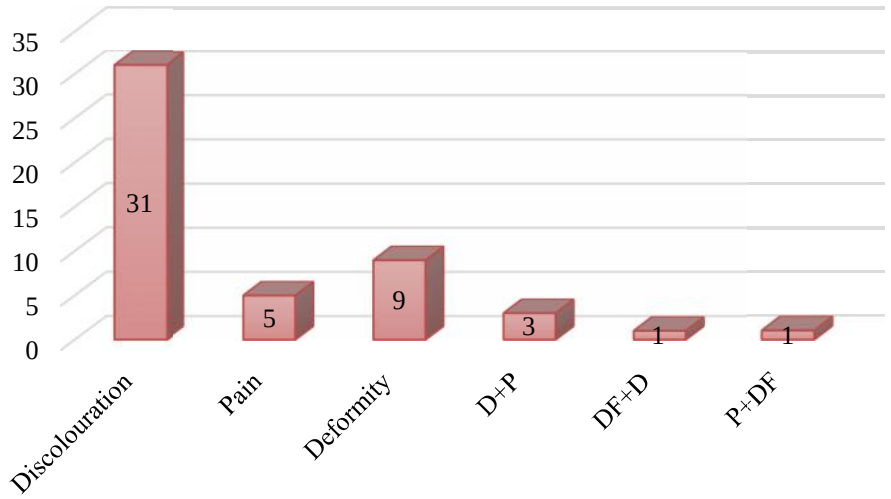
The above data suggests that hyperhidrosis and warmth are common aggravating factors.

Table 7.Presenting complaints:

In the present study patients presented with complaints like discolouration(D), pain(P), deformity(DF), also a combination of symptoms were present.

Presenting complaints	Number of patients	%
Discolouration	31	62
Pain	5	10
Deformity	9	18
D+P	3	6
DF+D	1	2
P+DF	1	2

Graph 7 Presenting complaints

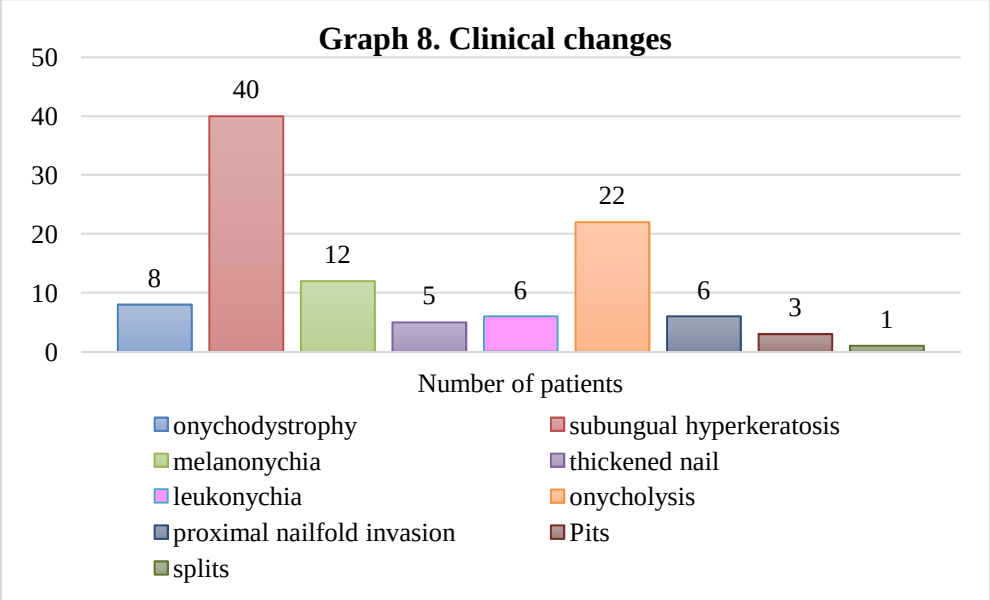


The above data suggests that discolouration of nails 31(62%) is the most common presenting complaint, followed by deformity 9(18) and pain 5(10%).

Table 8. Clinical changes

In the present study various clinical changes like onychodystrophy (OD), subungual hyperkeratosis (SH), melanonychia (M), thickened nail (TN), leukonychia (L), onycholysis (O), proximal nail fold invasion (PNF), pits(P), splits (S) were present.

Clinical change	Number of patients	%
Onychodystrophy	8	16
Subungual hyperkeratosis	40	80
Melanonychia	12	24
Thickened nail	5	10
Leukonychia	6	12
Onycholysis	22	44
Proximal nailfold invasion	6	12
Pits	3	6
Splits	1	2

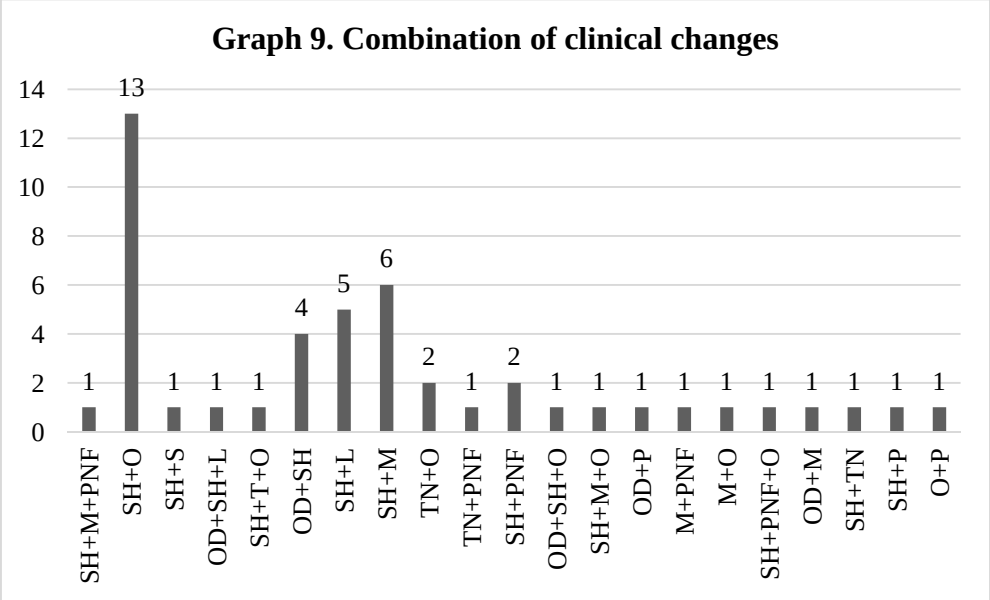


The above data suggests that, maximum number of patients presented with subungual hypertrophy 40(80%), followed by onycholysis 22(44%).

Table 9.Combination of clinical changes

In the present study various clinical changes like onychodystrophy (OD), subungual hyperkeratosis (SH), melanonychia (M), thickened nail (TN), leukonychia (L), onycholysis (O), proximal nailfold invasion (PNF), pits(P), splits (S) and a combination of various changes was observed.

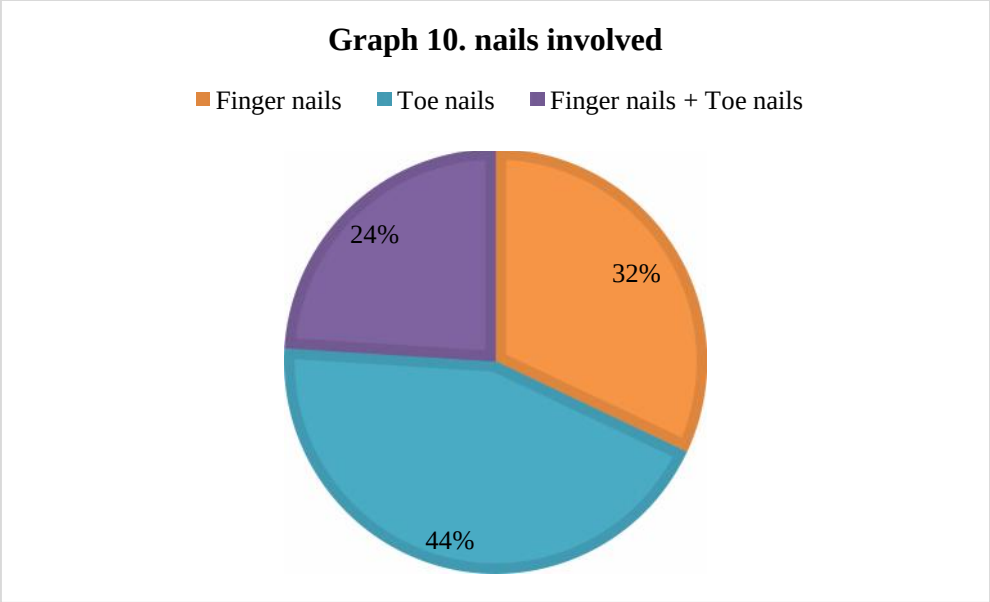
Clinical changes	Number of patients	%
SH+M+PNF	1	2
SH+O	13	26
SH+S	1	2
OD+SH+L	1	2
SH+T+O	1	2
OD+SH	4	8
SH+L	5	10
SH+M	6	12
TN+O	2	4
TN+PNF	1	2
SH+PNF	2	4
OD+SH+O	1	2
SH+M+O	1	2
OD+P	1	2
M+PNF	1	2
M+O	1	2
SH+PNF+O	1	2
OD+M	1	2
SH+TN	1	2
SH+P	1	2
O+P	1	2



In the present study, SH+O 13(26%) are the most common clinical change seen.

