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THE CAUSATIVE PATHOGENS OF TOENAILS ONYCHOMYOSIS IN AMMAN, JORDAN

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ABSTRACT

Abu Khadijeh F The causative Pathogens of toenails onychomycosis in Amman, Jordan ,NearEast University Institute of Health Sciences, M.Sc. Thesis in Medical Microbiology andClinical Microbiology Programme, Nicosia, 2020.

Onychomycosis is one of the most common types of infections in humans. It is seenbetween 20-25% in the world and it is one of the most common diseases . Affect thenails by 40% and is gradually increasing in developing countries. Althoughonychomycosis can affect everyone, some factors increase the likelihood ofinfection.. Treatment of such infections is expensive and takes a long time. This studywas conducted to determine the causative pathogens of toenails onychomycosis inAmman, A total of 300 study specimens were collected by convenient samplingtechniques. By using standard structured questionnaire socio-demographic data wascollected. Samples were isolated and identified by using KOH microscopicexamination and cultured on Sabouraud agar. Data were analyzed using SPSS andp-value was found as <0.05.Through 300 samples with ages from 1 to 77 years, theage group of (51_60) was the most prevalent. Non-Dermatophytes was the mostcommon fungus isolated with ratio 39.0% followed by Dermatophytes 33.0% andyeast with ratio 28.0%. Aspergillus spp is the most common species isolated fromnon-dermatophytes, while Trichophyton spp were among the dermatophytes andfrom the yeast Candida spp were isolated. The prevalence of fungal onychomycosisinfections seems to be related to age, sex and lifestyle. An exact diagnosis ofonychomycosis is based on direct microscopic examination and mycological culture.Our study shows that non-dermatophytes were the most common agents causing

onychomycosis

Keywords: Fungal disease, Onychomycosis, fungal, nail disease, culture

ÖZET

Abu Khadijeh F Ürdün, Amman'da ayak tırnaklarında onikomikoz etkeni patojenler. Yakın Doğu Üniversitesi Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı. Yüksek Lisans tezi Lefkoşa, 2020 .

Onikomikoz, insanlarda en yaygın enfeksiyon türlerinden biridir. Dünyada% 20-25 arasında görülür ve en yaygın hastalıklardan biridir. Tırnakları% 40 etkiler ve gelişmekte olan ülkelerde giderek artmaktadır. Onikomikoz herkesi etkileyebilse de, bazı faktörler enfeksiyon olasılığını arttırır ve bu tür enfeksiyonların tedavisi pahalıdır ve uzun zaman alır.. Bu çalışma Ürdün Amman'da ayak tırnaklarında onikomikoza neden olan patojenlerş belirlemek için yapıldı.Toplam 300 örnek uygun yöntemlerle toplandı. Standart yapılandırılmış anket kullanılarak sosyo-demografik veriler elde edildi. Numuneler KOH mikroskobik inceleme kullanılarak tanımlanmış ve Sabouraud agara ekildi. Veriler SPSS ve P değeri <0.05 kullanılarak analiz edildi. Yaşları 1 ile 77 arasında değişen 300 örnekten 51-60yaş grubu en yaygın olanıdır. Dermatofitler% 39.0 ile en sık izole edilen mantar olup bunu% 33.0 ile Dermatofit ve% 28.0 ile maya olarak değerlendirildi. Aspergillus spp, dermatofit olmayanlardan izole edilen en yaygın türken, Trichophyton Spp dermatofitler arasında ve maya olarak Candida spp izole edilmiştir. Onikomikoz enfeksiyonlarının prevalansı yaş, cinsiyet ve yaşam tarzı ile ilişkili görünmektedir. Onikomikozun kesin tanısı, doğrudan mikroskobik incelemeye ve mikolojik kültüre dayanır. çalışma, dermatofit olmayanların onikomikoza neden olan en fazla ajan olduğunu göstermektedir

Anahtar Kelimeler: Mantar hastalığı, Onikomikoz, mantar, tırnak hastalığı

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SYMBOL AND ABBREVIATIONS

CMC	Chronic mucocutaneous candidiasis
CW	Calcofluor under fluorescence
DSLO	Distal lateral subungual onychomycosis
EO	Endonyx onychomycosis
HIV	Human Immunodeficiency Virus
KOH	Potassium hydroxide
LASER	Light Amplification by Stimulated Emission of Radiation
LPCB	Lactophenol Cotton Blue
MIC	Minimum inhibitory concentration
NDM	Non-dermatophytes mould
PCR	polymerase chain reaction
PSO	Proximal subungual onychomycosis
REP-PCR	Repetitive Estrogenic Palindromic Sequence Polymerase Chain Reaction
SDA	Sabouraud's dextrose agar
SPP	Species
SWO	Superficial white onychomycosis
TDO	Totally dystrophic onychomycosis
USA	United States of America
SPSS	Statistical Package for the Social Sciences

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CHAPTER ONE: INTRODUCTION

1.1 Introduction

1.1.1 General Information

The onychomycosis is derived from the Greek word**"onyx"** meaning a nail and **"mykes"** a fungus. (Kaur R et a,.2008).

Infection of the nail unit name as onychomycosis, representing the foremost common nail disorder and accounting for five hundredth all nail diseases. Therefore, it's oftentimes encountered by physicians as a chief grievance, secondary concern, or an incidental finding seen on clinical examination. sadly, several patients area unit mismanaged, as physicians habitually treat onychomycosis through empirical observation, incorrectly basic cognitive process that they make the designation supported history and clinical examination alone (Lipner S.R et a,.2016). fungal infection of the nail that can be the causal agent by dermatophytes, yeasts or other non-dermatophytes moulds (NDMs) (Jennings et al., 2002) It possible to affect any part of the nail unit , like nail bed , nail matrix, or nail plate (Zaias, 1990).
Ungual onyxis" if the infection is by yeast-like fungi, or "ungula candidiasis" denominate if the infection is by fungi of the genus Candida. Ungual onyxis" if the infection is by yeast-like fungi, or "ungula candidiasis" denominate if the infection is by fungi of the genus Candida. "the name used to a causative pathogen and opportunistic fungus is "Ungual mycosis" (Elewski, B. E. 1998).

Nail fungi is an infection of the nails caused by dermatophytes, non-dermatophytes and yeast. The injury is the separation of the nail bed from the nail's plate and a change in the colour and texture of its outer surface (El-Tatawy et al ,.2015). 50% of cases are caused by skin fungi that include Trichophyton which the most common, Epidermophy and Microsporum(Souza et al ,.2014).

According to recent studies indicated that the rate of dermatophytes infection in the world is 5.5%, and the increase in the number of infections may be due to the increase in the number of patients suffering from chronic diseases such as diabetes and HIV, and the increase in patients suffering from obesity and immune deficiency.Onychomycosis represents approximately 50% of nail diseases, and it increases dramatically in developing countries, although the injury does not directly threaten life, it greatly affects the patient's psyche and social life, in addition to the long and expensive treatment costs, and onychomycosis occurs often By dermatophytes, but recently, non-dermatophytes have caused injuries significantly.(Gupta et al ,.2001).The distribution of onychomycosis differs according to the geographical location, where the wet areas differ according to the type of organisms cause and the extent of its spread from hot places(Scher et al,. 2003).

1.2Aims and Objectives of Research :

1.2.1 Aims

Knowledge of the prevalence of The causative Pathogens of toenails onychomycosis in Amman, Jordan.

To accomplish the aims following objectives were set.

1.2.2 Objectives of Research :

The study basic objectives were to investigate:

1. Isolate and identify the onychomychosis pathogens species in collected specimens in Amman , Jordan ..

2. To determine the distribution and prevalence of fungal nail disease in different age groups and gender.

3. To compare prevalence of dermatophytes and non-dermatophytes implicate in causing onychomycosis.

2. CHAPTER TWO: REVIEW OF LITERATURE

2.1 History

Descriptions of mycotic infections of the nail, identifiable intrinsically, The first case appeared in the 1829 disquisition on scalp ringworm by the Parisian empiric Mahon the younger. Mahon was the famous member of a family that contracted to take care of favus and allied scalp conditions in Paris at l'Hopital St Louis in the early decades of the 19th century (Crissey et a ,. 1990).

The alterations within the nails caused by favus manifest to result from a disturbance and an increase in the corneous secretion of That consist of it, because they increase in thickness and grow outward to an abnormal degree. The regularity and polish of the standard state provide a way to a longitudinal rugosity, they become frayed at the ends; but become more sensitive than normal; the
y assume the yellow colour characteristic of favus (Crissey et a ,. 1990).

The discovery of the dermatophytes in nail material belongs to George Meissner (the discoverer of the corpuscles That was named according to him), George Meissner who in 1853 observed hyphae in preparation of potassium hydroxide when adding 'thick fingernail, bent claw-like', taken from an 80-year-old man. The 'onychomycosis' appears to have been coined the term by Virchow 3 years later in 1856(Crissey et a ,. 1990). discovered Candida albicans infection of nails was first by Dubendorfer in1904 (Crissey et a ,. 1990)Onychomycosis caused by a species of Aspergillus was first confirm by Berseton and Keil in1941.In 1948 another cases of Onychomycosis because of Aspergillus were reported by Moore and Weises in USA(Crissey et a ,. 1990).within precocious work, dermatophytes grow on Sabouraud and pointed out that colony characteristics and under microscope different with different media, then Sabouraud's glucose Media turn out the most widely media used to diagnosis in medical mycology culture. After that, it was adjusted by the adding of antibacterial antibiotics (Gentamycin, Chloramphenicol) and Cycloheximide (Acti-Dione) for identification and selective member of pathogenic fungi(Merze et a,.2005) Arievich et al., insert 'emplastrum urea' to get rid of dystrophic nails at 1960 which was later adjusted and approved for the treatment of onychomycosis in 1978 by Farber(Singh et a .,1986)

During the last two decades, a lot of antifungal drugs developed to treat but Besides they are limited to relatively few clinical classes. Although a lot of antifungals was discovered, as we said, but there is a resistance in clinical isolates leads to failure in the treatment of mycosis. Tests prepared to confirm the minimal amount of antifungal drugs needed to inhibit the growth of fungal strains in culture (minimum inhibitory concentration or MIC) are broadly used to determine the relative efficiency of different antifungal drugs and to detect the new development of drug-resistant organisms that's very important for a future of develop a new resistant strain(Martinez-Rossi et a,.2008)

A new Classification of onychomycosis was proposed by Baran in 2011. the new classification mentioned a clinical subtype, in addition to onychomycosis Baran mentioned mixed onychomycosis, endonyx onychomycosis, secondary onychomycosis and dystrophic onychomycosis (Hay et a,.2011)

2.2 Anatomy of Nail :

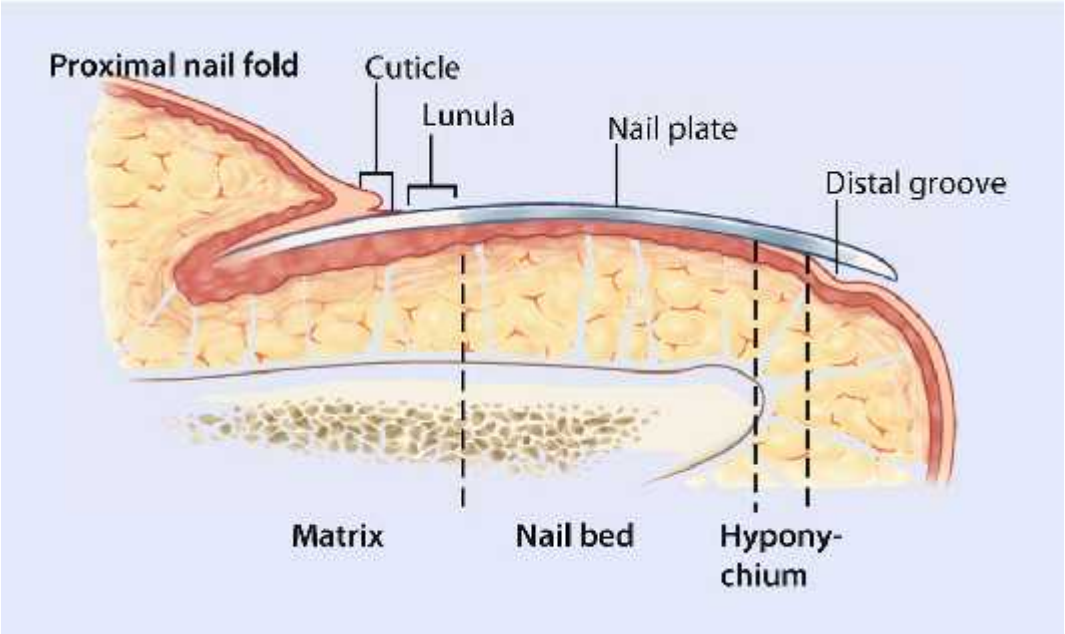


Fig (2.2.1) : Drawing of nail anatomy (Fleckman,.et al 2018)

2.2.1 Nail plate

Nail plate formation begins at 9 week when the nail growth is almost complete in the twentieth week and it is derivative from epidermis on the terminal phalanx(Samman,. et al 1995).foremost we must know the structure and the functional of each organization of nail unit and how is the process of nail growth to realize the infection on nails unit(Haneke,. 2006).

1.2.2 Nail unit :

The Nail Unit is formation of the generative part which is nail bed epithelium and matrix while the manufacture nail plate and it is surrounding with grooves and nail folds, while framing with hyponychium and eponychium (cuticles) and their supportive tissue name as nail unit fiber collagen. The framing and surrounding of the nail are referred to as (paronychium), the components forming, fasten and encirclement the nail plate are known as the (perionychium) (Morgan et al ,. 2001).