Table 10.Nails involved:

Nails involved	Number of patients	%
Finger nails	16	32
Toe nails	22	44
Finger nails + Toe nails	12	24

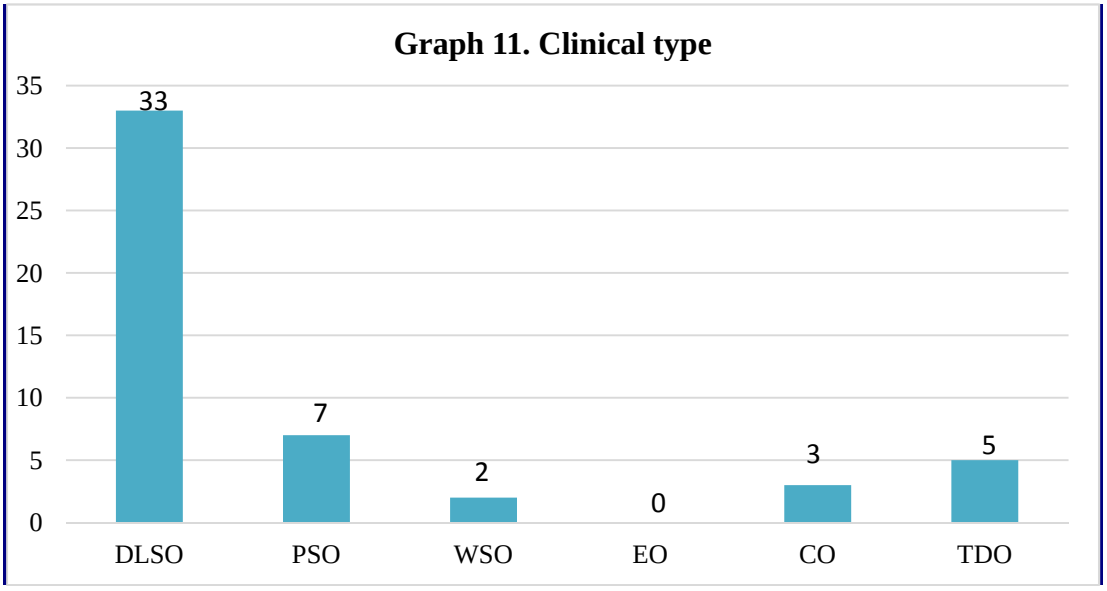


The above data suggests that toe nails 22(44%) are most commonly involved, followed by finger nails in 16(32%) patients and toe nails + finer nails in 12(24%).

Table 11.Clinical types of onychomycosis:

Different clinical types like distolateralsunungualonychomysosis (DLSO), proximal sunungualonychomysosis (PSO), white superficial onychomycosis (WSO), endonyxonychomycosis (EO), candidialonychomycosis (CO) and total dystrophic onychomycosis (TDO) were studied here.

Clinical type	Number of patients	%
DLSO	33	66
PSO	7	14
WSO	2	4
EO	0	0
CO	3	6
TDO	5	10



In the present study the most common clinical type of onychomycosis is DLSO(66%) is seen in 33 patients, the second most common is PSO(14%) seen in 7 patients, followed by TDOin 5(10%), COin 3(6%), WSOin 2(4%).

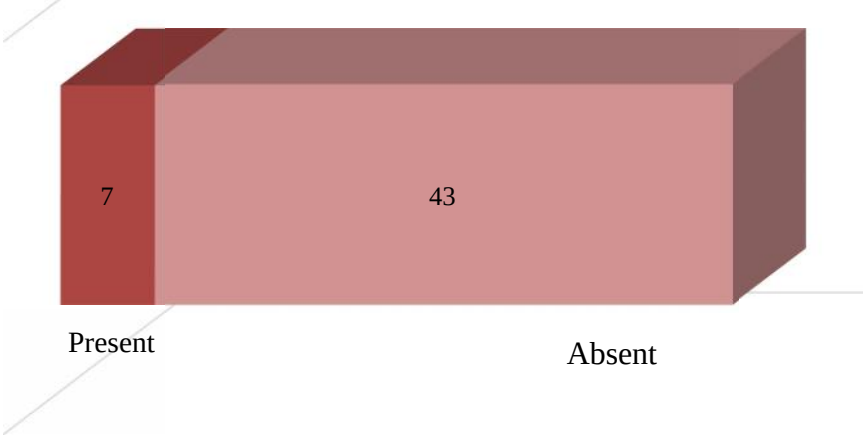
EO was not found in the present study.

Table 12.Associated fungal infections:

Associated fungal infections like tineacorporis (T.corporis), tineacruris (T.cruris), tineapedis (T.pedis), tineamannum (T.mannum) are present.

Associated fungal infections	Number of patients	%
Present	7	14
Absent	43	86

Graph 12. Associated fungal infections

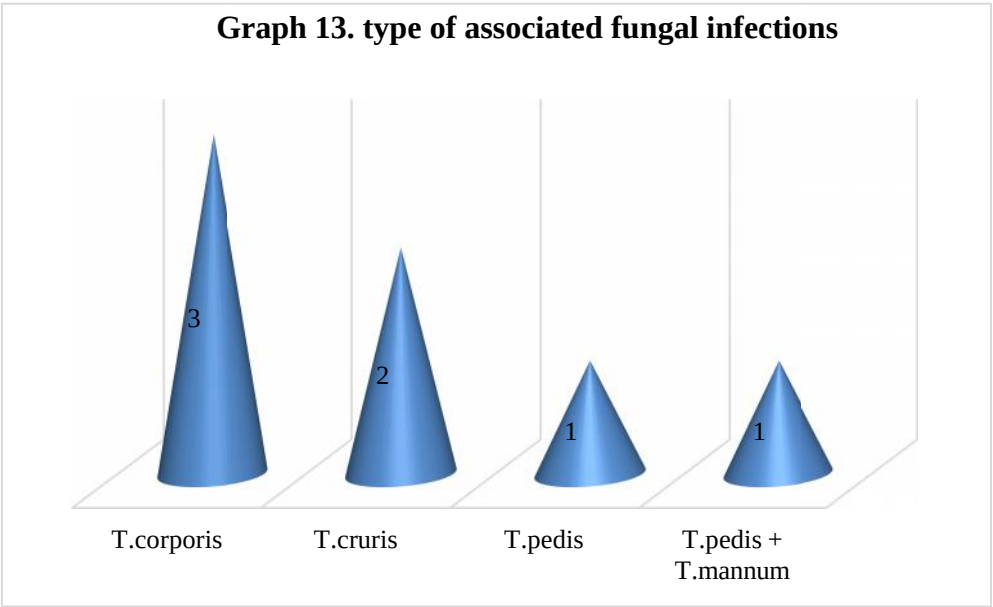


In the present study maximum number of patients i.e., 43(86%) had no associated fungal infections and 7(14%) had associated fungal infections.

Table 13. Type of associated fungal infections

Type of associated fungal infections	Number of patients	%
T.corporis	3	6
T.cruris	2	4
T.pedis	1	2
T.pedis + T.mannum	1	2

Graph 13. type of associated fungal infections

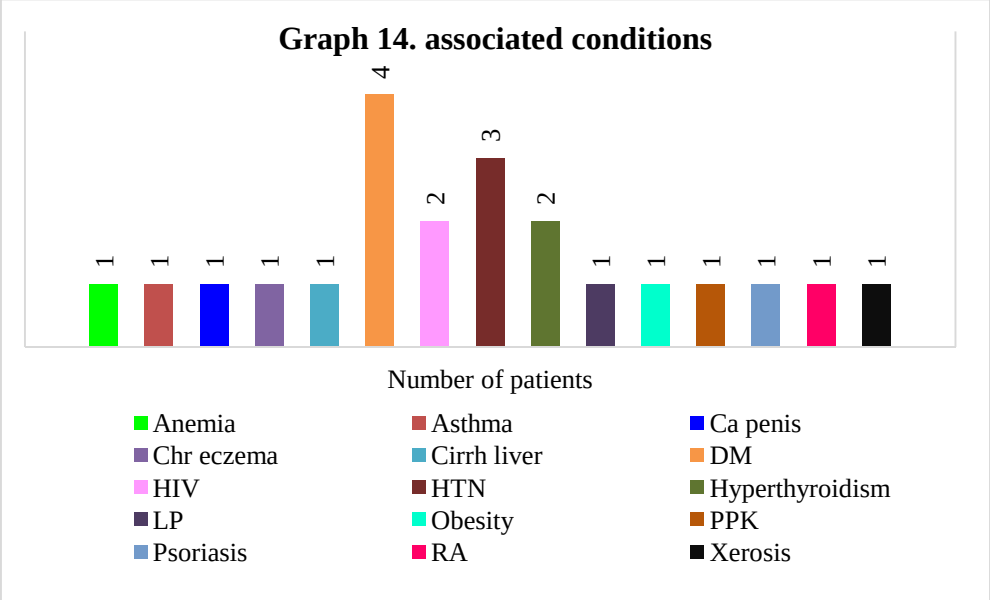


The above data suggests that the most common associated fungal infection is T.corporis, seen in 3(6%) patients, followed by T.cruris in 2(4%), T.pedis in 1(2%), T.pedis+T.mannum in 1(2%).

Table 14.Associated conditions:

In the present study associated conditions like anemia, asthma, carcinoma penis(ca penis), chronic eczema(chr eczema), cirrhosis of liver(cirrh liver), diabetes mellitus(DM), HIV, hypertension(HTN), hypothyroidism, lichen planus(LP), obesity, palmoplantarkeratoderma(PPK), psoriasis, rheumatoid arthritis(RA), xerosis are present.

Associated conditions	Number of patients	%
Anemia	1	2
Asthma	1	2
Ca penis	1	2
Chr eczema	1	2
Cirrh liver	1	2
DM	4	8
HIV	2	4
HTN	3	6
Hyperthyroidism	2	4
LP	1	2
Obesity	1	2
PPK	1	2
Psoriasis	1	2
RA	1	2
Xerosis	1	2



The above data suggests that DM (8%) was the most common associated condition.

Table 15.KOH and culture positivity:

Diagnostic test	Number of patients	%
KOH	25	50
culture	46	92

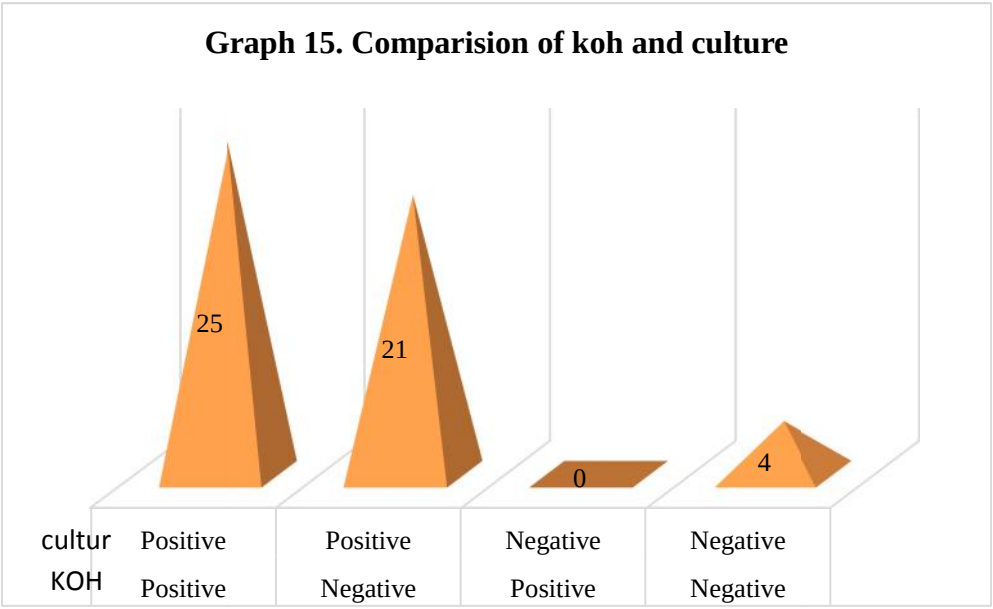
In the present study KOH positivity was recorded in 50% of cases and culture positivity in 92% of cases.

Out of 50 cases studied 46(92%) cases showed presence of fungus (either by KOH or/and culture).

Table 16.Comparision of KOH and culture:

KOH	Culture	Number of patients	%
Positive	Positive	25	50
Negative	Positive	21	42
Positive	Negative	0	0
Negative	Negative	4	8

Graph 15. Comparision of koh and culture



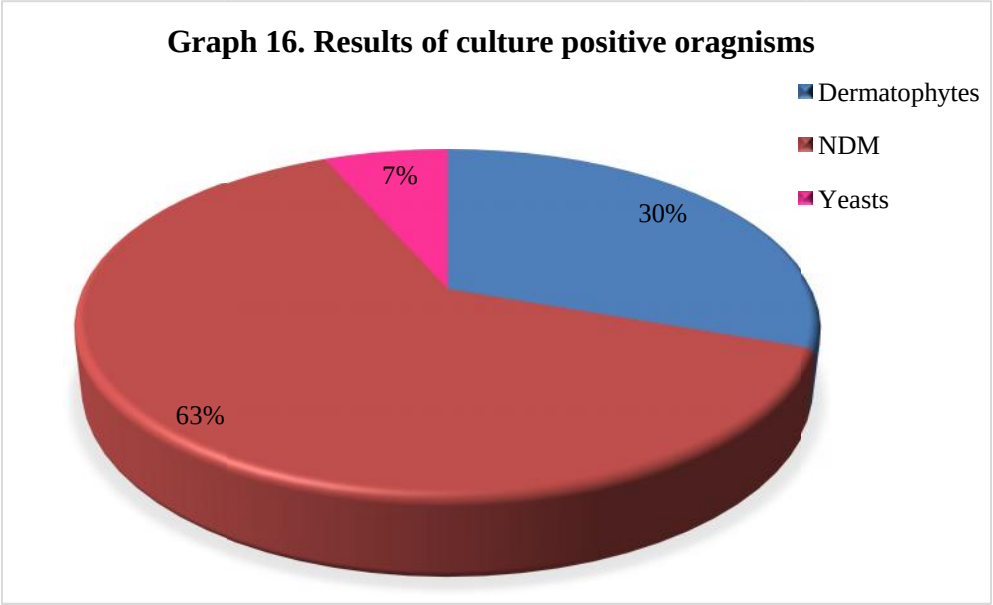
The above data suggests that both KOH and culture positive were found in 25(50%) of patients, only culture positive with KOH negative in 21(42%), both are negative in 4(8%).

False negative cases were 21(42%) with KOH, but there no false negative cases with culture.

Table 17.Results of culture positive organisms:

Fungus (n=46)	Number of specimens	%
Dermatophytes	14	30
NDM	29	63
Yeasts	3	6

Graph 16. Results of culture positive oragnisms

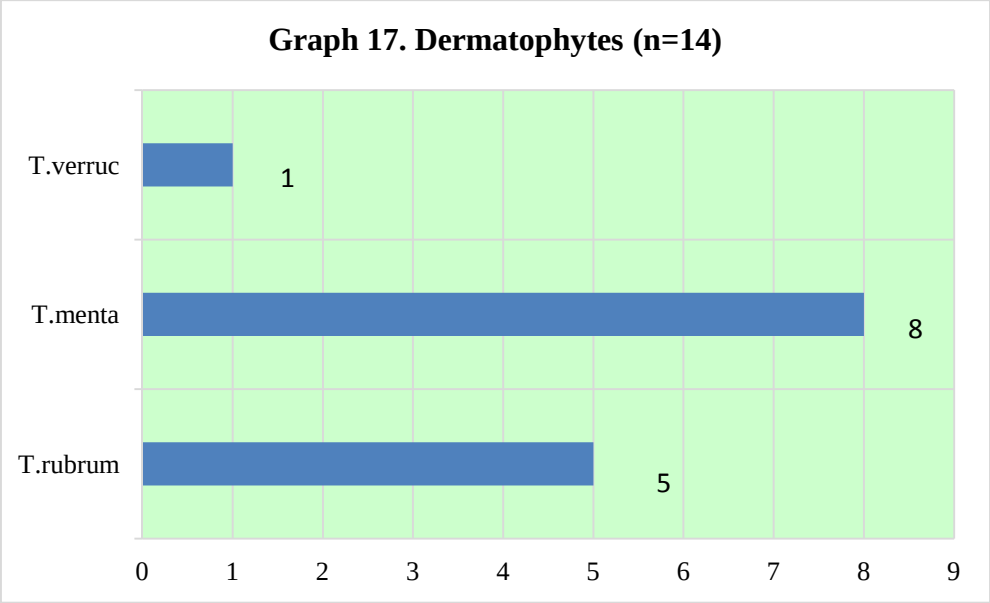


In the present study out of 46 culture positive specimens, the maximum were NDM (63%), followed by dermatophytes (30%) and the least were yeasts (7%).

Table 18.Frequency of dermatophytes isolated:

Dermatophyte species like Trichophyton rubrum (T.rubrum), Trichophyton mentagrophytes (T.menta) and Trichophytonverrucosum (T.verruc) were isolated.