1.2.3 Nail plate

Nail plate function as a shield for the distal digit and as exceptional self-renewal tool. the nail plate is a strong, partly diaphanous, keratinous structure that softly curves in different directions (transverse and longitudinal).The nail plate consists of three special layers anatomically, a thin layer that gives a smooth surface, medium-thick layer, ventral layer its show irregular surface with longitudinal deformations.Most

of uncoverd nail looks pink because of transition of pink color from the vascular nail bed that adherent . almost quarter of the nail is envelope by nail fold , the lateral nail fols coverd the narrow nail plate (Morgan et al,. 2001).

in adult, fingernail grows around 0.1 mm in day, in toenails nail growth 30% to 50% compared to fingernail. Decrease of nail growth it is due to several reasons like cytostatic drugs, cold, smoking and malnutrition. at variance, nail growth increase with psoriasis disease.) (Morgan et al,. 2001)

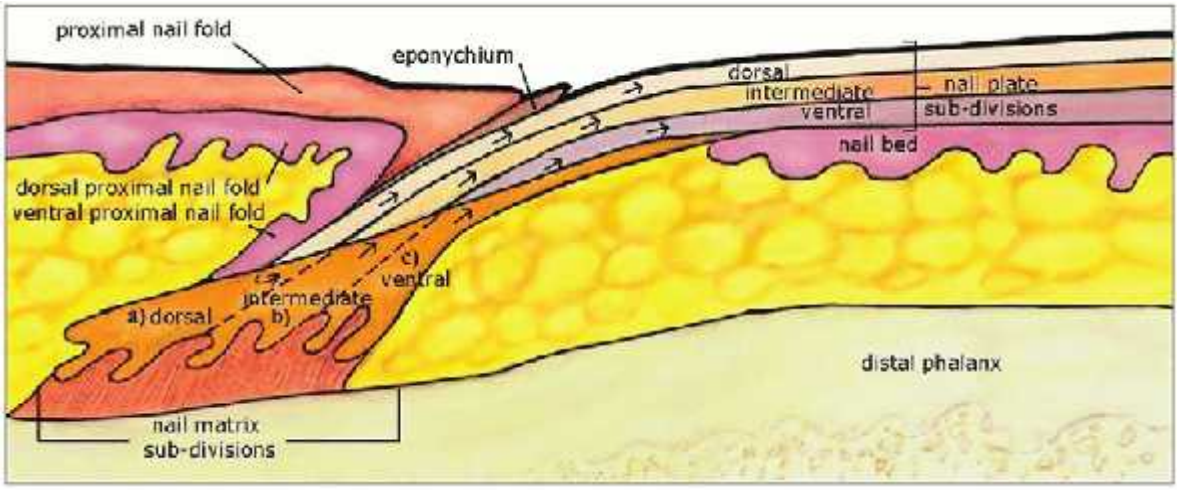


Fig (2.2.2) :Drawing of nail matrix and other parts of nail (Jiaravuthisan et al,. 2007)

2.2.4 Nail Matrix :

The nail matrix (intermediate and dorsal) is The growing layer of the nail system . like the case of the epidermis of skin, a lot of these cells divide like keratinocytes where found on the skin while Nail matrices produce this change without creating a layer of keratohyalin granular layer when the keratinocytes go out of basal layer then die and become incorporate to the nail plate.(Dawber R et al,. 1984)

2.2.5 Nail bed (ventral matrix) :

The nail bed is the located for vascular under the nail plate expanded from the lunula (half-moon) to the hyponychium. and It is consists of two layers, the superficial epidermis, which is the layer found under the nail plate that shift forward with the layer .while the second layer is the deeper dermis which is having a living tissue that work as a fixed to the bone and having also capillaries and glands.(De Berker et al,. 2007)

2.2.6 The nail folds:

From three sides on the nail plate, the nail folds surround. The two nail lateral folds integrate to the dorsal nail fold, And in this process, a pocket is made, and named proximal nail groove, for the root and matrix. Ventral and dorsal portion formation the PNF.(Morgan et al ,.2001)

2.2.7 Eponychium :

the dermal structure at the above of nail plate is named as Eponychium. it is a transitional region of skin than bind the epidermis to the matrix, and its products a thin stratum from keratin.(Scher et al ,.1997).

2.2.8 Onychodermal and Hyponychium Band :

onychodermal band typically extend (0.5 mm to 1.5 mm) , While its a narrow band and it's was explain as more faint than the pink nail bed and diaphanous quality .(Fleckman et al ,. 2018)

in order to there is no direct contact with nail plate and dermis , Onychodermal bands consort to the nearest point of relevance of the fingertip.(Morgan,et al 2001).While the hyponychium is a sign to the onset of the epidermis, and its hyponychium develops from the remote ridge and its initial located of the keratinization in the nail unit also of all epidermis in the embryo.

The first site of infected by dermatophytes is the hyponychium

it's the most popular species of onychomycosis. .(Fleckman et al,. 2018)

2.2.9 Blood and Nerve Supply of the Nail Unit :

The blood supply of the unit of nail originates from the side digital arteries. while these arteries emit medium-sized sections that pass through the dorsal interface of the Between the fingers provide a superficial arcade. so there are two exporters for blood supply, the apparent and the closest arcades.

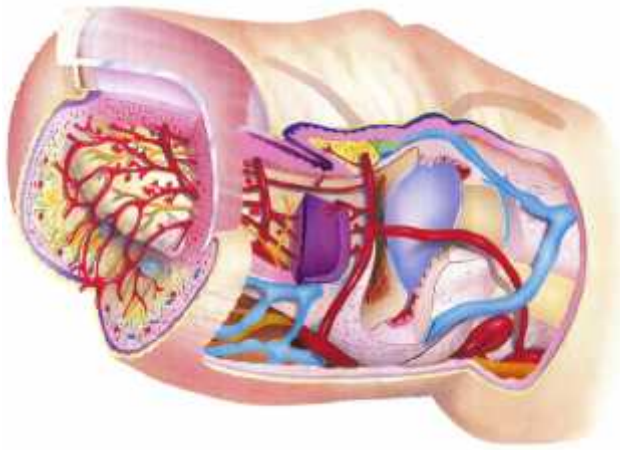


Fig (2.2.3) :Blood supply of the nail unit.(Fleckman et al ,.2018)

2.3 Epidemiology & Prevalence

Onychomycosis is the most popular nail disorder in the world. and its infected about 5% of the global population . while appearing for 20_40% of onychopathies and 30% of mycotic skin infections.(Kaur,.et al 2008).

Even though onychomycosis is not a life menacing disease, but because of its a high widespread, that makes it a real global health problem.(Kaur et al,. 2008). Reports shows of spread inconsistency of onychomycosis with evaluation ranging from 2-3 % to 13% in the western population.As opposed to western countries where the onychomycosis is high prevalence and cause of nail disorders, the prevalence of onychomycosis is low in South Asia.(Kaur et al,. 2008).In North America, they reported around 2% to 13% of onychomycosis cases(Araújo,et al., 2003). About Canada, the incidence of onychomycosis showed 6.5%. (Summerbell,. 1997).while previous studies about spread rates of onychomycosis in the United Kingdom, Spain, and Finland the around 3% to 8%.(Iorizzo et al., 2007).In European countries, They did an (Achilles) project which is a big project for scanning of onychomycosis in 20 countries and the result showed a spread rate of about 29%.(Burzykowski et al., 2003).In turkey as mentioned in the previous study, in 1999, the proportion was approximately 41% of infected with onychomycosis (Kiraz et al ,.1999).In the 1990s in South East Asia at the Extensive survey of onychomycosis show that in Tropical countries is 3.8% and about subtropical countries its around 18%(Bramono ,.2001).In developing countries, important primary procedures geared towards social and economic concerns and health issues for other diseases have led to a decrease in the attention of onychomycosis by medical doctors and public people.(Kaur et al,. 2008).because of that , not that much data is obtainable regarding the spreading of onychomycosis there(Seebacher et al., 2008).According to the previous studies, the spread varies of onychomycosis from 4% to 18% rely on the geographic distribution, age, and population studies.(Erbagci ,.et al 2005).Also, the spread varies according to social class, climate, travel, living environment, occupation.(Kaur,.et al 2008).

2.3.1Age :

Previous studies pointed out the highest age group can be infected is from (61_70)years while the lowest incidence age group is from (21_30) years(Kaur et al,. 2008).Estimates show that the age group from (40_60) may infect in rate range from 15% to 20% of onychomycosis .(Jesudanam et al,. 2002).onychomycosis infection on children is rare its less than 30 times than adult due to some reasons like they didn't spend much time in pathogen environment(public changing rooms, sharing showers), the size of nail surface for invasion, and nail growth faster than an adult . In the USA the range of onychomycosis infection of children was 0% while in Guatemala was 2.6%(Gupta.et al ,.1997).onychomycosis increase related by age according to some reasons like poor peripheral circulation, weakened immune function, some disease like diabetes and lack of attention in nail hygiene and trim (Hapcioglu et al,. 2006).

2.3.2Sex :

Onychomycosis are more common in males than females, according to several factors. Young people are more exposed to some sports and recreational activities that damage nails.(Baran et al,. 1999).In Indonesia, a few studies indicated that women are more susceptible to infection, depending on certain conditions such as dealing with cleaning materials and exposure to water and moisture at a ratio of 1.2 to males.(Bramono et al,. 2005).while Common infections Candida of in the toenail in women more than men and frequently, perhaps due to wearing more closed and narrow shoes.(Gupta et al,. 1997).

2.4 Causing factors:

Among the factors contributing to the occurrence of infection in the nail include several reasons such as the geographical area, health status and profession, genetic diseases, safe diseases and lifestyle. (Scher et al,. 2003).

2.4.1 Genetics :

The latest studies indicate a genetic relationship to infection in the onychomycosis(Thomas et al,. 2010).In a previous study in America, researchers reported family patterns of T. rubrum infection in the nails, which were shown no related to be transmitted between families. While it was found that there was an increase in the possibility of infection in the onychomycosis while one of the parents was infected.(Faergemann et al,. 2005)

2.4.2 Smoking :

Another factor that may contribute to increasing the possibility of infection is smoking, in a study conducted on a group of smokers that showed that it is a direct cause of increasing the infection by 49.2%, and in patients who smoke two packets or more, the chances of infection are greater than others (Guptaet al,. 2000).

2.4.3 Immunodeficiency :

The possibility of onychomycosis increases in people with viruses such as patients with HIV, Trichophyton Rubrum is the main cause often in the infection is more prevalent in them as it affects mostly the fingernail and toenail, while onychomycosis is one of the signs of the presence of HIV, and the second type of The fungi that cause infection in patients is T. mentagrophytes. as it gives rise to superficial white onychomycosis.(Thomas et al,. 2010).

2.4.4 Sports :

It turned out that there was a higher possibility among athletes to infected with onychomycosis than others. In previous studies, it was highlighted that sports contribute significantly to nail fungus infection, as they provide fertile conditions such as plastic shoes that cause moisture, sharing the surfaces, swimming pools and showers, also some sports without footwear such as gymnastics.(Thomas et al ,.2010).

2.4.5 Diabetes :

In diabetics, the risk of infection increases significantly, which may reach three times that of others, as it was found that 34% of diabetics have onychomycosis, and this infection poses a great danger to them as complications with it, such as infection with bacteria and other fungi, and because wound healing is less in these patients, The doctor may amputate the extremities in case of severe injury.(Thomas et al,. 2010).

2.4.6 Environmental :

The probability of getting infected with nail fungus in urban areas increases more than others due to several factors, including overpopulation, an increase in the number of hours wearing closed shoes, sharing of spare clothing rooms, sports clubs and swimming pools. An example of an environmentally affected nail fungus is the Scytalidium dimidiatum.(Thomaset al,. 2010).

2.5 ETIOLOGY :

Onychomycosis consists of three groups of fungi : dermatophytes, non-dermatophytes moulds and yeasts.(Kaur et al,. 2008).Every year the number of patients suffering from nail fungi increases, and on the other hand the number of microorganisms that are classified as causes of nail infections around the world increases. (Greer,.1995) With the apparent increase in the spread of onychomycosis infection, it is very important not to forget to take each filamentous and pseudohyphal of these as potential causes of infection.(Bassiri-Jahromiet al,. 2010).The geographical location affects the type causer of infection with nail fungi. The type of fungus that most causes nail fungus is the skin fungus, which is very high in the toenails in rate 90% and It is estimated at about 50% in fingernails. Then followed by yeasts, it was recently considered to be an effective cause of fungi and molds.(Midgleyet al,. 1994).Non-dermatophytes mould It appears frequently in the elderly people also in patients with a skin disease that can affect the nails, patients with immunodeficiency and patients with chronic diseases, which is more common in the toenails than the fingernail and its prevalence are estimated from 1.5 _ 6% of the fungi.(Greer,.1995).

2.5.1 Dermatophytes :

Dermatophytes are a group of fungi that affects both skin, hair and nails. And this type of fungi is characterized by its high ability to use and digest keratin. These fungi also produce the keratinase enzyme. Most skin fungi are distinctly similar
many respects and characteristics, such as surface antigens. And with time, methods of diagnosis and identification have become easier to refer to each arrangement, the morphology of conidia and colonial properties. (Rippon,. 1988).0
Dermatophytes fungi are classified according to each of these properties:
Genetic characteristics, morphological and ecological.(Taha,.2011).

Morphological Classification:

Dermatophytes reproduce Asexually, and therefore they produce macroconidia and micro .while they differ in the conidial form. Therefore, dermatophytes is classified into types, which are: Microsporum, Epidermophyton and Trichophyton.(Summerbell,. 1997)

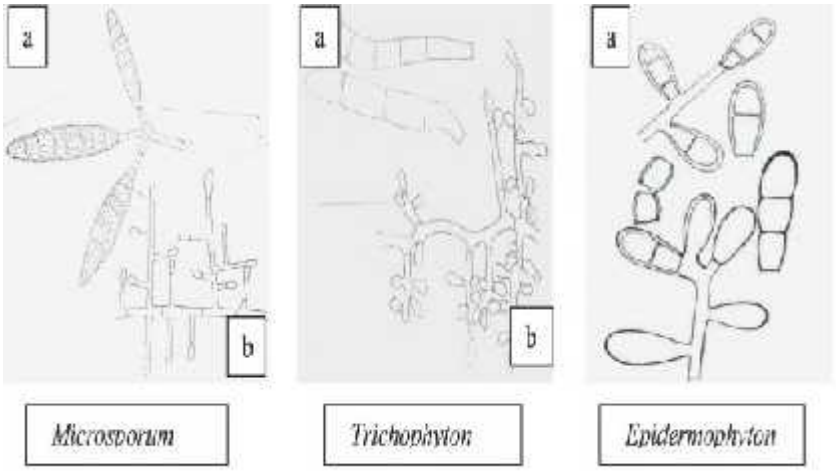


Fig (2.5) : Conidial types in the three dermatophytes. (a) Macroconidia; (b) Microconidia (Aneja et al,. 2013)

Microsporum genus :

Macroconidia are a spindle structure , have a coarse thick or thin wall, also its multicellular with an rate 2_12 cells as stated by species.The main distinguishing feature of this type is a (echinulations) on a cell wall of macroconidia. (Simpanya,. 2000).however it is around 2-3µm in Microconidia and it is also a pyriform.(Taha,.2011).

Fig (2.5.1) : Microsporum macroconidia **(**Mazón,. et al 1997)

The colonies are configuration singly along the hyphae in culture. They are often flat and white and yellow.(Ellis et al,. 2007).

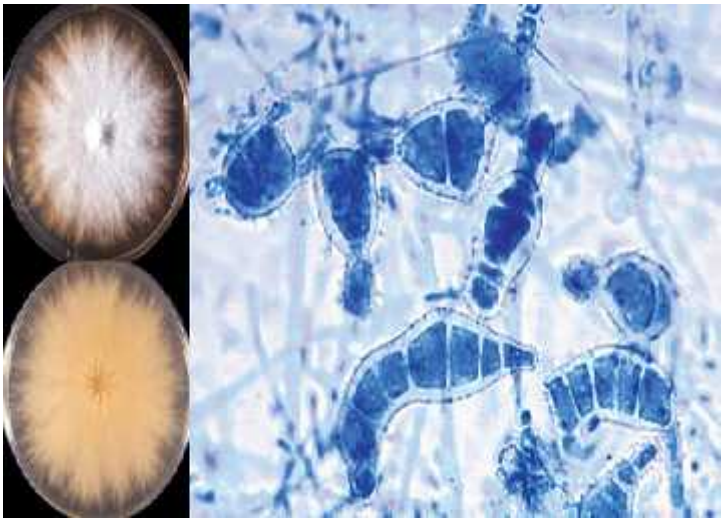


Fig (2.5.2) : Microsporum colonies in culture.