Fungus	Number (n=14)	%
T.rubrum	5	35
T.menta	8	57
T.verruc	1	7



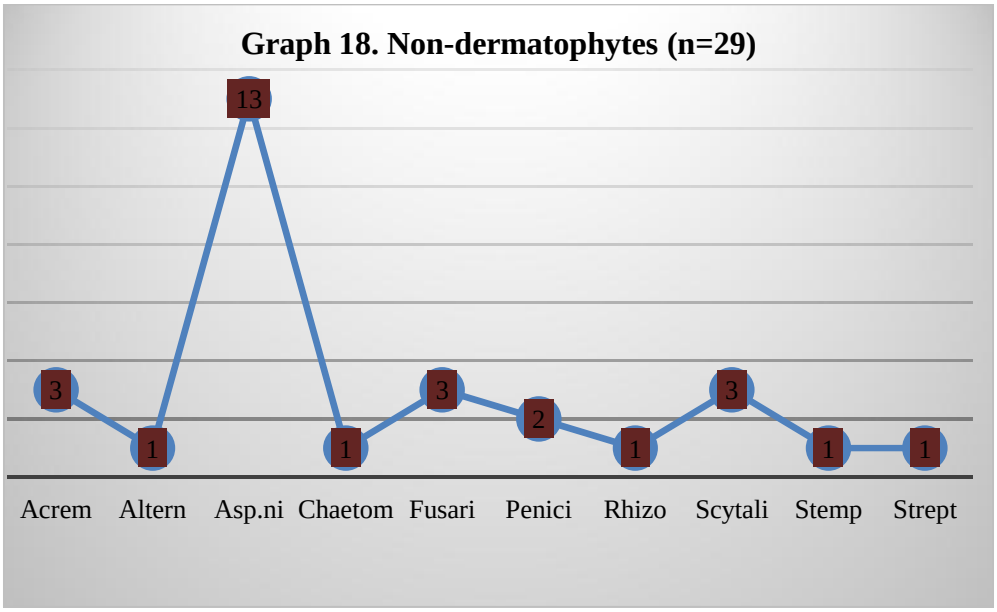
The above data suggests that the most common dermatophyte isolated is Trichophyton mentagrophytes (8)

Frequency of NDM isolated:

Table 19. Frequency of NDM isolated:

In the present study non dermatophytic moulds(NDM) like Aspergillusniger (Asp.ni), Acremonium sp.(Acrem), Alternaria sp.(Altern), Chaetamonium sp.(Chaetom), Fusarium sp.(Fusari), Penicillium sp.(Penici), Rhizopus sp.(Rhizo), Scytalidium sp.(Scytali), Stemphyllium sp.(Stemp), Streptomyces sp.(Strept) were isolated.

NDM (n=29)	Number of specimens	%
Acrem	3	10
Altern	1	3
Asp.ni	13	44
Chaetom	1	3
Fusari	3	10
Penici	2	6
Rhizo	1	3
Scytali	3	10
Stemp	1	3
Strept	1	3



The above data suggests that the most common NDM isolated is Aspergillu sniger 13(44%).

Aspergillus niger was the most common fungal pathogen isolated in the present study.

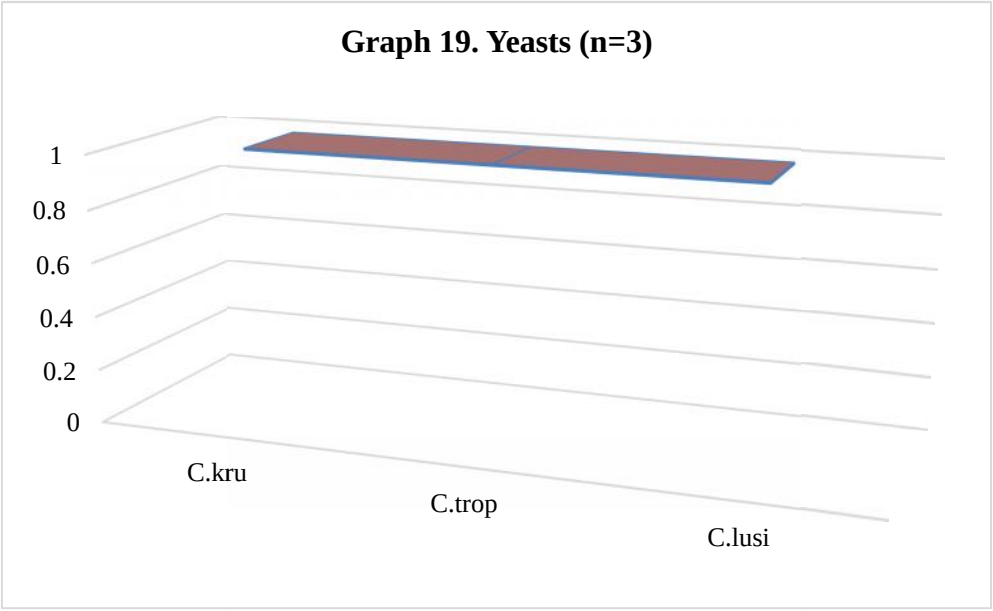
Other common NDM isolated were Acremonium species (3), fusarium species (10) and scytalidium species (10)

Table 20.Frequency of yeasts isolated:

In the present study yeasts like Candida kruzei(C.kru), Candida tropicalis(C.trop), Candida lusitanea(C.lusi), were isolated.

Yeasts (n=3)	Number of specimens	%
C.kru	1	33
C.trop	1	33
C.lusi	1	33

Graph 19. Yeasts (n=3)

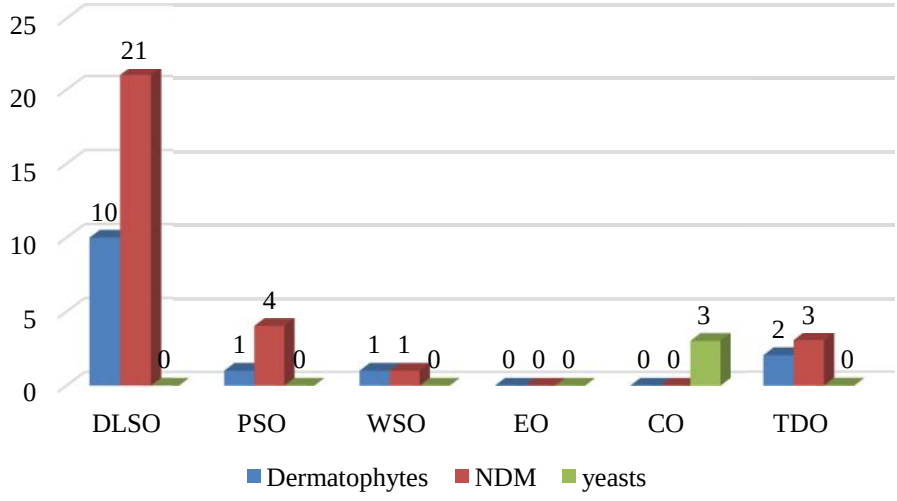


In the present study three candida species (1 each) were isolated i.e., Candida kruzei(C.kru), Candida tropicalis(C.trop), Candida lusitanea(C.lusi).

Table 21.Distribution of various fungal species:

Clinical type	Dermatophytes	NDM	Yeasts	Total
DLSO	10	21	0	31
PSO	1	4	0	5
WSO	1	1	0	2
EO	0	0	0	0
CO	0	0	3	3
TDO	2	3	0	5
Total	14	29	3	46

Graph 20. Distribution of various fungal species



The above data suggests that DLSO, PSO and TDO are most commonly caused by non-dermatophytic moulds.

In our culture positive specimens of DLSO in 31 cases, NDM dominated (21 cases) followed by dermatophytes (10 cases).

Out of 5 culture positive cases of PSO, NDM dominated (4 cases), followed by dermatophytes (1 case).

Out of 2 culture positive cases of WSO, 1 was caused by NDM and 1 was caused by dermatophyte.

Out of 5 culture positive cases of TDO, NDM dominated (3 cases) followed by dermatophytes (2 cases)

DISCUSSION

In the present study 50 clinically suspected cases of onychomycosis were studied during the period of January 2015 to December 2015.

Onychomycosis and age:

In the present study, onychomycosis was seen to affect almost all age groups ranging from 12 to 85 years. The highest incidence was seen in the age group of 51-60 years. There were mixed reports about the prevalence of onychomycosis in different age groups. Some authors reported highest prevalence in the age group of 20-40 years, [124, 125, 126] while others have reported highest prevalence above 55 years of age.[127, 128]

In the present study the lowest incidence was seen in 81-90 years. The actual incidence of onychomycosis may be higher in elderly in India, but the disease being usually asymptomatic and the elderly being dependent upon others for medical and social help, they may not be presenting to the hospital.[129]

In the present study there are no children less than 10 years old. Prevalence of onychomycosis among children has been reported to be low (0.44%).[129] The reason for the low prevalence of onychomycosis in children may be attributed to rapid growth rate of nail plate with subsequent elimination of fungi, difference in structure of nail plate and lack of cumulative trauma.[125]

Onychomycosis and sex prevalence:

In the present study higher incidence was seen in males (52%) than females (48%) with a ratio of 1.08:1.

Name of the study	**Male : female ratio**
Grover 2003[127]	1.63:1
Ahmed.et al 2010[129]	2.5:1
Reddy.et al 2012[131]	1.85:1
Saroj.G.et al 2012[130]	1.4:1
Present study	1.08:1

The higher incidence in males could be attributed to occupation related subclinical trauma and occlusive footwear. Lower incidence of onychomycosis among females as compared to males may be more apparent than real because of underreporting.[128]

Onychomycosis and occupation:

In the present study, maximum number of patients were housewives 17(34%) similar to the study of Reddy et. al. 2012 (26.6%) and this may be associated with constant trauma associated with wet work. The second highest number of patients were farmers who usually work with their cows to milk them.[1]

Duration of infection:

In the present study, maximum number of cases 28(56%) had duration of illness <1 year, as seen in the study done by Neupane.et al 2009 (65% had duration <1year).[132] Most of the other studies (Sujatha V.et al 2000, Jesudanam.et al 2002.) also showed duration of illness <1year.[133, 134]

Precipitating factors:

In the present study, hyperhidrosis, warmth, occlusive footwear and trauma were the common predisposing factors observed. Almost similar predisposing factors were observed in other studies by Neupane.et al 2009, Reddy.et al 2012.[131, 132]

Presenting complaints:

In the present study discolouration of nails 31(62%) was the most common presenting complaint, followed by deformity 9(18) and pain 5(10%). In a similar study done by Reddy.et al 2012, out of 60 patients of onychomycosis, all of them (100%) showed discoloration of nails.

Clinical changes:

In the present study, maximum number of patients presented with subungual hypertrophy 40(80%), followed by onycholysis 22(44%). A similar finding was seen in Reddy.et al's study (93% of patients showed subungual hypertrophy) and Ramesh V.et al 1982's study (95.5%).[126]

Nails involved:

In the present study, toe nails 22(44%) were most commonly involved, followed by finger nails in 16(32%) patients and, both toe nails and finger nails in 12(24%). A higher incidence of onychomycosis of toe nails have been reported by various authors (Gupta.et al 1997).[135] The reason for this could be, in India most of the people walk barefoot.

But most of the studies (Sujatha V.et al 2000:finger nails-48.57%, toe nails-31.43%; Reddy.et al 2012: finger nails- 55%, toe nails- 40%) showed more finger nail

involvement than toe nails.[131,133] This may be because of the patients concern, driving them to seek medical treatment.

Clinical type of onychomycosis:

High incidence of DLSO pattern was seen in various studies (Sujatha V.et al 2000(90.57%), Grover 2003(41%), Neupane.et al 2009(80%).[127, 132, 133] Similarly, in the present study, most common clinical type of onychomycosis observed was DLSO, seen in 33(66%) patients.

In the present study, the second most common type was PSO seen in 7 patients (14%). Among these 7 patients with PSO (a marker for HIV), 2 patients were positive for HIV antibodies. In the areas of high HIV endemicity, onychomycosis is recognised as a marker of HIV infection.[131]

EO was not found in the present study. This may be attributed to underreporting due to absence of signs of inflammation in the nail bed like onycholysis or subungual hyperkeratosis and presence of normal nail plate.

In the present study, CO is seen in 3(6%). Candida onychomycosis was observed in studies like, Neupane.et al 2009(14.2%), Ramesh v.et al 1982(8.88%), Reddy.et al 2012(20%).[126, 131, 132]

Associated fungal infections:

In the present study maximum number of patients i.e., 43(86%) had no associated fungal infections and 7(14%) had associated fungal infections. The most common associated fungal infection is T.corporis, seen in 3(6%) patients, followed by T.cruris in 2(4%), T.pedis in 1(2%), T.pedis+T.mannum in 1(2%). Similar findings

were observed in Ramesh.et al 1982's study (T.pedis and T.mannum each in 10% of patients).[131] Many patients selected in our study came with presenting complaints of skin infection whose nails changes were observed and noted.

Other associated conditions:

In the present study DM (8%) was the most common associated condition similar to Gulcan A et al. 2011.[136] Diabetes as a common associated condition with onychomycosis was also mentioned in Kaur.et al 2008, Reddy.et al 2012, Ahmad.et al 2010, Das.et al 2010. [58, 129, 131, 137]

KOH and culture:

In the present study out of 50 cases studied 46(92%) cases showed presence of fungus (either by KOH or/and culture). Other studies from different geographical locations showed even lower isolation rates. 51.76% in Das.et al 2010, 82.2% in Sujatha.et al 2000, 63.3% in Gupta.et al 1997, 45.53% in Jesudanam.et al. 2002.[133, 134, 135, 137] This shows that, diagnosis of onychomycosis solely based on clinical features is often misleading.

Comparison of KOH and culture:

In the present study sensitivity of KOH against culture was 54.3% and specificity was 100%.

False negative cases were 21(42%) with KOH, but there was no false negative cases with culture.

In the present study KOH positivity was recorded in 50% of cases and culture positivity in 92% of cases.

Study	KOH positivity (%)	Culture positivity (%)
Ramesh V.et al. 1982[126]	100	68.8
Sujatha V.et al.2000[133]	77.14	60
Das.et al 2010[137]	63.64	95.45
Grover 2003[127]	55.9	62.7
Present study	50	92

The higher incidence of culture positivity in the present study may be attributed to many factors:

- The scrapings were cultured irrespective of KOH report.
- Low visibility of scattered and scanty fungal filaments on KOH examination.
- The scrapings were cultured and sub cultured more than once in some cases.
- The samples were collected from the area close to the nail matrix where the fungus is in most viable form.
- The samples were collected in black sterile paper which absorbs the moisture and reduces the rate of contamination. This produces pure cultures in the isolates.

So direct microscopy should always be coupled with culture for accurate diagnosis and species identification.

Distribution of various fungi:

In the present study, out of 46 culture positive specimens, the maximum were NDM (63%), followed by dermatophytes (30%) and the least were yeasts (7%).

Study	Dermatophytes (%)	NDM (%)	Yeasts (%)
Reddy.et al[131] 2012	70	10	20
Grover 2003[127]	23.7	22	16.8
Vijaya. D.et al 2004[138]	39.02	12.19	90.47
Saroj .G.et al 2012[130]	54.8	22	23.3
Present study	30	63	7

Most of the studies showed predominance of dermatophytes in contrast to the present study.

Aspergillus niger was the most common fungal pathogen isolated in the present study (44%). Similar to the present study, Sujatha V.et al 2000, (47.6%) showed highest isolation rate of Aspergillus niger. This higher incidence of Aspergillus niger may not be significant as they are ubiquitous in nature and common contaminants in cultures.[139]

Banerjee.et al.1990, from India, reported a higher isolation rate of dermatophytes in their study in contrast to the present study.[128] Achten G.et al.1978, reported higher isolation rate of candida (56.74%) in their study in contrast to the present study.[7]

In the present study, most common dermatophyte isolated is Trichophyton mentagrophytes (8). Scytalidium species, which causes onychomycosis not only in previously damaged nails, but can act as primary pathogen, is isolated in the present study. Not many cases of Scytalidium species infecting the nail are reported so far.[140]

Other NDM like Acremonium sp.(Acrem), Alternaria sp.(Altern), Chaetamonium sp.(Chaetom), Fusarium sp.(Fusari), Penicillium sp.(Penici), Rhizopus sp.(Rhizo), Scytalidium sp.(Scytali), Stemphyllium sp.(Stemp), Streptomyces sp.(Strept) were also isolated in the present study in contrast to other studies where Aspergillus species are most commonly isolated. (Grover 2003, Reddy.et al 2012).[127, 131]

CONCLUSION

A detailed clinical and mycological study was done in 50 cases of onychomycosis presenting to the Department of dermatology, KLE hospital and MRC, Belgaum.

To conclude our study revealed:

- Commonest age group was 51-60 years (22%) with male preponderance (52%).
- The maximum number of patients were housewives (34%) and most of them presented with in 1 year (56%).
- Hyperhidrosis and warmth were common aggravating factors.
- Discolouration (62%) of nails was the most common presenting complaint.
- Subungual hypertrophy (80%) and onycholysis (40%) were the most common clinical changes observed.
- Toe nails (44%) were most commonly involved.
- DLSO (66%) was the most common morphological pattern observed.
- Tinea corporis (6%) and diabetes mellitus (8%) were the most common associated conditions.
- Isolation rate of fungus was 92% (46 out of 50).
- The higher incidence of culture positivity (92%) than KOH positivity (50%) was observed.
- Out of 46 culture positive specimens, the maximum were NDM (63%).
- The most common NDM isolated was Aspergillus niger (13).

- The most common dermatophyte isolated was Trichophyton mentagrophyte (8).
- Yeasts like Candida kruzei (C.kru), Candida tropicalis (C.trop), Candida lusitanea (C.lusi), were isolated.
- DLSO, PSO and TDO were most commonly caused by NDM.

Overall there was wide variation in the clinical and mycological profile of onychomycosis. Further KOH examination for fungus and culture play an important role in the diagnosis of dermatophytosis.

SUMMARY

The present one year cross-sectional study was done in KLES, Dr Prabhakar Kore Hospital and MRC, Belgaum from January 2015 to December 2015. A total of 50 patients presenting with clinical features of onychomycosis were subjected to KOH examination and culture for fungi.

Commonest age group was 51-60 years (22%) with male preponderance (52%). The maximum number of patients were housewives (34%) and most of them presented with in 1 year (56%). Hyperhidrosis and warmth are common aggravating factors. Discolouration (62%) of nails was the most common presenting complaint. Subungual hypertrophy (80%) and onycholysis (40%) were the most common clinical changes observed. Toe nails (44%) were most commonly involved. DLSO (66%) is the most common morphological pattern observed. Tinea corporis (6%) and diabetes mellitus (8%) were the most common associated conditions. Isolation rate of fungus was 92% (46 out of 50). The higher incidence of culture positivity (92%) than KOH positivity (50%) was observed. Out of 46 culture positive specimens, the maximum were NDM (63%). The most common NDM isolated was Aspergillus niger (13). The most common dermatophyte isolated was Trichophyton mentagrophyte (8). Yeasts like Candida kruzei (C.kru), Candida tropicalis (C.trop), Candida lusitanea (C.lusi), were isolated. DLSO, PSO and TDO were most commonly caused by NDM.