Microsporum contains 18 types that affect both hair, skin and it is seldom in nails, an example we can see some species.

- Microsporum canis
- Microsporum persicolour
- Microsporum ferrugineum
- Microsporum gypseum
- Microsporum audounii
- Microsporum nanum and others (Aly R,. 1994).

Trichophyton genus :

Trichophyton appears under a microscope: Fist the Microconidia, it is irregular in shape, ball-shaped and with size range from 2 _4.While macroconidia are cigar-shaped, smooth and fluffy walled septate from 0 to 10 septa.(Khurana et al., 2011).

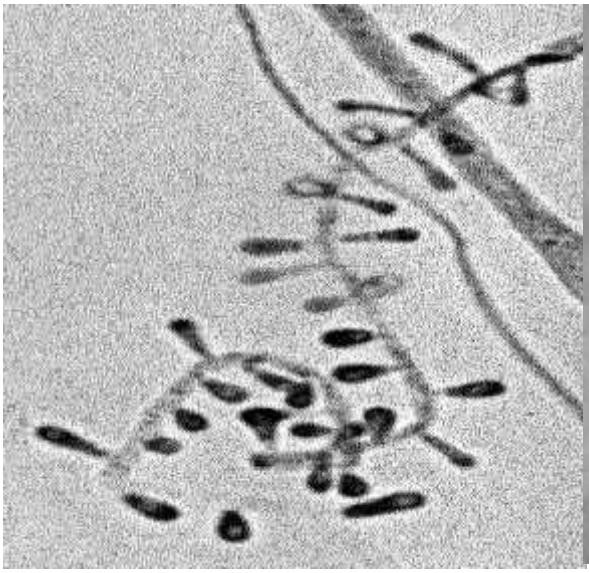


Fig (2.5.3) : Clavate microconidia. Fig (2.5.4) : macroconidia (Brasch ,.et al 2005)

Some Species of **Trichophyton** .

- Trichophyton equinum Trichophyton fiavescens
- Trichophyton georgiae Trichophyton gloriae
- Trichophyton gourvilii Trichophyton longifusus
- Trichophyton. rubrum Trichophyton schoenleinii
- Trichophyton simii Trichophyton soudanense
- Trichophyton terrestre Trichophyton tonsurans
- Trichophyton verrucosum Trichophyton violaceum

Trichophyton species infect each of hair, skin and nail. onychomycosis can be caused by some of Trichophyton group species and Epidermophyton species.(Lewis,. et al 1958).

From 30 species of Trichophyton, the most popular of them are Trichophyton rubrum and Trichophyton mentagrophytes.(Kaur et al., 2008).While Trichophyton tonsurans is one of the species produce many of macroconidia and seldom produced macroconidia.(Ellis et al,. 1996).

Fig (2.5.5) : Trichophyton rubrum colonies (Taha, 2011).

Epidermophyton :

The pathogenic species of Epidermophyton members is Epidermophyton floccosum. under microscope microconidia are absent while about the macroconidia are structure thin-walled, multiple club-shaped and smooth, abouthyphae it is bifurcated. (Ellis et al,. 1996).

Fig (2.2.6): Macroconidia of Epidermophyton floccosum (Mazónet al,. 1997).

Primary culture growth is slow, the top is green to yellow coloured, flat, folded Centre, pleomorphic tuft, while it is the yellow-brown colour seen in the reverse side.(Taha,.2011).

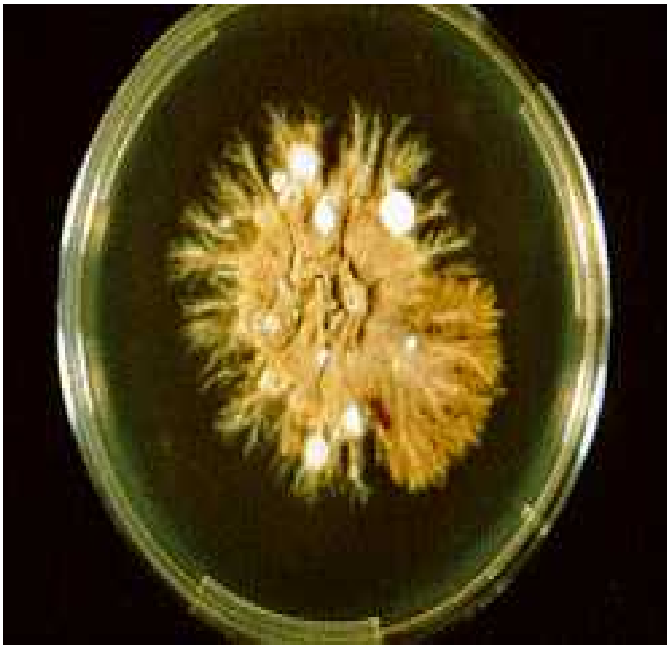


Fig (2.2.7) : Colonies of Epidermophytonfloccosum (Mazón,.et al 1997).

2.6 Ecological Classification:

According to the natural environment, dermatophytes classified into three groups of epidemiologically name as ecological groups and it is a Zoophiles, Anthropophiles and Geophiles. the distinction between there by the hosts and the keratin of the hosts (Taha, 2011).

2.6.1Zoophilic:

Zoophilic live in animals The most common members of this type group are : M. canis, T. verrucosum, T. equinum and M. canis. it is transmitted from animals like Pets to humans by direct contact or by contaminated products. (Taha, 2011).

2.6.2 Anthropophylic:

Anthropophylic is only in human, The most common members of this type group are T.rubrum, E.fluccosum and T.mentagrophytes. it transmitted from person to person by direct contacting or touch a contaminated matter.(Taha ,2011).

2.6.3 Geophylic:

Geophylic exist in soil , The most common members of this type group are M. nanum,M. cookei , M. gypseum and others . it transmitted by direct touch.(Hainer, 2003).

2.7 Non –Dermatophytes Moulds

Non-dermatophytes moulds are fungi infect the skin, it is found in soil and The remains of decaying plants.(Tosti et al,. 2000).The non-dermatophytes different from dermatophytes by location colonized, moulds colonize in the external stratum of the nails. And it is popular in toenails more than fingernails. because of all non-dermatophytes moulds produce mycelium and this frond help fungus to grow among at the stratum of hard keratin. like Aspergillus species, we can see the spores on direct microscopy by a nail specimens.(English,.1976).

species of moulds can cause onychomycosis :

- Aspergillus spp
- Scytalidium dimidiatum
- S. hyalinum
- Fusarium spp
- Acremonium spp.
- Onychocola canodiensis
- Scopulariopsis brevicaulis. (Summerbell et al,. 2005)

The most common of Non-dermatophytes species are Scopulariopsis, Acremonium and Fusarium, after that Aspergillus species and Scytalidium species.(Ghannoum et al,. 2000).

2.8 Yeast

Yeast is a member of normal microbial flora in our bodies, we can found it in mucous membrane and cuticle, while it is unicellular and it is reproduced by budding.(Fisher and Cook,. 1998).Yeasts are one of the microorganisms used for technology in our lives. It is used in the production of some foods, yeast, beer and a wide range of biochemical industries, and despite its great benefits, it causes damage to drinks, food and some diseases. At this time, there are about 700 species that are recognized and studied. Yeasts are unicellular and multiply by fission or budding (Boekhout.et al,. 1996).Numerous types of yeast have been found to grow in nail tissue and where we cannot primarily detect it through direct examination under a microscope, but we use other methods. Candida is usually considered a second infection in the nail layer infected withparonychia. Candida causes nail damage and harbouring the inoculum yeast prepared for the nail invasion.(English,.1976). The most common species caused infection of the nail (onychomycosis) is Candida Albicans(Seebacher et al ., 2007)while another species like Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei can cause onychomycosis also but it less widespread.(Faergemann ,.1996).The infection with candidiasis is opportunistic, meaning that there must be a change in immunity and a deficiency to break down the keratin and penetrate the nail.(Nardo Zaias ,. et al 1996).The most common species of candida than cause onychomycosis is candida Albicans that is produced pseudohyphae, hyphae and chlamydoconidia clusters and blastoconidia clusters , while in culture it produce a creamy colour and smooth colony(Taha, 2011).

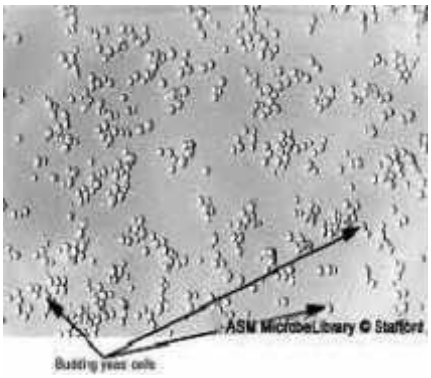


Fig (2.8): Candida Albicans under microscope (Mazón,. et al 1997)

Fig (2.8.1): Candida Albicans on saburauds dextrose agar(Mazón et al,. 1997)

2.9 Onychomycosis Classification:

onychomycosis infections constitute severalthree types, which are: superficial white nail onychomycosis, Distal and lateral subungual onychomycosis and proximal subungual onychomycosis. And the person can develop more than one type-together.(Hay,.et al 2011)

2.9.1 superficial white nail onychomycosis :

superficial white nail onychomycosisis the least common type among other types, but it is distinguished at the site of injury as it infects the nail plate and these fungi invade the nail through the dorsal surface causing small white spots with edges that are distinguished clinically, and then these spots unite and cover the entire nail surface Gradually**.(** Baranet al,. 2011).This group includes many types of fungi, the main cause of the type of dermatophytes is T.mentagrophytes, and non-dermatophytes also cause infection with white superficial onychomycosis.(Piraccini.,et al 2004)Candida can also cause infection, especially Candida A Albicans .(Faergemann, .1996).T. rubrum infection is on the surface of the nail plate and not in the nail bed, and is common especially in children who suffer from blockage of the nail plate.(Baran et al., 1998).

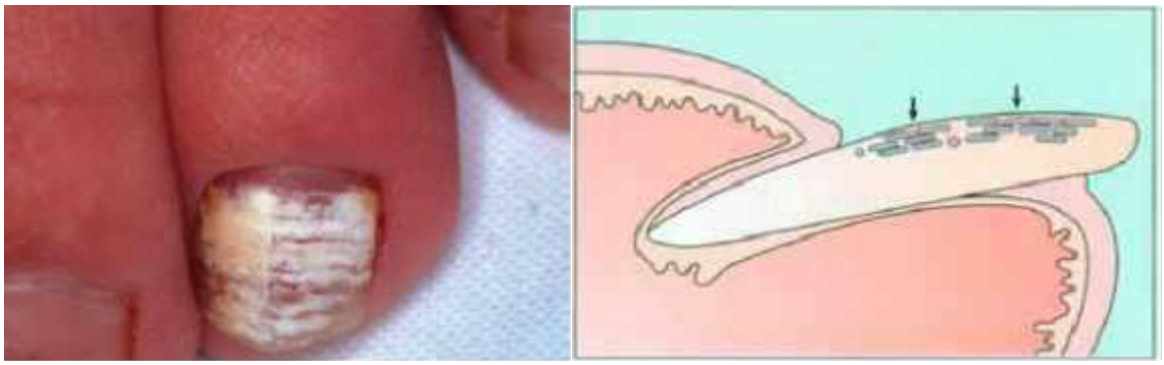


Fig (2.9) :case of superficial white nail onychomycosis (Piraccini,. 2004)

2.9.2 Distal and lateral subungual onychomycosis :

This is the most common type of infection in the nail plate, as these organisms invade the far surface of the nail plate and lateral as well. They are characterized by pigmentation and keratinization where the colour changes to yellowish-white and can also become brown, orange and black.(Ha.et al,. 2011).This infection results in the separation and fragmentation of the nail plate.(Roberts,.2003)

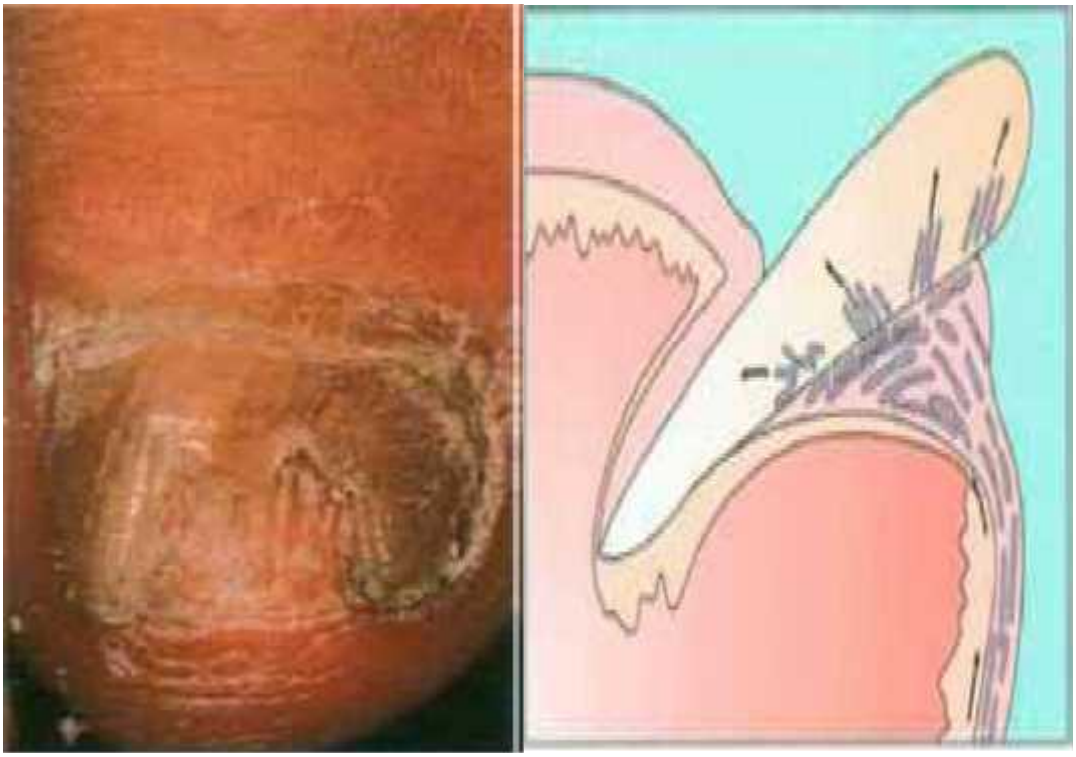


Fig (2.9.1) : Case of DLSO.(Piraccini,. 2004)

2.9.3 Proximal subungual onychomycosis :

This type is not very common, and infection appears under the nail and nearby nails. The infection spreads and slowly expands the nail. This infection is very common in patients with HIV. Some organisms that cause this type of infection are Fusarium, Aspergillus, T rubrum and C.Albicans. This infection is difficult to treat as it requires both oral and topical treatment, in addition to some cases, to surgery.(Hay,.et al 2011).