The present study highlights the necessity of microbiological confirmation in case of onychomycosis and also stresses that diagnosis of onychomycosis solely based on clinical features is often misleading. Culture was considered the gold standard for the diagnosis of onychomycosis. It is cheaper and routinely available test.

Monitoring the incidence of the fungal species enables the detection of emerging organisms and may be helpful in the treatment and adequacy of current pharmacologic regimens. In this study current knowledge of pathogenesis, diagnosis and treatment of onychomycosis with newer modalities was reviewed.

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ANNEXURE I

INFORMED CONSENT FORM

I.D.NO. | | | |

A ONE YEAR CROSS-SECTIONAL CLINICO-MYCOLOGICAL STUDY OF ONYCHOMYCOSIS IN KLE DR.PRABHAKAR KORE HOSPITAL & MRC, BELGAUM

The study is conducted by _____ Post graduate student in M.D Dermatology under guidance of _____ Dermatology, J N Medical College, Belgaum.

Respected Sir/Madam, we invite you to participate in our study as, you are eligible for the same. During the study you will be asked some questions in detail regarding your present complaints.

Purpose of the study:

The purpose of this study is to find out the prevalence and etiological agent of Onychomycosis . You are being asked to participate in this research because you have been diagnosed to have Onychomycosis. All patients attending the outpatient department, who are diagnosed to have this disease, will be requested to participate in this study during the period of one year. Use of appropriate diagnostic techniques including direct microscopy and fungal culture is important to ensure correct diagnosis and treatment of onychomycosis for better results.

Procedure and treatment:

1. Should you choose to participate, you will be asked to give a detailed history of your disease, undergo a physical examination, and consent to a few routine investigations.

2. Samples will include nail clippings and subungual debris. All collected samples will be analyzed by direct microscopy and fungal culture.

Risks and benefits:

You may undergo some amount of discomfort during the process of investigations, which may include slight pain. However all necessary steps and precautions will be taken to ensure your safety. The result of you taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care

Alternatives:

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease.

Privacy and confidentiality:

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

Relations with the Institutional policy:

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project.

Financial incentives:

You shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results:

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will be maintained confidential at all times.

Voluntary participation:

Your participation in this study is voluntary. Your decision whether or not to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. . In the event if you suffer any physical injury as the result of your participation in this study, you may contact

In case you need further information regarding your rights as a study participant, you may please contact Dr. Ganga.S.Pillai, chairman of the ethical committee, J N Medical College, Belgaum.

STATEMENT OF CONSENT:

I.D.NO: | | | |

I Mr/Ms/Mrs _____

Volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept to participate in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participant's name:

Signature or left thumb print of participant:

Witness name:

Signature of witness:

Signature of the investigator:

Date:

ANNEXURE I

INFORMED ASSENT FORM

I.D.NO. | | | |

A ONE YEAR CROSS-SECTIONAL CLINICO-MYCOLOGICAL STUDY OF ONYCHOMYCOSIS IN KLE DR.PRABHAKAR KORE HOSPITAL & MRC, BELGAUM

The study is conducted by _____ Post graduate student in M.D Dermatology under guidance of Dr. B.SIDDRAMAPPA$_{MD}$., Professor of Dermatology, J N Medical College, Belgaum.

Respected Sir/Madam, we invite your child to participate in our study as, your child is eligible for the same. During the study you will be asked some questions in detail regarding your child's present complaints.

Purpose of the study:

The purpose of this study is to find out the prevalence and etiological agent of Onychomycosis. Your child is being asked to participate in this research because your child has been diagnosed to have Onychomycosis. All patients attending the outpatient department, who are diagnosed to have this disease, will be requested to participate in this study during the period of one year. Use of appropriate diagnostic techniques including direct microscopy and fungal culture is important to ensure correct diagnosis and treatment of onychomycosis for better results.

Procedure and treatment:

1. You will be asked to give a detailed history of your child's disease, let us undergo a physical examination, and consent to a few routine investigations.

2. Samples will include nail clippings and subungual debris. All collected samples will be analyzed by direct microscopy and fungal culture.

Risks and benefits:

Your child may undergo some amount of discomfort during the process of investigations, which may include slight pain. However all necessary steps and precautions will be taken to ensure your child's safety. The result of your child taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care

Alternatives:

Your child will still be receiving the usual standard care for the disease even if your child do not participate in this study,

Privacy and confidentiality:

Your child's privacy will be respected and all information collected about your child during the course of this study will be kept confidential. Your child's identity will remain undisclosed.

Relations with the Institutional policy:

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project.

Financial incentives:

Your child shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results:

The results of this study may be published for scientific purpose or presented to a scientific group. Your child's identity, however, will be maintained confidential at all times.

Voluntary participation:

Your child's participation in this study is voluntary. Your decision whether or not to let your child participate will neither affect the care of your child's current disease, nor your future relations with the doctor or the hospital. . In the event if your child suffers any physical injury as the result of participation in this study, you may contact.

In case you need further information regarding your child's rights as a study participant, you may please contact Dr. Ganga.S.Pillai, chairman of the ethical committee, J N Medical College, Belgaum.

Statement of Consent:

I.D.NO: | | | |

My child Mr/Ms _____

Volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept my child to participate in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participant's name:

Participant's Father/ Mother: Name:

Signature or left thumb print of participant's Father/ Mother:

Witness name:

Signature of witness:

Guardian's name:

Guardian's signature:

Signature of the investigator:

Date:

ANNEXURE - II

PROFORMA

ONE YEAR CROSS-SECTIONAL CLINICO-MYCOLOGICAL STUDY OF ONYCHOMYCOSIS IN KLES DR PRABHAKAR KORE HOSPITAL AND MRC, BELGAUM.

Case No. ☐☐ **OP/IP No.** ☐☐☐☐☐☐☐

Name: First name ☐☐☐☐☐☐ Middle name ☐☐☐☐☐☐☐

Last name ☐☐☐☐☐☐

Age: ☐☐

Sex:

1. Male
2. Female

☐

Occupation:

1. Business
2. Housewife
3. Professional
4. Farmer
5. Student
6. Any other

☐

Income: Monthly income (In Rs.):

1. Above poverty line 2. Below poverty line

☐

Address with phone number:

Presenting complaints and duration:

History of present illness:

Onset:

Acute ☐ Insidious ☐

Factors initiating the disease: season others

Associated skin diseases (if any):

Fungal infection on any other site in the body:

Precipitating Factor (IF ANY):

1. Hyperhidrosis: ☐
2. Trauma: ☐
3. Personal Hygiene: ☐
4. Poor nutrition: ☐
5. Contact with person suffering from fungal infections : ☐
6. Warm, humid climate: ☐
7. Obese: ☐
8. Diabetes: ☐

9. Endocrine disorder: ☐

10. Topical applications: ☐

11. Common bathrooms: ☐

12. Peripheral vascular disease: ☐

13. Sporting Activities: ☐

14. Concomitant Fungal Infections: ☐

15. Occlusive footwear: ☐

Past History:

History suggestive of similar illness: ☐

1. Present
2. Absent

History of any other medical disorders: _____

Family History:

Personal History:

Diet

1. Veg
2. Mixed ☐

Appetite ☐

1. Normal
2. Poor

Bowel/ Bladder ☐

1. Normal
2. Altered

Sleep

1. Normal
2. Altered ☐

Alcohol

1. Present
2. Absent ☐

Smoking

1. Present
2. Absent ☐

Treatment History

Oral antifungal 1-On treatment ☐

2-Not on treatment

Topical antifungal 1-On treatment ☐

2-Not on treatment

Alternative Treatment if any ☐

CLINICAL EXAMINATION OF NAILS:

SITE OF LESION:

Finger nail involvement:

Toe nail involvement:

INSPECTION:

- Onychodystrophy ☐
- Subungual hyperkeratosis ☐
- Melanonychia ☐
- Thickening of nail plate ☐
- Leukonychia ☐
- Marginal thickening ☐
- Onycholysis ☐
- Proximal nail fold invasion ☐
- Pits ☐
- Lamellar splits ☐

SECONDARY INFECTION

1. Present
2. Absent

INFLAMMATION

1. Present
2. Absent

DISCHARGE

1. Present
2. Absent

SYMMETRY

1. Present
2. Absent

ODOUR

1. Present
2. Absent

PAIN

1. Present
2. Absent

PALPATION:

- Surface texture of nail:
- Tenderness:

General physical examination:

Built

1. Poor
2. Moderate
3. Good

☐

Vitals ☐☐☐

Pulse / min

☐☐☐

BP(mm/hg): Systolic

Diastolic ☐☐☐

Temperature ^{0}F ☐☐☐

Weight(Kg) ☐

Pallor ☐

1. Present
2. Absent

Icterus ☐

1. Present
2. Absent

Cyanosis ☐

1. Present
2. Absent

Clubbing ☐

1. Present
2. Absent

Lymph nodes ☐

1. Palpable
2. Non palpable

Edema ☐

1. Pitting
2. Non Pitting
3. Absent

Systemic Examination:

Cardiovascular system: Heart sounds

1. Normal ☐
2. Abnormal; if abnormal specify the finding_____

Respiratory system: Breath sounds

1. Normal ☐
2. Abnormal; if abnormal specify the finding_____

Per abdomen:

1. Normal ☐
2. Abnormal; if abnormal specify the finding_____

Central nervous system: Neurological examination

1. Normal ☐
2. Abnormal; if abnormal specify the finding_____

Investigations:

KOH:

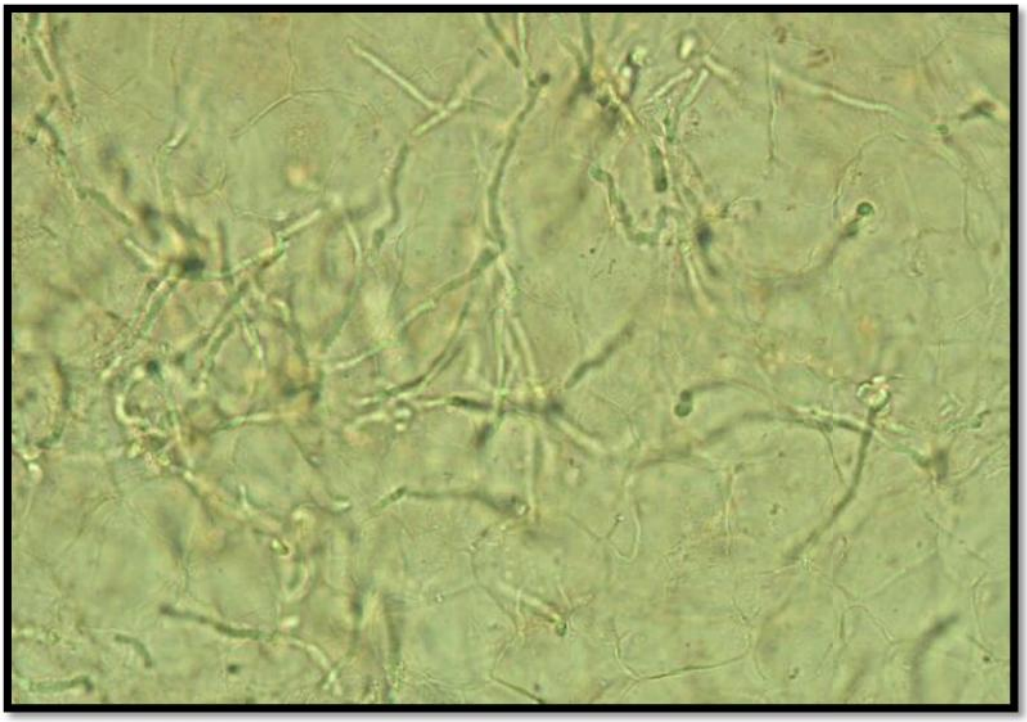
FUNGAL CULTURE:

DIAGNOSIS:-

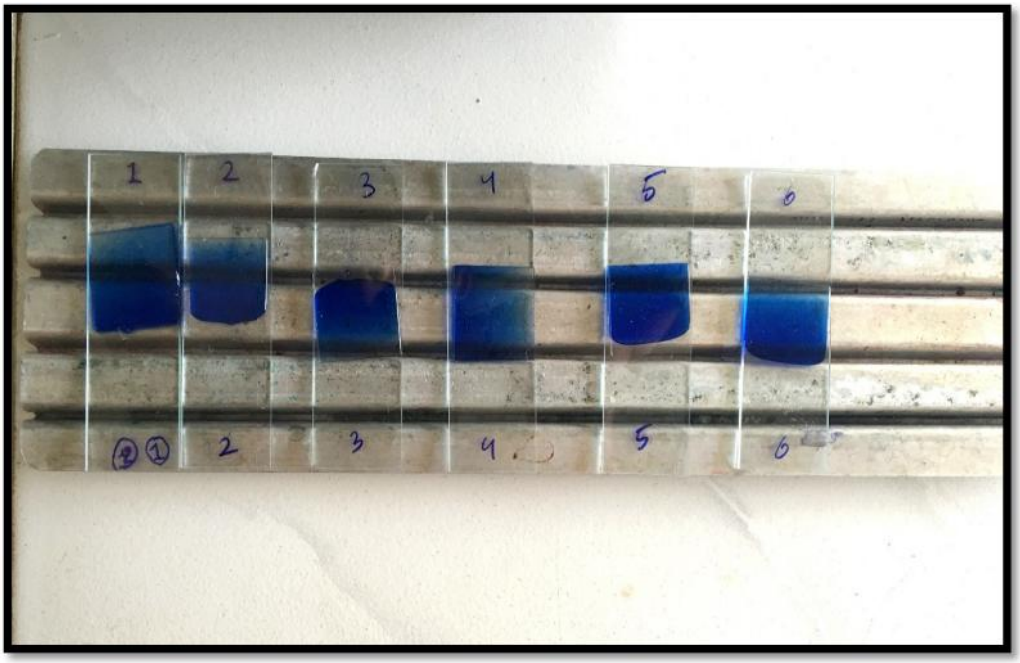
Signature:

Guide's Signature:

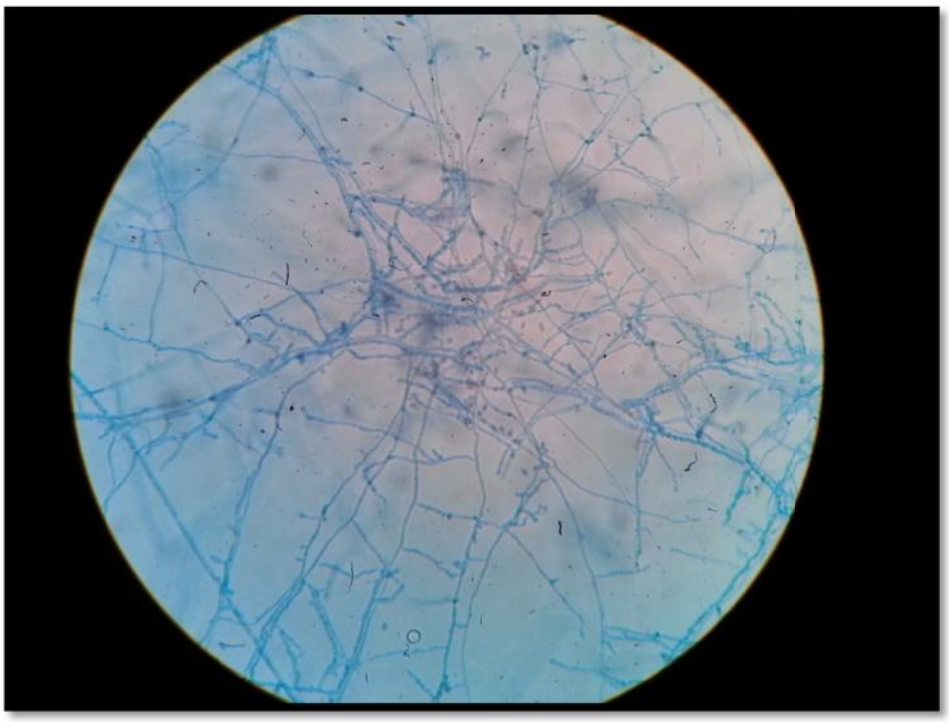
ANNEXURE III – PHOTOGRAPHS



Photograph 1. Branched fungal hyphae on KOH examination.



Photograph 2. LCB preparation from different colonies



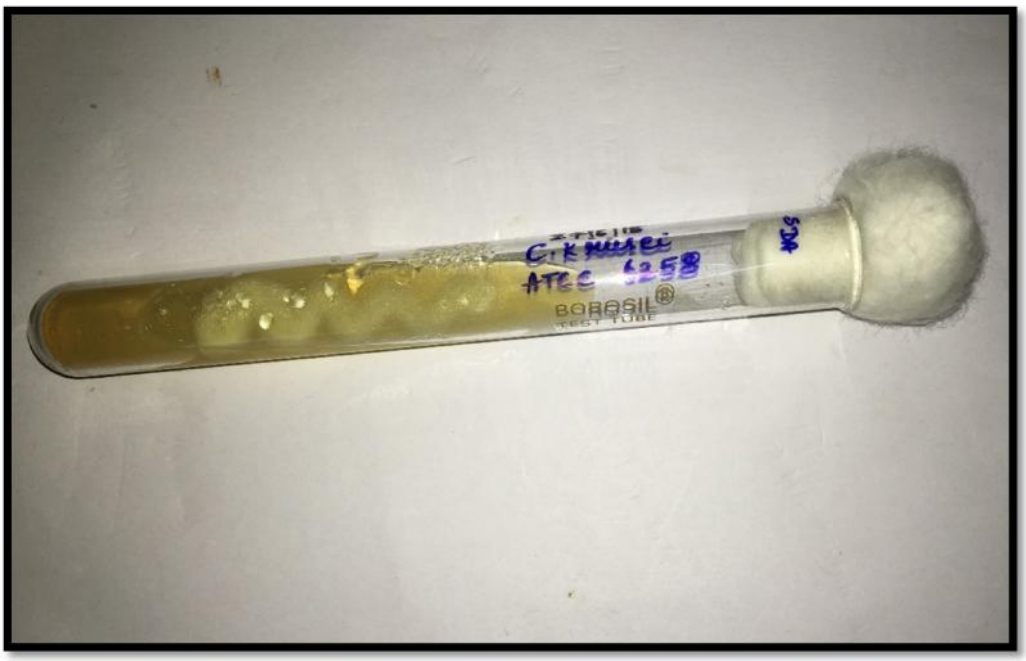
Photograph 3. LCB preparation of T.rubrum -Tear shaped microconidia