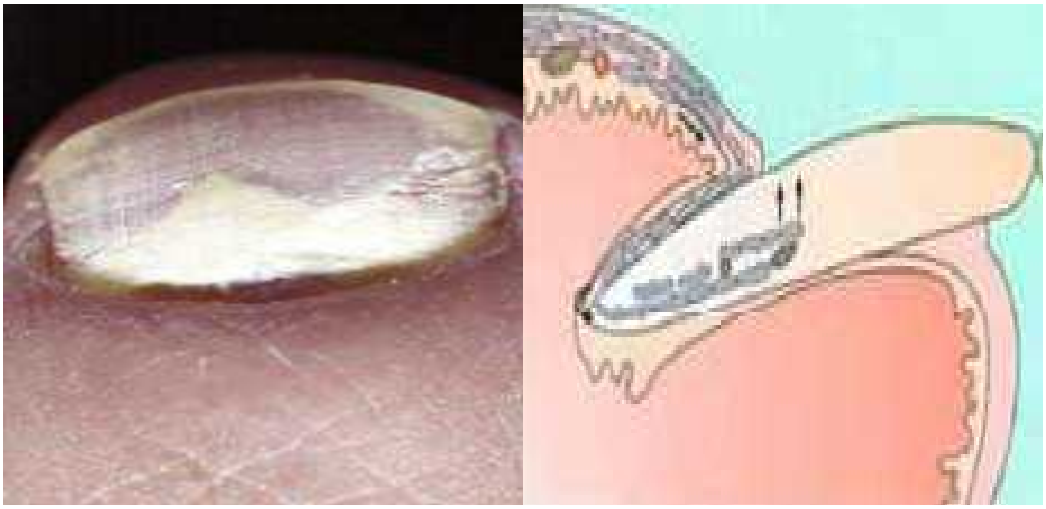


Fig (2.9.2) : case of PSO.(Habif,.2010) (Piraccini,. 2004)

2.9.4Mixed infections :

It is an infection caused by both dermatophytes and non-dermatophytes together and needs further research and scrutiny to determine their status(Summerbell, 1997).

2.9.5Endonyx onychomycosis:

This type of infection is characterized by the fact that it only invades the nail plate and does not invade the nail bed, which leads to separation of the nail plate and its colour change together.(Tosti et al,. 1999)

While these fungi invade the nail plate as we said, the nail penetrates through the skin and deeply invades the plate without keratosis.(Gupta,.2001) Among the most important speciesthat cause infection are T. violaceum Your action and T. soudanense cause scalp inflammation.(Baran et al,. 1998).

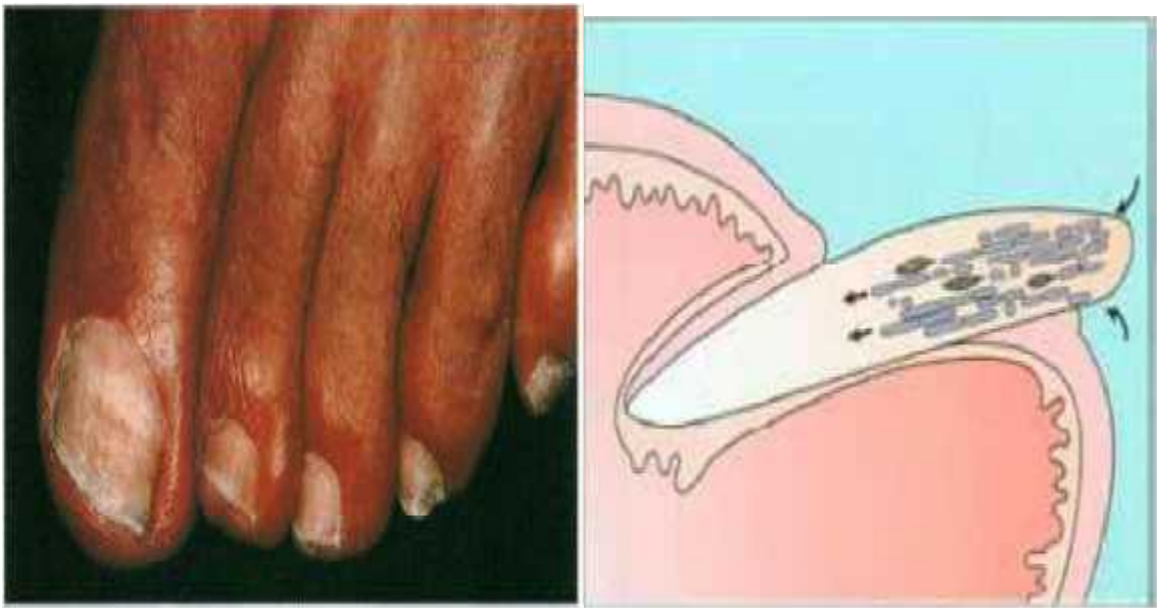


Fig (2.9.2) :Case of EO .(Piraccini,. 2004)

2.9.6Totally dystrophic onychomycosis :

When any type of nail fungus develops after the nail plate has been invaded, it develops, causing complete atrophy of the nail plate(Elewski, 1998). This appears as a paired colour change to brown or yellow.(Baran et al,. 1999)

Fig (2.9.3) : dystrophic onychomycosis &chronic paronychia(Singalet al,. 2011).

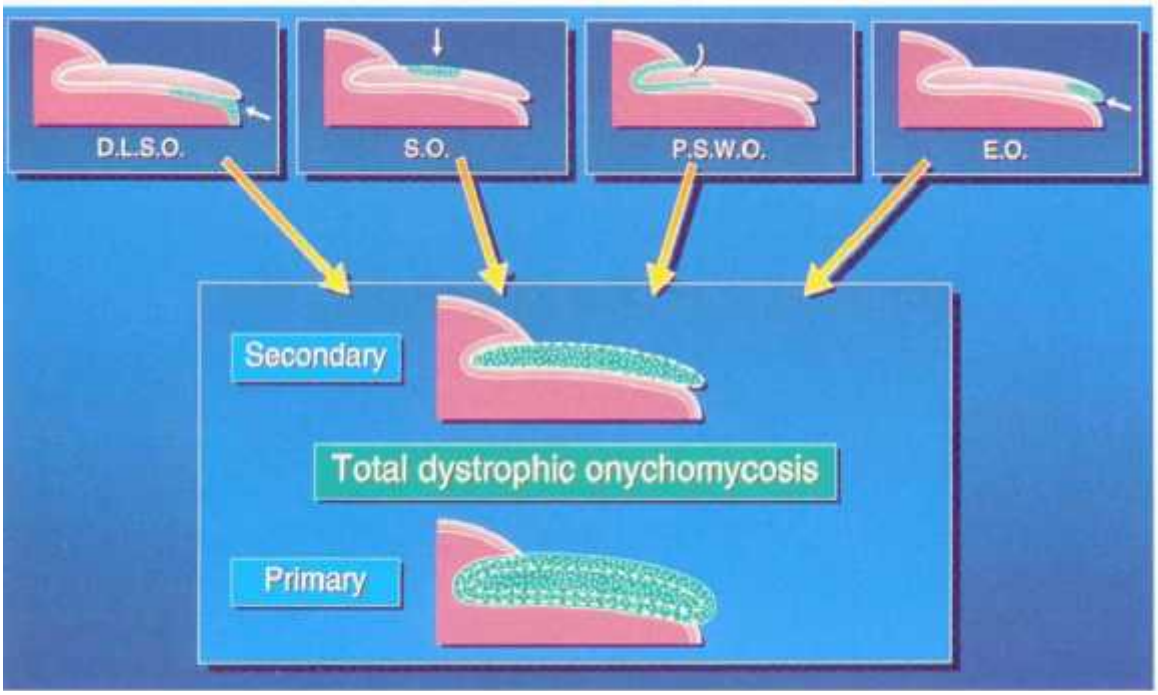


Fig (2.9.4) : Total dystrophic onychomycosis .(Hay et al,. 2011).

2.9.7 Secondary onychomycosis :

These fungi invade the nail plate and the surrounding tissue, causing hyperkeratosis Which leads to the thickness of the nail plate and the formation of what is called psoriasis, and the other form of injury is nail atrophy, which is diagnosed clinically and by the laboratory to prove that the cause is fungi.(Hay,.et al 2011)

2.9.8Candidal Onychomycosis :

The superficial candida causes infection of the nails and the surrounding skin, causing the Candidal onychomycosis.(Jayatilakeet al,. 2009).
Candida invades the nail in four different forms which: secondary nail dystrophy, chronic paronychia, distal nail infection, secondary candidiasis and chronic mucocutaneous candidiasis. (Roberts et al,. 2003)
And Candida mostly affects women more than men, depending on the lifestyle, and it is also frequently found in patients with immunocompromised.(Tasi et al,. 2001).

Secondary nail dystrophy and Chronic paronychia :

This infection occurs as a result of moisture in the water, as it affects patients who work in wet conditions and their nails are exposed to water for a long time, which causes the skin to separate from the nail plate, so bacteria and fungi enter the nails, creating an actual infection in the near and side folds that appear as inflammatory signs and skin separation.(Roberts et al., 2003).

Chronic mucocutaneous candidiasis :

This type of acute infection causes granulomatous candidiasis, as it causes swelling of the lateral and proximal nail that causes strong deformation of the nail thickness and is called (chicken stick).(Elewski,.1998).

Distal nail infection :

This type of infection is rare because it is directly related to patients with Raynaud's disease and is characterized by nail decay.(Gary, . 2007).

Secondary candidiasis :

It is an infection of infected nails, causing a form of psoriasis(Baran,.et al 1998).

3.Onychomycosis Diagnosis :

Fungi infect nails and it is changed in their shape and composition, and to diagnose this type of infection clinically and diagnostically through the laboratory.((Roberts et al., 2003).Diagnosis of infection begins with knowledge of the medical history, nature of life and work because it affects the type of infection, then a clinical examination is carried out, the extent of damage and change in the nails are determined, and the last stage is to take a sample and examine it in the laboratory.(Elewski, 1998).During the clinical examination, the infection is recognized and distinguished from changes that are not related to onychomycosis, while the type of infection can also be determined by changes in the nail whether it is decay or change in thickness or near skin, dry nail plate, keratinization, and surface leukemia.(Scher et al,. 2007).The most common types that simulate mycosis of psoriasis nail are the most common and diseases that affect nails. Also, idiopathic nail dissolution, traumatic dystrophy, flat lichen, yellow nail syndrome (rare), contact dermatitis and bacterial infections.(Elewski,.1998)

3.1 Differential diagnosis of onychomycosis :

Features suggesting the diagnosis for each infection :

3.1.1 Psoriasis :

Patient family history, rash spreading to places such as the knee, nail etch and change in color.(de Berker ,. 2009).

Fig (3.1) : Case of psoriasis (Jiaravuthisan,.et al 2007).

3.1.2 Lichen planus :

Caused atrophy of the nail and white streaks on the side near the nail, with scars(de Berker ,. 2009).

Fig (3.2): Case Lichen planus.(Piraccini.,et al 2010)

3.1.2 Yellow-nail :syndrome :

In this syndrome, a defect coincides with the length of the nail, where the problem appears in slow growth and longitudinal and transverse curvature as well, and infection can happen with no pathologic relevance. (de Berker ,. 2009)

Fig (3.3) :Case of Yellow-nail :syndrome (Piraccini.,et al 2014)

3.1.3 Trauma :

This infection affects one of the fingers and changes in its shape with a regular change in the colour of the nails.

3.1.4 Periungual squamous cell carcinoma :

This infection affects one nail within the fingers , and it is represented by a wart in one side of the nail.(de Berker ,. 2009)

3.2 Laboratory Diagnosis of onychomycosis:

A final diagnosis of nail fungus After a clinical diagnosis is made in several ways, one of them can be used: histological, fungal and molecular techniques. Examining onychomycosis through mycological studies, first, pay attention and make sure the sample is taken correctly, then we start examining it with potassium hydroxide (KOH), and culture is one of the best standard methods to diagnosis onychomycosis, while also calcofluor white under fluorescence is a method can use it.

3.2.1 Sampling Techniques:

It is important to ensure that the sample was taken accurately and correctly, as it affects the accuracy of direct examination under a microscope using KOH and cultivation, and the samples are collected by scraping or puncturing the nail plate vertically or horizontally.(Rios-Yuil,. et al 2017).

4 **Table (3.2) :<u>Method of sampling for each type of onychomycosis</u>** : (de Berker,. 2009).

Distal and lateral subungual onychomycosis	The affected nail is scraped and cut from the base of the nail and the underside of the nail plate from the affected tip near the nail bed .
Proximal subungual onychomycosis	The sample is collected by cutting on the surface of the nail plate and collecting debris using a sterile blade in the deep part of the nail plate and the base of the nail.
superficial white nail onychomycosis	The sample is collected by scraping the white spots on the nail surface and then taking away the debris under them.
Totally dystrophic onychomycosis	The sample is collected from anywhere on the nail plate or bed in the infected nail
Candida spp	The sample is collected in the case of nail onycholysis by scraping the raised nail layer beneath the nail plate or by using the side near the edge of the proximal nail and the side of the nail in other candidiasis infections.

For a more accurate and quality sample, Dermatoscopy can be used.(Rios-Yuil,. et al 2017).

3.2.2 Specimen Analysis:

Direct Microscopy :

The direct examination method under the microscope usingKOH is a practical, fast and inexpensive method .(Weinberg et al,. 2003).

The cause was not determined by this examination but is there a fungal infection or not in general by seeing strings and spores in the structure of the fungus .while This method may show false negative results, up to 15%.(Weitzman,.et al 1995).This error occurs due to several reasons, including the wrong sampling method, an incorrectly equipped mixture of KOH, the insufficient time during preparation and an inappropriate amount of hyphae. (Daniel,.1991).It can also give false-positive results as a result of the presence of a mosaic or saprophytic (Markus et al,. 2001).The nails are prepared by soaking them in a KOH or NaOH solution from 10 to 30% with the addition of 5% glycerin(Weitzman,.et al 1995).Then, using a clean glass

slide, put the sample on it and wait from fifteen minutes to twenty minutes before examining it under a microscope(Roberts et al., 2003).Then it is placed under a microscope at 10x and a force of 40 x until we see the formation of fungi such as spores, septate hyphae and pseudohyphae as well as budding cells (Zaias et al ,.1996)

Culture :

The method that used To isolate fungi, by using custom media called Sabouraud dextrose agar, and also use Sabouraud dextrose agar with antibiotics. After culturing, the sample should be incubated at a temperature between 25 and 30 ° C. Colonies can be formed within 72 hours, but the usual protocol is to leave the sample in the incubation degree for three to four weeks until we have clear colonies. The type of fungi is determined by placing it under the microscope and reading it with a specific technique. The type can also be determined by biochemical analysis. For the diagnostic method under the microscope, changes are recorded in the form and colour of the colonies at first, and the possibility of forming pigments as well, then taking a swab from the colonies that were formed and coloured with lactophenol cotton blue and then read microscopically. It should be known that not all growth causes disease. There is also a saprophytes. To distinguish between them, it is necessary to have more than three inoculations.(Rios-Yuil et al,. 2017).