Photograph 4. LCB preparation of T.mentagrophytes - Spiral hyphae of T.mentagrophytes

Photograph 5. LCB preparation of Asp.niger - Black colour phialide of Aspergillus niger

Photograph 6. T.mentagrophytes colony on SDA – White cottony growth

Photograph 7. Asp. Niger growth on SDA– Black velvety growth



Photograph 8. C.kruzei colony on SDA – White creamy growth

Photograph 9. Disto-lateral subungual onychomycosis

Photograph 10. Proximal subungual onychomycosis

Photograph 11. White superficial onychomycosis

Photograph 12. Candidial onychomycosis

Photograph 13. Total dystrophic onychomycosis

Photograph 14. Onychomycosis with Tinea mannum

Photograph 15. Onychomycosis with Tinea pedis

Photograph 16. Onychomycosis with T.pedis with T.mannum - Involvement of two feet and one hand

Sl No	AGE	SEX	OCCUPATION	DURATION (MONTHS)	PRESENTING C/O NAILS	AGGREVATING FACTORS	CLINICAL CHANGES	NAILS	CLINICAL TYPE	ASSOCIATED FUNGAL INFECTION	ASSOCIATED CONDITIONS	KOH	CULTURE
1	20	F	Student	7	D	W	SH	F	DLSO	-	-	Positive	Asp.Ni
2	20	M	Farmer	8	D	W	SH+M+PNF	F	PSO	-	Asthma	Positive	Asp.Ni
3	70	M	Farmer	24	D+P	W	SH+O	T	DLSO	-	Xerosis	Negative	T.Ment
4	50	M	Business	12	D	H	SH+O	F+T	DLSO	-	Chronic eczema	Negative	Asp.Ni
5	63	M	Farmer	63	D+P	T+D	SH+S	T	WSO	-	Diabetes mellitus	positive	Acrem
6	38	F	House wife	2	P	H	SH+O	F+T	DLSO	-	Lichen planus	positive	Acrem
7	58	M	Farmer	24	D	O	OD+SH+L	F+T	TDO	T.Pedis+T.Mannum	Obesity	Positive	T.Rubr
8	50	M	Farmer	2	P	T	SH+T+O	T	DLSO	-	Hypertension	Positive	T.Rubr
9	55	F	House wife	12	D	W	OD+SH	F	DLSO	-	-	Negative	Negative
10	32	F	House wife	24	D	H	OD+SH	F	TDO	-	-	Positive	Chaetom
11	69	M	Farmer	4	D+P	W+D	SH+L	F+T	DLSO	-	Diabetes mellitus	Negative	Strept
12	56	F	House wife	16	DF	H	SH+O	T	PSO	-	-	Negative	T.Rubr
13	54	F	House wife	6	DF	H	ODSH	F	DLSO	-	-	Negative	T.Ment
14	34	F	House wife	20	D	W	SH+M	T	DLSO	-	-	Positive	Asp.Ni
15	50	F	House wife	12	D	T	SH+M	F	DLSO	-	-	Negative	T.Ment
16	49	F	House wife	8	D	ED	SH+L	F+T	DLSO	-	Hypothyroidism	Negative	T.Rubr
17	51	M	Business	12	DF	H	SH+O	T	PSO	T.Pedis	-	Negative	Negative
18	22	F	House wife	3	D	H	SH+M	F+T	DLSO	T.Corporis	-	Negative	Negative
19	72	M	Professional	8	p	PVD	SH+L	F	DLSO	-	Hypertension	Negative	Fusari
20	53	M	Business	9	DF+D	OF	TN+O	T	DLSO	-	-	Negative	Asp.Ni
21	50	F	House wife	2	P	T	TN+PNF	F	CO	-	-	Positive	C.Kru
22	68	M	Farmer	3	D	D	SH+L	T	CO	-	Diabetes mellitus	Positive	C.Trop
23	35	F	House wife	8	D	H	SH+O	T	DLSO	-	-	Positive	Asp.Ni

24	24	M	Business	9	D	O+D	SH+PNF	T	CO	-	Diabetes mellitus	Negative	C.Lusi
25	46	M	Farmer	36	D	H+W	SH+PNF	T	PSO	-	-	Positive	Asp.Ni
26	55	M	Professional	24	P+DF	ED+OF	OD+SH+O	F+T	TDO	-	Cirrhosis of liver	Negative	Sytali
27	13	F	Student	8	D	PN	O	F	DLSO	-	Anemia	Negative	T.Ment
28	12	M	Student	3	D	OF	SH+M+O	F	DLSO	-	-	Positive	Asp.Ni
29	85	M	Farmer	9	D	H	OD+P	F+T	TDO	-	Psoriasis	Positive	T.Ment
30	55	M	Business	4	D	OF	SH+M	T	DLSO	-	-	Positive	Asp.Ni
31	71	M	Farmer	24	D	PN	M+PNF	F+T	PSO	T.Corporis	HIV	Negative	Asp.Ni
32	23	F	Student	2	D	PN+OF	SH+O	T	DLSO	-	-	Negative	Fusari
33	20	F	Student	16	D	W	SH+M	T	PSO	-	-	Positive	Asp.Ni
34	53	M	Farmer	7	DF	W	SH+O	F	DLSO	-	-	Negative	Asp.Ni
35	32	F	House wife	2	D	T	M+O	F	DLSO	-	-	Positive	Rhizo
36	28	F	House wife	12	D	PN	M	T	PSO	T.Cruris	HIV	Negative	Negative
37	55	F	House wife	11	D	H	SH+O	T	DLSO	-	-	Positive	Asp.Ni
38	13	F	Student	13	D	W	SH+O	F	DLSO	-	-	Negative	Altern
39	14	M	Student	12	DF	W	SH+O	F+T	DLSO	-	-	Negative	T.Rubr
40	70	M	Farmer	12	D	ED	SH+M	T	DLSO	-	Hypothyroidism	Positive	Fusari
41	39	F	House wife	24	DF	W	SH+L	F	DLSO	-	-	Positive	T.Ment
42	65	F	Business	18	DF	H	OD+SH	T	DLSO	-	-	Positive	T.Ment
43	72	M	Farmer	10	D	PN	SH+PNF+O	F+T	TDO	-	Ca penis	Negative	Sytali
44	35	F	Business	3	DF	T	OD+M	T	DLSO	-	Hypertension	Positive	T.Verruc
45	43	M	Professional	14	D	OF	SH+TN	T	DLSO	-	-	Positive	Stemph
46	22	F	House wife	24	D	W	TN+O	F	DLSO	-	PPK	Negative	Penici
47	58	M	Business	6	DF	O	SH+P	F+T	DLSO	-	Rheumatoid arthritis	Positive	Penici
48	50	M	Farmer	6	D	H	SH+O	F	WSO	T.Corporis	-	Positive	T.Ment
49	32	M	Professional	2	D	OF	SH+O	T	DLSO	-	-	Negative	Acrem
50	28	F	House wife	6	P	W	O+P	T	DLSO	T.Cruris	-	Negative	Sytali

MASTER CHART KEY

1. Sex:

   Male - M

   Female - F

2. Presenting complaints of nails:

   Deformity - DF

   Discolouration - D

   Pain - P

3. Aggravating factors:

   Hyperhidrosis - H

   Trauma - T

   Occlusive footwear - OF

   Diabetes - D

   Obesity - O

   Endocrine disorders - ED

   Warmth - W

4. Clinical changes:

   Onychodystrophy - OD

   Subungual hyperkeratosis - SH

   Melonychia - M

   Thickened nail plate - TN

   Leukonychia - L

   Onycholysis - O

   Proximal nail fold invasion - PNF

Pits - P

Splits - S

5. Nails affected :

   Finger nails - F

   Toe nails - T

6. Clinical type of onychomycosis:

   Disto-lateral sunungual onychomysosis - DLSO

   Proximal sunungual onychomysosis - PSO

   White superficial onychomycosis - WSO

   Endonyx onychomycosis - EO

   Candidial onychomycosis - CO

   Total dystrophic onychomycosis - TDO

7. Associated fungal infections:

   Tinea cruris - T.cruris

   Tinea pedis - T.pedis

   Tinea mannum - T. Mannum

   Tinea corporis - T.corporis

8. Culture:

   Aspergillus niger - Asp.ni

   Trichophyton mentagrophytes - T.men

   Trichophyton rubrum - T.rub

   Trichophyton verrucosum - T. Ver

   Chaetomonium species - Chaet

   Streptomyces species - Strept

   Acremonium species - Acre

Fusarium species - Fusa

Candida lusitaniae - C.lust

Candida krusei - C.kruz

Candida tropicalis - C.trop

Sytalidium species - Sytal

Stemphillium species - Stemp

Penicillium species - Penici

Rhizopus species - Rhizo