Fig (3.2): Colonies' of Candida on SDA (Stefanetti,.et al 2014).

3.2.3 Calcofluor white under fluorescence :

The method of detection with Calcofluor white under fluorescence helps in overcoming problems that may occur during the dyeing stage during the detection with a microscope (Ovrén et al,. 2016).The examination is carried out by CW by placing the sample taken from the nail on a slide fixed with the oxidation of polyvinylpyrrolidone and left for ten minutes with a mixture of 10% KOH, after time passage the slide is washed with distilled water and allowed to dry at room temperature and then placed under a fluorescent light microscope with measuring absorbed from 380 410 nm (Blake et al,. 2015)The result is positive when round or filamentous shapes appear with blue peripheral fluorescence (Bonifaz et al,. 2013).One of the disadvantages of detecting fungi with this method is the high cost of testing and also a weak signal over time (Ovrén., et al 2016).

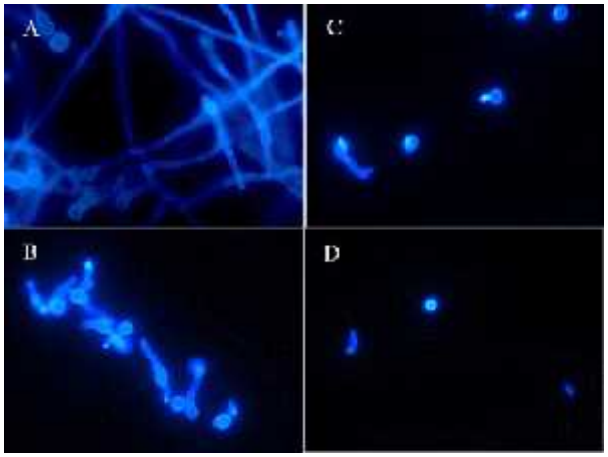


Fig (3.2.1) : Detected Spore of T. rubrum by CW (Foss,.et al 2014).

3.2.4 Histopathology:

Nail fungus examination by histological examination requires a specific type of sample as it depends on nail bed samples and nail plate, and the sample is taken by a perforator, shear or scalpel, but this method is not preferred due to risks that can be caused, such as nail dystrophy (Suarez et al,. 1991). After taking the sample by cutting, it is the best method or other methods of the nail plate, while it is fixed by formalin at 10% and then treated by adding phenol 4% to soften the nails and from other soften that can be used (in 10% formalin, 5% trichloroacetic acid Acetic. It is then taken on glass slides for examination after stain (Stephen S et al,. 2015).Dyes used in preparing the nail sample for histological examination include (Toluidine blue, Blankophor, McManus, Grocott, Haematoxylin and Eosin stain) (Saurez et al ,.1991).The result is positive when fungus leads appear like red dots(Lemont,. 1997) which is the preferred method for detecting fungi due to their high accuracy and the scarcity of false results (Weinberg et al., 2003)

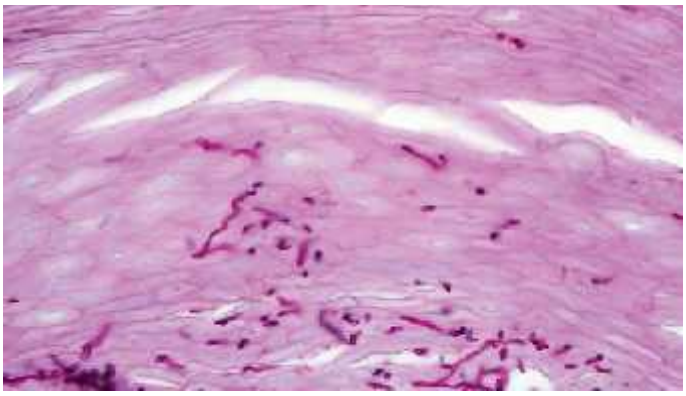


Fig (3.2.2) : Onychomycosis hyphae by Histopathology.(André et al ,. 2013)

3.2.2 Molecular Diagnostic Techniques :

Because of the existence of defects in the method of culturing and Direct microscopy, the technique of detection by molecular diagnostics has been developed to overcome these defects and give more accurate results. can obtain results using this technique even if the sample is insufficient or low quality, while its principle is a polymerase chain reaction with PCR in real-time and post-PCR analysis or without.The required sample can be obtained directly from the nail or a swab from culture. The PCR is the fastest method, but there are some disadvantages such as the high cost of the examination and the inability to follow the patient during treatment because it gives positive results even with the presence of dead fungal cells (Verrier et al,. 2017).

DNA extraction:

It is the first step in the way of molecular techniques and this is done by disrupting the structure of keratin with or without enzymes (Kotrotsiou et al ,. 2017).

PCR & Post-PCR analysis:

Detection by PCR enables us to diagnose one or more types of fungi without the need for other tests. Detection by PCR enables us to diagnose one or more types of fungi without the need for other tests. As for post- PCR, one or several steps can be used to increase accuracy in detecting dermatophytes, while the disadvantages of this method of detection is to increase the possibility to have contamination during PCR and the long time to obtain a result and manipulation.(Rios-Yuil et al ,.2017)This screening method Restriction fragment length polymorphism is used to diagnose specific fungal species and can be used to identify two types of onychomycosis by interpreting the band profiles. The method of detection by (A PCR terminal restriction fragment length polymorphism) was developed until non-dermatophytes were detected with mixed infection and identification of the types of Trichophyton with species. The advantage of this method is fast and automatic, and a large number of samples can be diagnosed. To reveal a greater number of fungi, A pan-fungal PCR microarray assay was developed to examination, as it detects twenty-six species of fungi. Most of the commercially available products detect dermatophytes and others, such as PCR-based, which also detects Candida and its species. (Verrier et al,. 2017).

3.3.7 Real-time PCR:

One of the main advantages of detecting fungi with real-time PCR assay in is the detection of more than one type of fungus together with one tube and this also helps in reducing the risk of contamination (Verrier et al,. 2017) It also shows an accurate result even with the poor quality of the sample, unlike the traditional method of detection (the golden method) (Petinataud, et al,. 2016)

3.3 Treatment of onychomycosis

Treatment options include topical, oral , chemical, surgical and laser treatment (Gupta et al,. 2006).

3.4.1 Oral antifungal agents :

One of the most effective oral fungi treatment methods is one of the most effective. The US Food and Drug Administration have approved each of these medications to treat onychomycosis: itraconazole, griseofulvin and terbinafine, in addition to previous treatments used such as fluconazole. (Gupta et al,. 2006).

3.4.2 Surgical :

While the treatment of onychomycosis with surgery is carried out by the method of debridement or surgery that includes two partial and total forms, in the case of preparation the doctor reduces the thickness of the nail plate and give an objective and oral adjuvant treatment that relieves the patient's pain, while in the case of partial surgery which is better, the affected part of the nail is removed either as a whole It removes the nail plate completely to relieve pressure on the nail bed, and this process should be followed by auxiliary treatments and cleaning.(Gupta et al ,.2006).

3.4.3 Topical treatment :

One of the methods used in treatment is topical treatment: it is applied to the affected area and the solvent solution is reported, which increases the concentration of the active substance that reaches the nail plate at a high concentration. Examples of these treatments include ciclopirox and amorolfine (Gupta et al,. 2006).

3.4.4 Chemical :

This method is similar to partial surgery until you remove the affected part by applying a chemical compound such as urea 40% for a specific period time that may extend for a week, making sure to protect the rest of the nail (Gupta et al,. 2006).

3.3.5 Laser Treatment :

The use of lasers to treat nail fungus is not new, as it was common in the 1980s. The method was then introduced by creating punctures with the affected nail to increase the effectiveness of topical treatments(Rothermel et al ,.1987)Over time, a laser treatment method, such as a diode laser that works along a double optical length, has evolved 870 to 930nnm (Landsman et al,. 2011).

CHAPTER FOUR : MATERIALS AND METHODS

4.1 Patients :

A study of 300 patients infected with onychomycosis Retrospective and prospective, patients with Change in Nails, change in nail plate colour and thickening of outpatient department in Dermatology clinic and privet hospitals in Amman , Diagnosis were in Al Basheer hospital , Amman, Jordan.
preparation for project from January 2019 while collecting samples after collecting patients contact in a period of 5 months from July 2019 to December 2019.A history was recorded for patient regarding the sex, age, onset of disease and various treatment. Inclusion guide: All clinical Sample of onychomycosis of sex, age and health status of outpatient. Medical history and family history, as well.samples excluded : Patients who have taken antibiotic tablet or Topical treatment before 3 weeks.
Data collected from University hospital

4.2Materials:

4.2.1 KOH :

potassium hydroxide (KOH) solution 20% prepared by mixed 20 gm of potassium hydroxide in 100 ml distilled water, After that gently mixed while KOH is fused after this process we must keep it at temp 2-8 C.

4.2.2 Culture Media :

a selective medium used for the isolation of dermatophytes and other types of yeast and fungi is Sabouraud Dextrose Agar (SDA), this media inhibit the growth of bacteria and we can also add antibacterial to improve the inhabit . while pH of this media is around (5.0 acidic ph).

4.2.3 Diagnosis Media :

To detect and classification fungi after growth for microscopic examination the stain used is Lactophenol Cotton Blue (LPCB). it is stained the fungi blue. CHROMagar Candida used to isolated Candida.

4.2.4 **Other material**:

- Nail clipper
- Petridishs (plastic)
- Sterile glass slides and cover
- Bunsen Flame and loop
- Glass tubes

4.3 Methods:

4.3.1 Specimen collection :

All samples were collection after clinical diagnosis and by dermatologist .

Table (4.3) : Specimen collection for each clinical type .

Distal and lateral subungual onychomycosis	scraped and cut from the base of the nail
Proximal subungual onychomycosis	cutting on the surface of the nail plate and collecting debris
superficial white nail onychomycosis	collected by scraping the white spots on the nail surface and debris under them.
Totally dystrophic onychomycosis	collected from nail plate or bed in
Candida spp	collected by scraping the raised nail layer near to the proximal and lateral

4.3.2 Samples process:

Move a drop of KOH solution to a clean slide.. place the sample (small pieces nail pieces should not be more than 2 mm) or crushing of nail to the drop of KOH, Then cover with clean glass. Place the slide damp piece of filter paper or cotton wool to stave off the preparation from desiccate out. immediately examine it microscopically using the 10X and 40X objectives. if light source in microscope used the contrast intensive will not be satisfactory for see the unstained fungi.

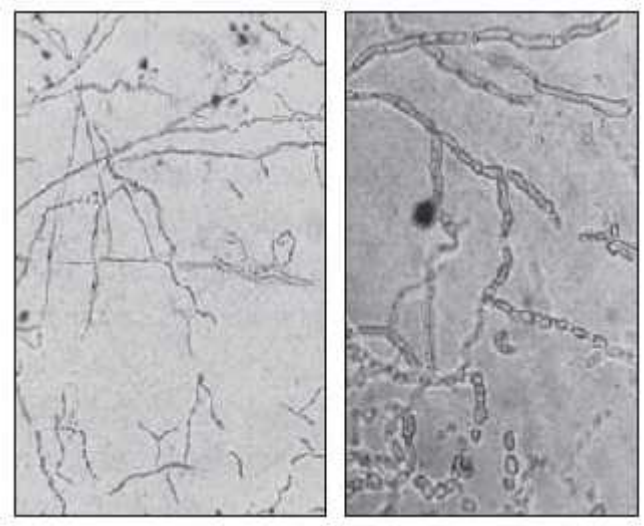


Fig (4.3) : Left: Fungal hyphae in a (KOH) preparation of skin scales as seen with the 10x objective. Right: Hyphae and arthroconidia as seen with the 40x objective.

4.3.3 Culture :

The medium used in this study was Sabouraud's Dextroseagar containing chloramphenicol (50mg/lt) and cycloheximide (500 mg/lt).the nail scrapings sample were inoculated into the medium and were incubated at 25-28°C for growth for 10-14 days.

4.3.4 Examination of culture :

To study the colony that grow on Sabouraud's dextrose agar after period time of incubation, we look for the morphology, surface of colony and color .

colonies were obtained after a 48 hours to one 14 days incubation period at 25°C.Colonies are different in colour, surface and shape , as shown in Figures

Fig (4.3.1) : Candida colonies on SDA

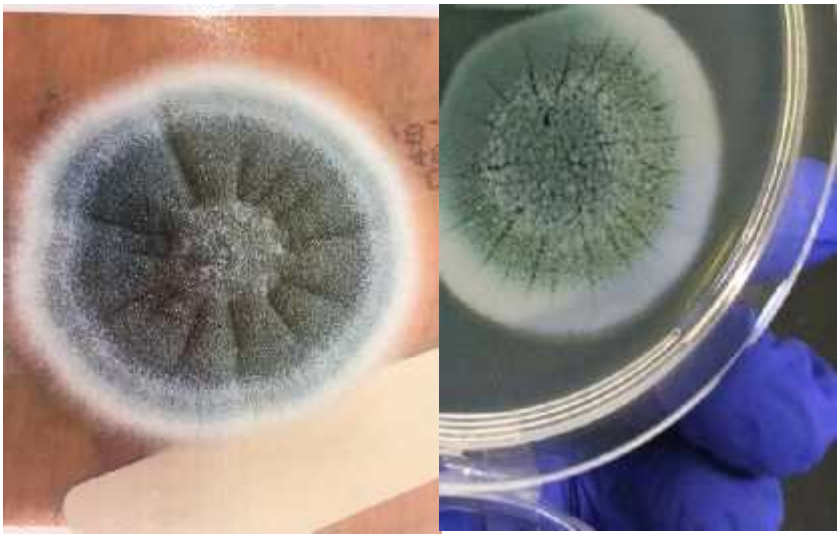


Fig (4.3.2) : Penicillium colonies on SDA

Fig (4.3.3) : Aspergillus Colonies on SDA

Fig (4.3.4) :Trichophyton colonies on SDA

To Identification must take a small amount of colony detected on SDA and add it on a clean glass slide then add a Drop of lactophenol cotton blue (LPCB) and examination under a microscope. Then we identify the fungi under a microscope and we used the atlas of important fungi to characterize each type of fungi.

- In dermatophytes: the sample study under microscope according to size, shape and positioning for microconidia and macroconidia, then variation of hyphae (Taha,.2011).
- non- dermatophytes moulds: study of hyaline and coloured hyphae .
- About Candida: we look to study pseudohyphae and the shape of culture.

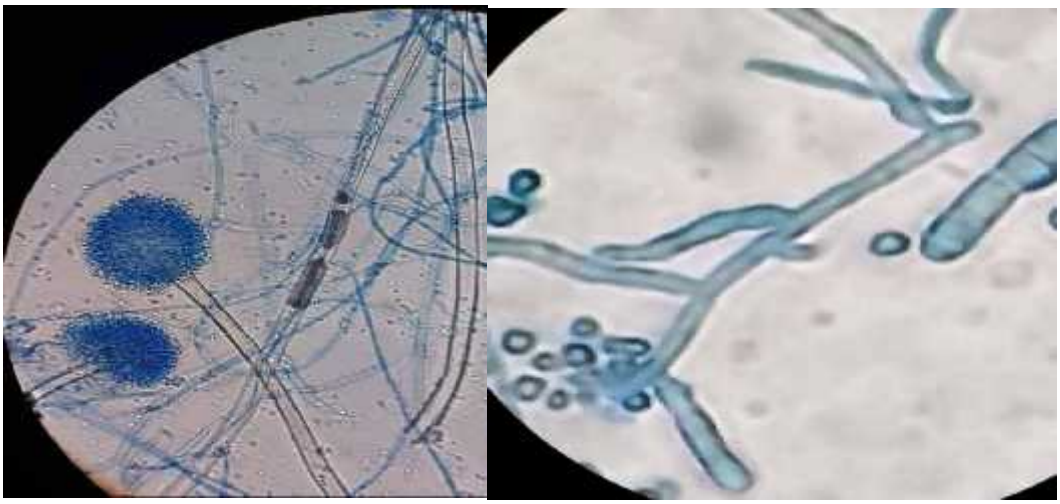


Fig (4.3.5) : Aspergillus spp , Trichophyton spp in LPCB stain

4.4 Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ±standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. A P<0.05 was deemed significant. All statistical analyses were done using statistical packages SPSS version 25.0

According to sample collection to study 300 of samples show topography, surface and rate of growth from 478 samples were collected .

CHAPTER FIVE :RESULTS

According to sample collection total collect 478 samples from Toenails , were 300 samples showed positive result, 149 were male patients (49.6 %) and 151 were female patients (50.4%).

Table (5.1) Age and Sex Distribution

Age in Year	Number of Male	Number of Female
0 _10	1	1
11_20	7	13
21_30	15	8
31_40	22	19
41_50	40	32
51_60	27	48
61_70	27	22
71-77	10	8
Total	149	151

As shown in the previous table, the most affected category was age group from (51_60) years with 74 patients, followed by age group from (41_50)years with 72 patients, then from (61_70) years with 49 patients, from (31_40) years with 41 patients, age group under 30 show 22 patients and the last age more than 71 show 18 patients.

Previous studies mentioned that the age group from 40 to 60 is the most susceptible to infection according to some reasons in the lifestyle, exposure to humid environments, the increase of chronic diseases, obesity and smoking.((Jesudanam,. et al 2002).

- **youngest age was 8 while the oldest age was 77**.
- **63 of patient with diabetes 21.0%** .

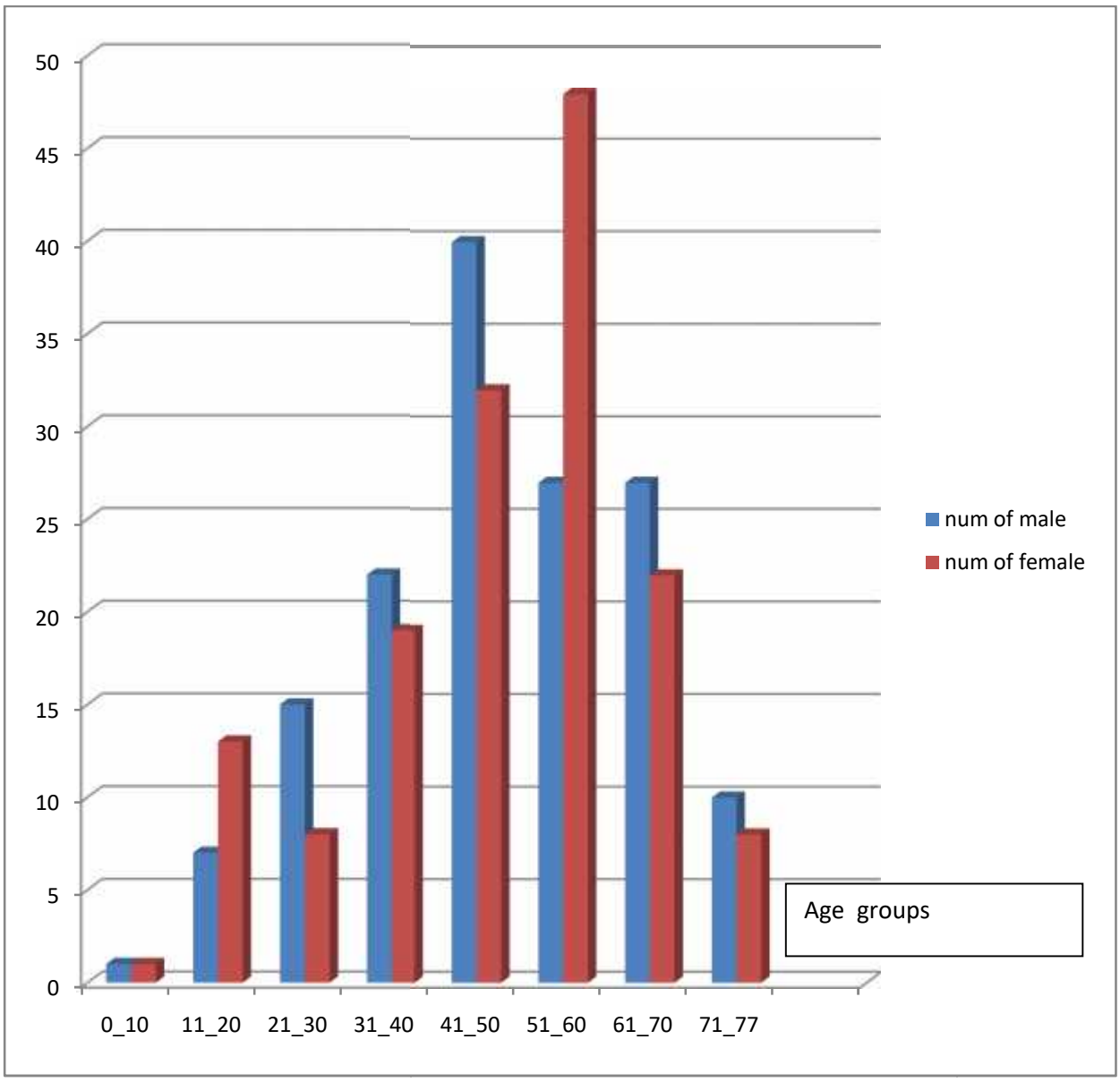
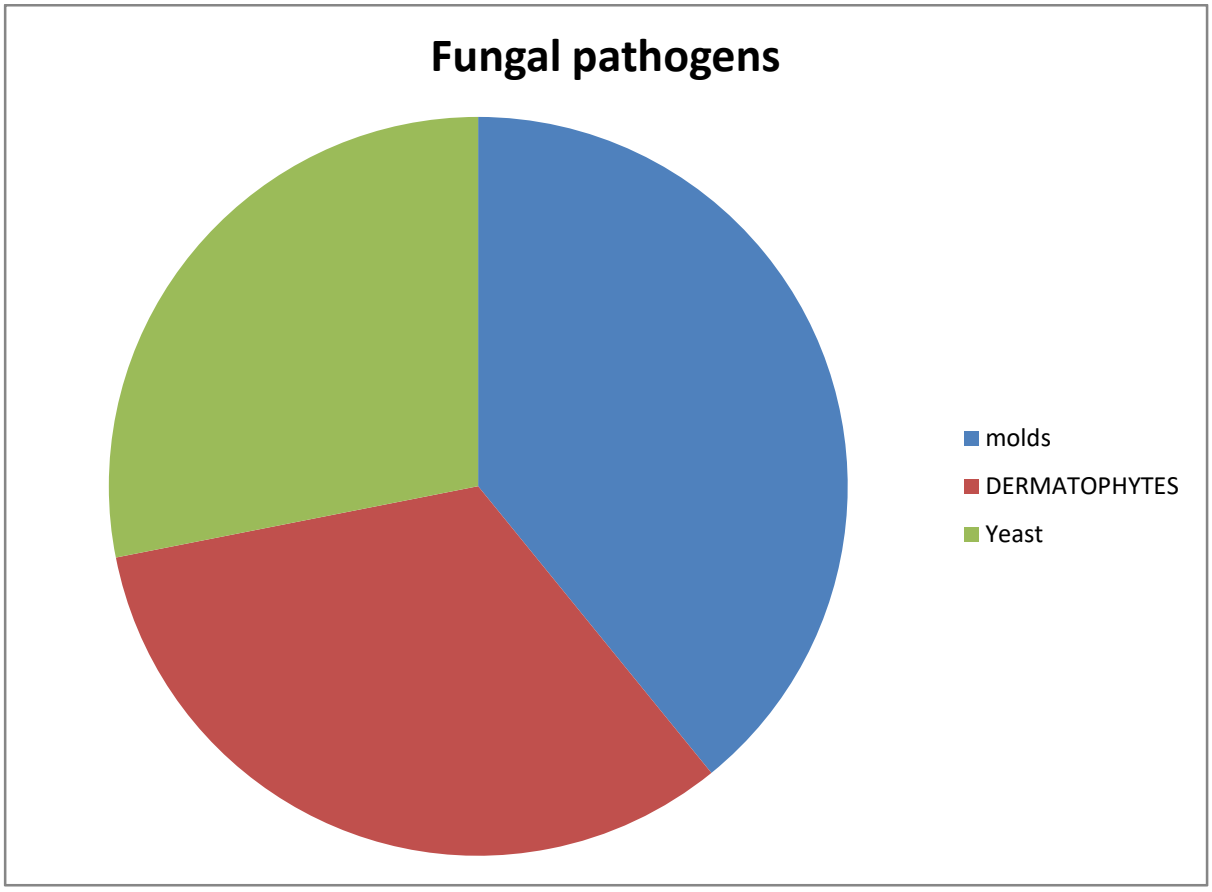


Fig (5.1) : This figure show that female infected more than male with ratio 50.3%

Fig (5.2) : Fungal Pathogens Identification by culture



- Nondermatophytes molds were isolated in 117 specimens 39.0% .
- Dermatophytes were isolated in 99 specimens 33.0 %.
- Yeast were isolated in 84 specimens 28%.

Dermatological, non-dermatophytes and yeast fungi cause onychomycosis, while non-dermatophytes are more common in toenails, and this cause is more noticeable than in older patients in addition to patients suffering from other nail diseases besides chronic diseases and immune deficiency (Greer,.1995).

5.1 Isolated Fungal Pathogens :

Table (5.1.1): Dermatophytes :

Organisms	Number of patients	Ratio
Trichophyton Spp	92	30.6%
Epidermophyton	6	2.0%
Microsporum	1	0.3%

The most organism isolated from Dermatophytes was Trichophyton Spp (30.6%).

According to recent studies indicated that the rate of Trichophyton infection in the world is 5.5%, and the increase in the number of infections may be due to the increase in the number of patients suffering from chronic diseases such as diabetes and HIV, and the increase in patients suffering from obesity and immunocompromised patients.(Gilaberte et al,.2017).

Table (5.1.2) :Non-dermatophytes molds:

Organisms	Number of patients	Ratio
Aspergillus spp	59	19.6%
Penicillium spp	47	15.6%
Cladosporium spp	10	3.33%
Nigrospora spp	1	0.3%

The most organism isolated from Non-Dermatophytes was Aspergillus spp(19.6%) .

Onychomycosis infection caused by Aspergillus spp is very prevalent these days, as it is considered the dominant cause of onychomycosis resulting from non-dermatophytes by 77% for other species and its prevalence ranges between the population in the world to reach 35%.while Aspergillus spp often affects the

toenails 25 times more than the hand, and the reason is due to frequent exposure to soil, water and moisture factors, and is often a type of onychomycosis distal–lateral subungual onychomycosis . (Bongomin et al ,.2018).

Table (5.1.3) : Yeast :

Organism	Number of patients	Ratio
Candida Spp	73	24.3%
Trichosporon spp	7	2.3%
Geotrichum candidum spp	1	0.3%
Rhodotorula spp	3	1.0%

The most organism isolated from Yeast was Candida Spp (24.3%).

Onychomycosis infection caused by candidiasis is found largely in patients suffering from immune diseases such as HIV and chronic diseases such as diabetes and vascular diseases, as well as in patients who take immunosuppressant's and antibiotics for a long period in addition to exposure to detergents and soaps frequently(Jayatilake et al,.2009).

Table (5.1.4) : Sex and Fungal Isolated

Organisms	Male	Female
Trichophyton spp	50	42
Aspergillus spp	33	26
Candida spp	32	41
Penicillium spp	23	24
Trichosporon spp	2	5
Cladosporium spp	6	4
Epidermophyton spp	3	3
Nigrospora spp	0	1
Geotrichum candidum spp	0	1
Rhodotorula spp	0	1
Microsporum spp	0	3

This table shows that dermatophytes, especially the type of Trichophyton, are more common in males than females, in addition to non-dermatophytes represented by the type of Aspergillus, depending on the nature of the work that requires wearing plastic shoes for a long time and sharing the pool and the surfaces of clubs more and some work such as agriculture, while in women we observe It is largely a yeast infection, due to the lifestyle and home jobs, the greater exposure of women to detergents and the use of cosmetics frequently on nails.

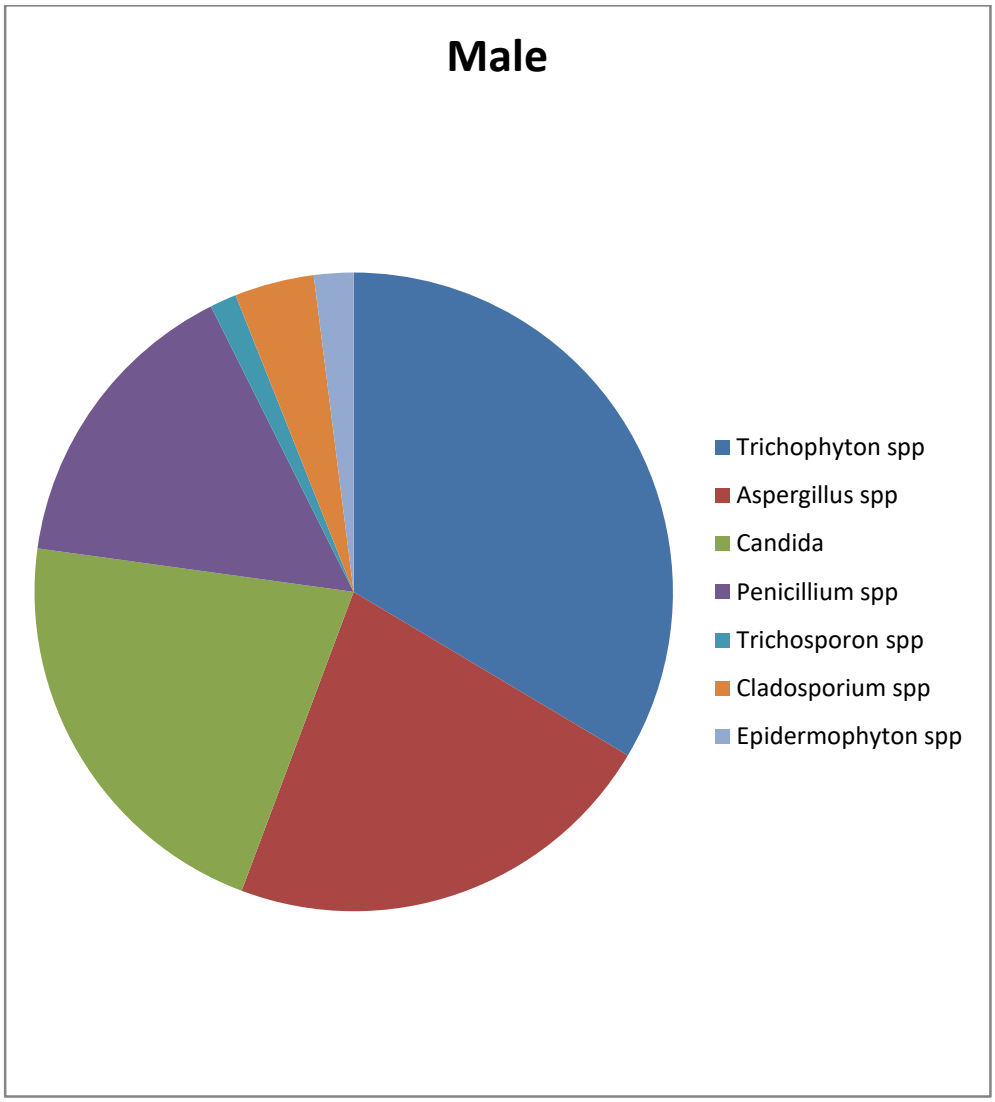


Fig (5.3) : The most species isolated in man was Trichophyton spp with ratio 33.5% , Aspergillus spp 22.1% , Candida spp 21.4% , Penicillium spp 15.4% , Trichosporon spp 1.3% , Cladosporium spp 4.0% , Epidermophyton spp 2.0% , and no isolated for Nigrospora spp, Geotrichum candidum spp, Rhodotorula spp and Microsporum spp.

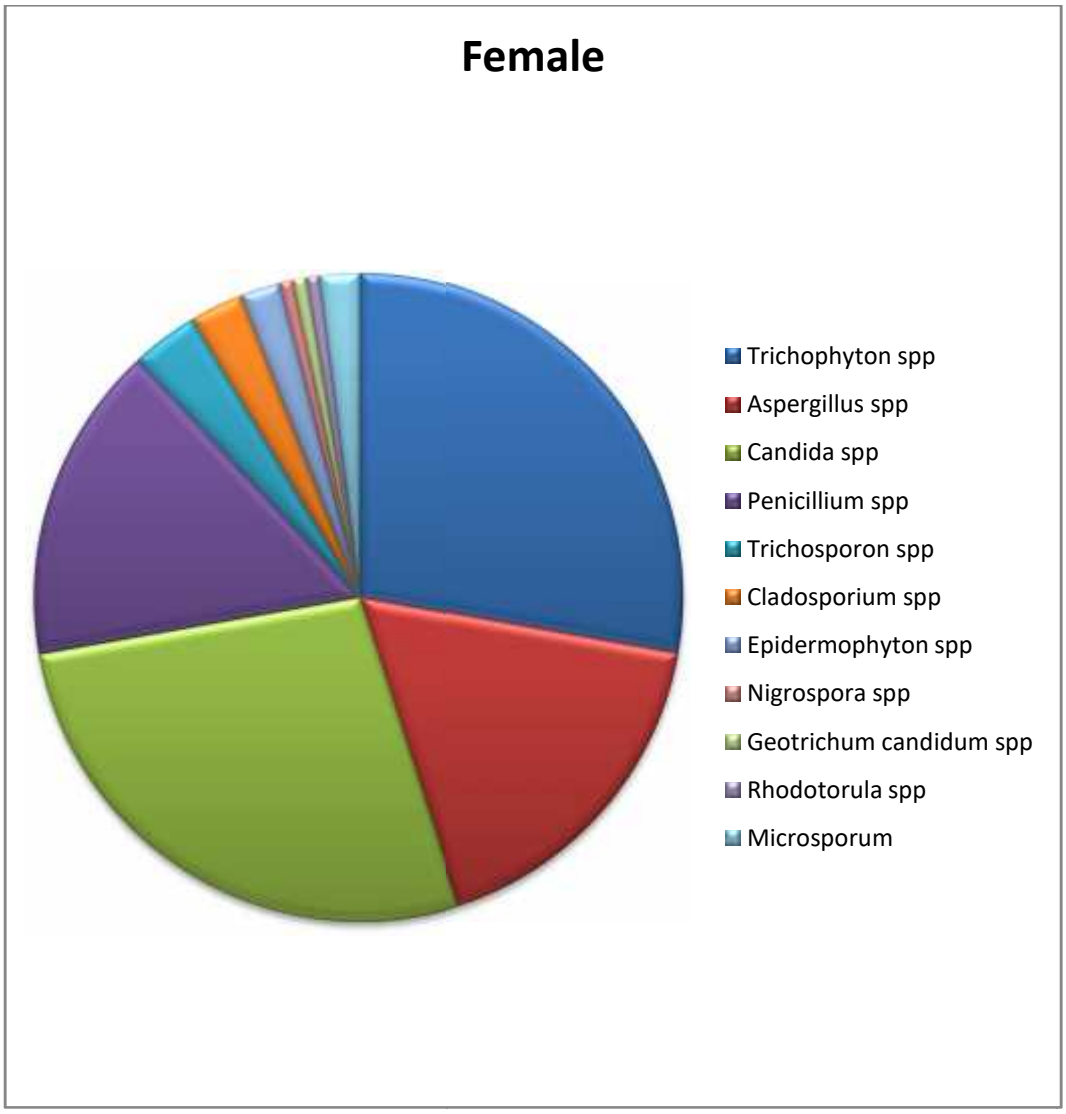


Fig (5.4):In women the most species isolated was Trichophyton spp with ratio 27.8% , Aspergillus spp 17.2% , Candida spp 27.1% , Penicillium spp 15.9% , Trichosporon spp 3.3% , Cladosporium spp 2.6% , Epidermophyton spp 2.0% , Nigrospora spp 0.7%, Geotrichum candidum spp0.7 %, Rhodotorula spp 0.7 % and Microsporum spp2.0%.

Table (5.1.5) : Age and Fungal Isolated

Organisms	1_10	11_20	21_30	31_40	41_50	51_60	61_70	71_77
Trichophyton spp	**0**	**4**	**6**	**16**	**20**	**30**	**11**	**5**
Aspergillus spp	**0**	**8**	**1**	**10**	**16**	**8**	**10**	**6**
Candida spp	**1**	**3**	**9**	**5**	**16**	**19**	**12**	**8**
Penicillium spp	**0**	**3**	**3**	**5**	**13**	**11**	**11**	**1**
Trichosporon spp	**1**	**0**	**0**	**0**	**0**	**2**	**2**	**1**
Cladosporium spp	**0**	**0**	**4**	**1**	**2**	**1**	**2**	**0**
Epidermophyton spp	**0**	**0**	**0**	**2**	**2**	**1**	**1**	**0**
Nigrospora spp	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Geotrichum candidum spp	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Rhodotorula spp	**0**	**0**	**0**	**0**	**2**	**1**	**0**	**0**
Microsporum spp	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Total	**2**	**20**	**23**	**41**	**72**	**75**	**49**	**18**

This table show the most age group infected from (51 _60) , while the less age group infected for children with age from (1_10).Specimens collected from patient with age group (1-77).

Kaur et al reported in previous studies The high rate of occurrence of onychomycosis in a certain category is the result of a relationship with the job and a common lifestyle and the reason for the low incidence of this disease in young people and those who are younger as a result of awareness in terms of aesthetics and the lack of factors that help its emergence(Kaur et al 2008).

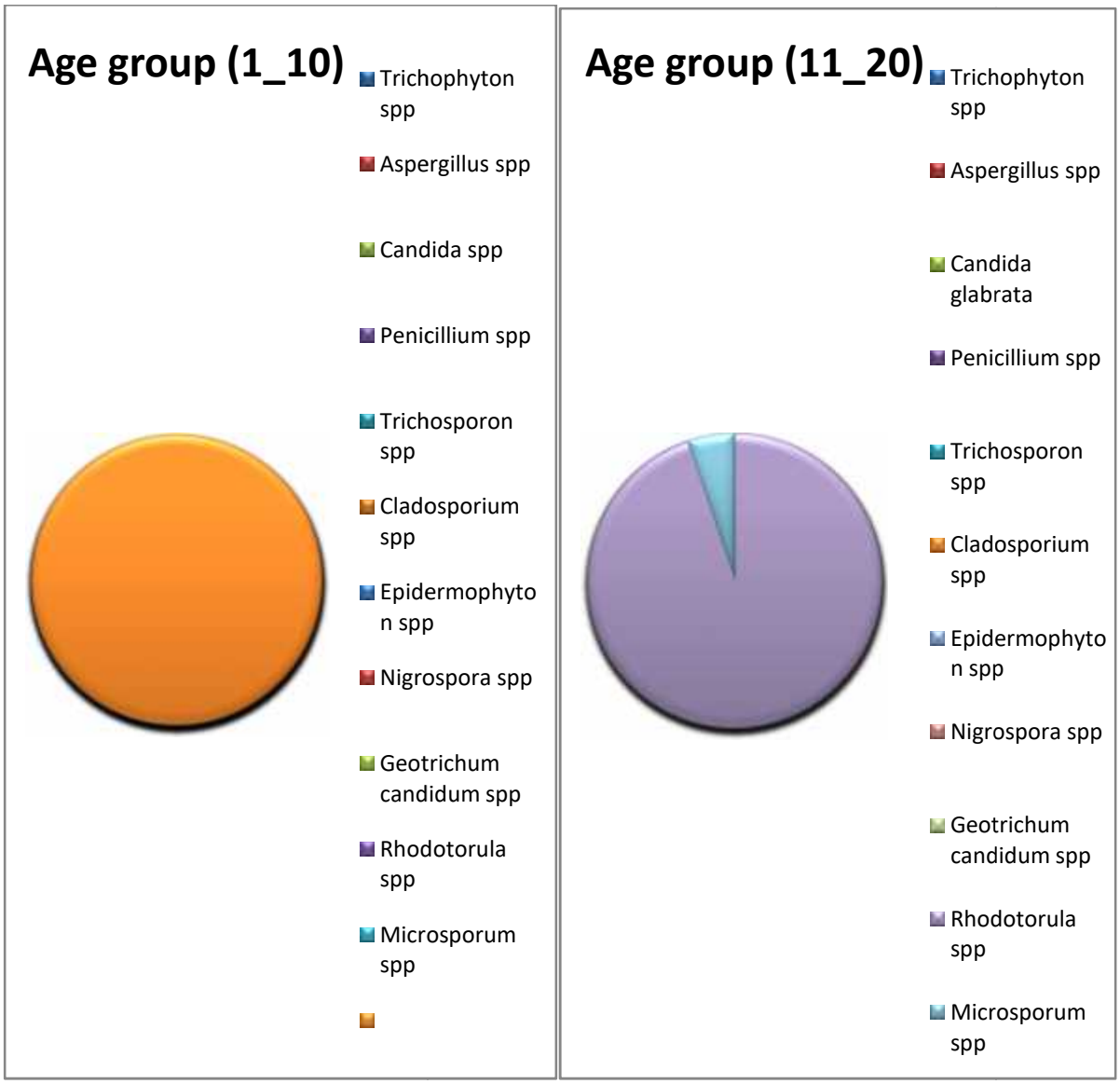


Fig (5.5) :This is graph show that the children and those under the age of twenty are the least affected category, depending on the good health condition they have and not being exposed to the conditions that lead to the disease.

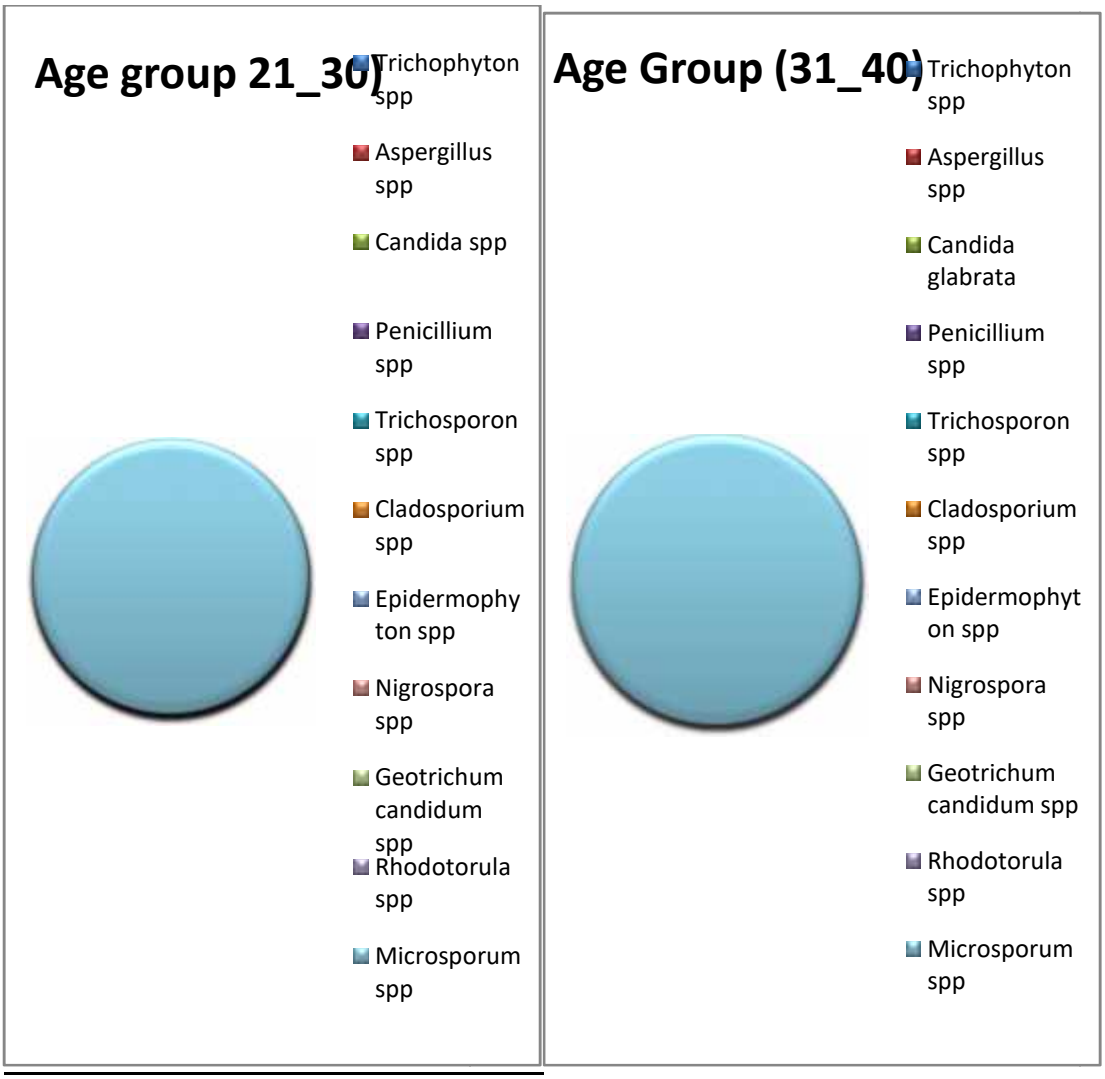


Fig (5.6) :As for the youth category, which mostly represents the category of workers in which infection is more common than adolescents and children , depending on working conditions and other environmental conditions, and in age group (21-30) candida spp is more common than other , while age group (31_ 40) Trichophyton spp is more common .

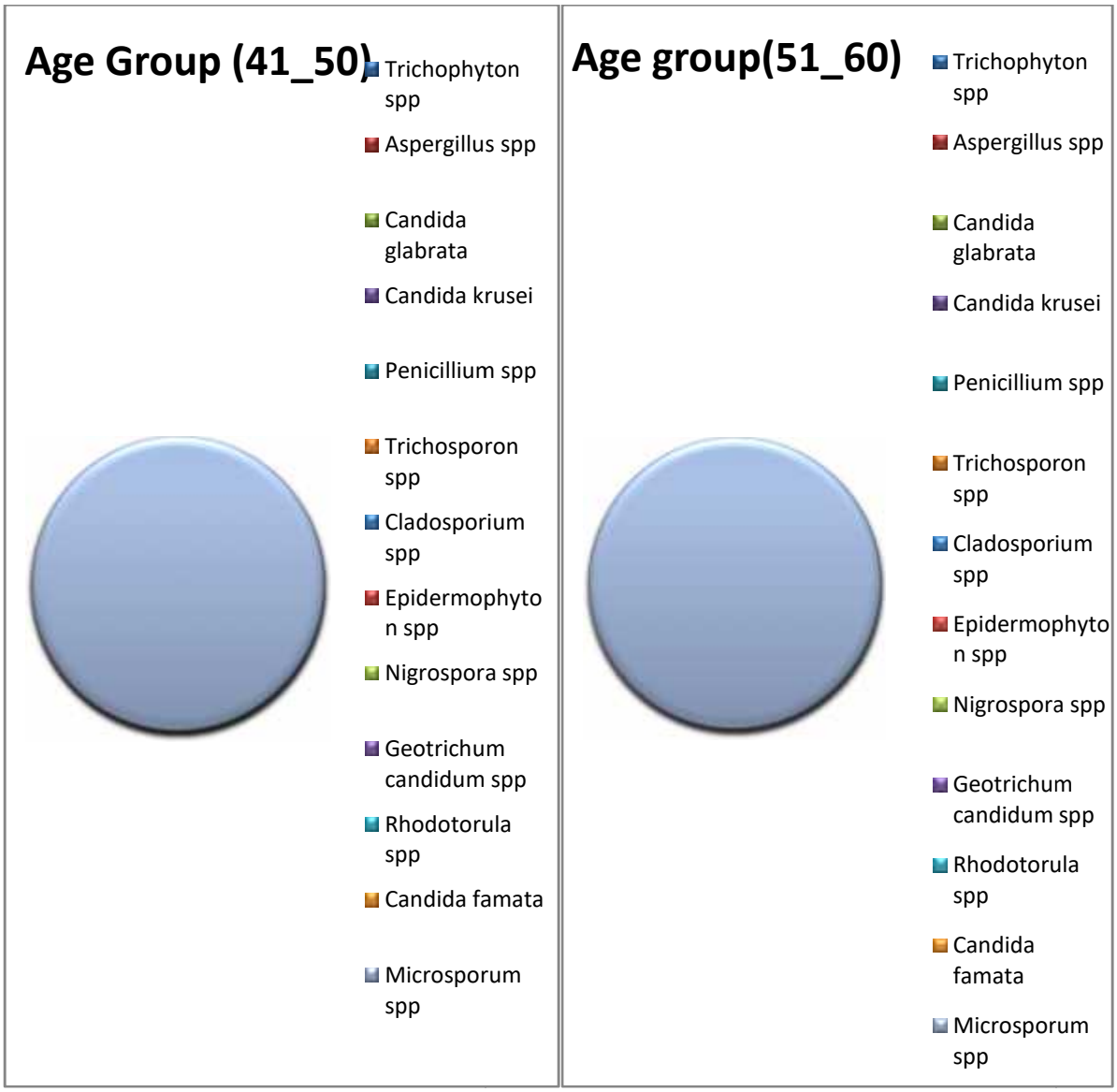


Fig (5.7) As for the age group (41-61), they are more likely to have‘ the infection than other age groups, and Trichophyton spp is the most isolated species from their nails.

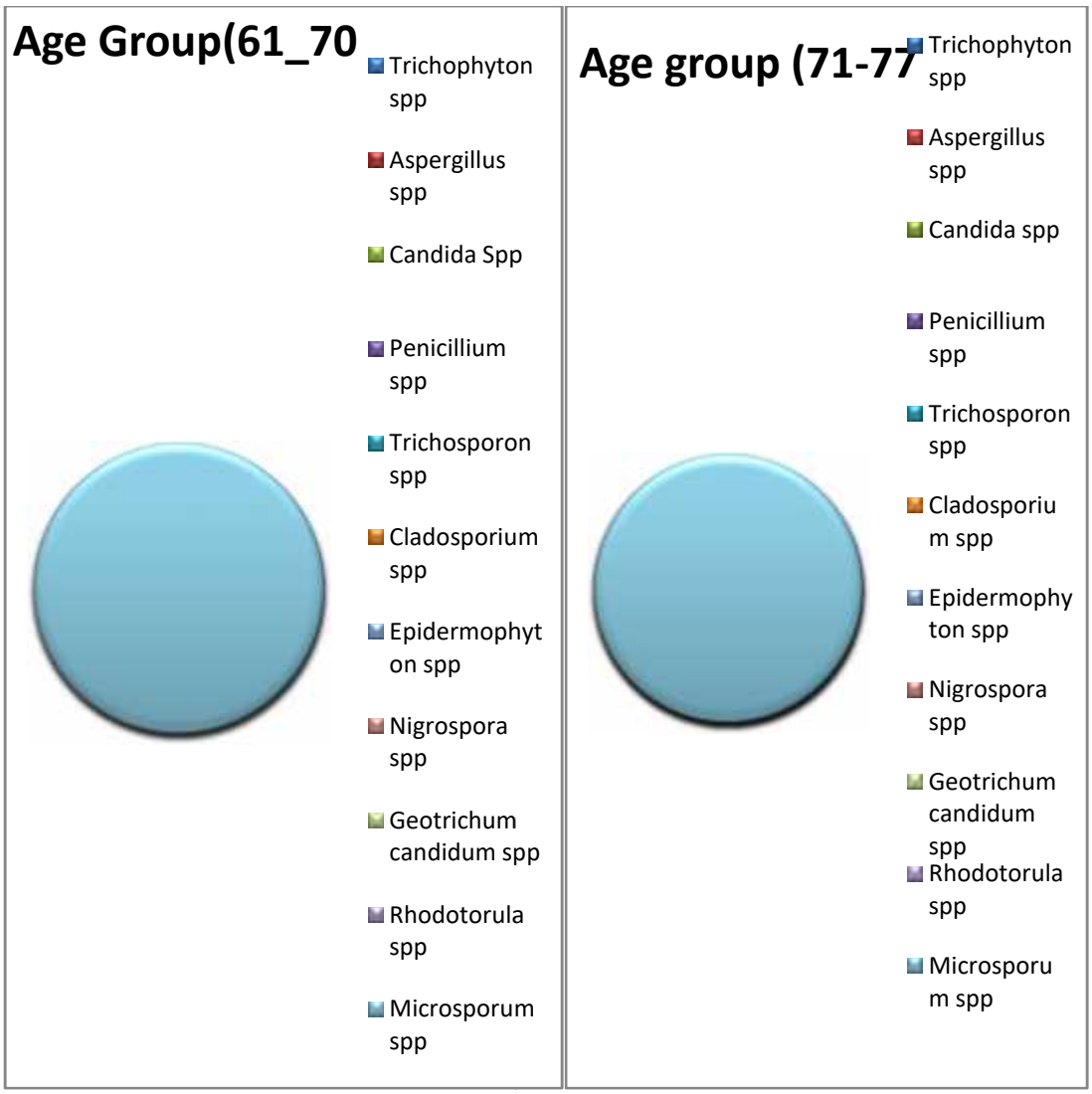


Fig (5.8) : As for the age group 61 to 77, they are also highly susceptible to infection as a result of changes in the immune system and the appearance of some diseases. Candida infections are more common in this group than in other types.

Chapter SIX : Discussion

Discussion

While we see the development of methods of care and hygiene, onychomycosis is still common, dermatophytes , non- dermatophytes and Candida cause nail injuries, a change in the structure and colour of, which leads to psychological problems as it is the biggest cosmetic problem.

Causes conditions such as the surrounding environment in this study patients were living in urban area , working conditions, and sharing sites such as swimming pools and bathrooms. Significantly surfaces and spread of chronic diseases are a serious threat to increased incidence. Work was done with dermatology clinics in Amman and three private hospitals to transfer samples to the reference laboratory at Al Bashir Hospital, the largest government hospital in the Jordanian capital, with the period from August 2019 to December 2019 the largest government hospital in the Jordanian capital, while 478 samples were collected from patients who visited the clinics They are suspected of being infected with fungi. 300 samples showed positive results after the examination.

6.1 Age distribution :

In this study, the most infected age group is (51_60) years, with a mean age of 55 years. previous studies said that the age group from 40 to 60 is more likely to be infected with onychomycosis by a rate ranging between 15 to 20%.(Jesudanam,. et al 2002)This is often due to the circumstances of life, where more attention is paid to preserving the beauty, increased exposure to humid climates and the work that creates these climates more than others who are less old.

6.2 Sex distribution:

In this study, we found that females were 50.3% more likely to be infected, while males were 49.6%. As mentioned in a previous study, females are more likely to be infected than males(Rigopoulos,. et al 1998).This may be due to women working in humid climates at home, such as cooking and washing clothes, as well as going to the salon and sharing nail clippers with others.

6.3Fungal isolates :

In this study, Non-Dermatophytes was the most common with ratio 39.0% then Dermatophytes 33.0% and yeast with ratio 28.0%.

In non-dermatophytes the most species isolated were Aspergillus spp 19.6%, then Penicillium spp with 15.6%, Cladosporium 3.3% and Nigrospora 0.3% About Dermatophytes the most species isolated were Trichophyton spp 30.6%, Epidermophyton 2.0% and Microsporum 0.3%.the most species isolated from yeast were candida spp with 24.3%, Trichosporon 2.3%, Geotrichum Candidum 0.3% and Rhodotorula 1.0% .In this study DLSO was most commonly caused by Trichophyton .spp followed by Aspergillus spp

,then Candida spp , and Epidermophyton spp . In Mo were most commonly caused by Trichophyton spp, then Aspergillus spp and candida spp.In TDO, most common were isolated Trichophyton spp followed by Candida spp , Epidermophyton spp and Aspergillus spp .

CHAPTER SEVEN: Conclusions and summary

- Onychomycosis that affect nails are a common disease, with increasing numbers of cases from previous, It is clinically classified into the main types distal and lateral subungual onychomycosis and it is the most common, proximal subungual onychomycosis, superficial white onychomycosis, total dystrophic onychomycosis, Endonyx onychomycosis and Candidal onychomycosis.

- onychomycosis is a serious problem not only cosmetic, and it is difficult for the patient and doctors, as it can hinder certain functions and functions such as tailors. Because of that, it is a serious problem that should not be neglected.

- The duration of treatment of onychomycosis is long and expensive, which costs the patient time and money for treatment, as well as drug interactions and side effects of treatment, are high because of that it is difficult to treat.

- In this study, the incidence of onychomycosis was 62.7% of total
- specimen collected .

- infection of onychomycosis more common than man with ratio 50.3% to 49.6% in male . Their age from 1 years to 77 years.

- All patients live in urban areas .

- The most common age group infected with onychomycosis were from (51-60) , while the less age group from (0-10) .

- out of 300 sample , 63 cases (21%) were have diabetes

- Non-dermatophytes most isolated , followed by dermatophytes , then Yeast

- the most common organism isolated Trichophyton Spp 30.6%

- From non-dermatophytes the most organism isolated was Aspergillus spp 19.6%

- Candida 24.3% the most common organism isolated from
  yeast .

- DLSO was most commonly .

CHAPTER EIGHT :Reference

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CURRICULUM VITAE

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Educational Level

Name of the Institution where was graduated		**Graduation year**
Postgraduate/Specialization	Near East University, Northern Cyprus.	**2020**
Masters	Near East University, Northern Cyprus.	**2020**
Undergraduate	Al- Balqa' Applied University (BAU), Jordan.	**2015**
High school	The Ministry of Education, Jordan.	**2011**

Master's Thesis	
Title:	THE CAUSATIVE PATHOGENS OF TOENAILS ONYCHOMYOSIS IN AMMAN, JORDAN
Supervisor	Assist. Prof. Dr. EŞREF ÇELİK

Job Experience

Duty	Institution	Duration (Year - Year)
Medical Lab Technician Trainee	Prince Hamza Hospital, Jordan	**2014**
Medical Lab Technician	Al Gardens Hospital , Jordan	**2015-2016**
Medical Lab Technician	Icon medical labs , Jordan	**2015-2016**
Medical Lab Technician	Al Arab labs , Saudi Arabia	**2017-2018**

Courses and Certificate

Name	Name of the Institution where take place	year
Medical Lab Technician Trainee Certificate	**The Ministry of Health, Jordan.**	**2014**
Professional Accreditation Certificate	**The Ministry of Health, Saudi Arabia**	**2017**
Quality control Certificate	**The Ministry of Health, Saudi Arabia**	**2018**

Foreign Languages	Reading comprehension	Speaking*	Writing*
English	**Excellent**	**Very Good**	**Very Good**

Foreign Language Examination Grade

YDS	ÜDS	IELTS	TOEFL	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
*								

Computer Knowledge

Program	Use proficiency
SPSS	**Excellent**
Microsoft office	**Excellent**
Other Computer Programs and Skills	**Excellent**

وزارة الصحة

الرقم ______
التاريخ ______
المرافق ______

Al-Bashir Hospital

Meeting code : —

Ref.No : 00212

Date : 1 / 7 / 2019

RESEARCH ETHICS COMMITTEE APPROVAL SHEET

Title of the project : The causative Pathogen of toenails onychomycosis in Amman, Jordan

Principle Investigator : Farah Abu Khadijeh

Co-investigators : Dr. Moath Badawi , Dr. Mohammad Abdullah

المملكة الأردنية الهاشمية
مستشفى البشير
دائرة المختبرات

2019/7/1

The causative pathogens of toenails onychomycosis in Amman, Jordan

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