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Characterization of dermatophytes and non-dermatophytes isolated from patient with Onychomycosis, attending at Rank dermatology clinic, Addis Ababa, Ethiopia.

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A thesis submitted to the department of medical laboratory sciences, college of health science, Addis Ababa University, in partial fulfillment of Master of Science degree in clinical laboratory sciences (diagnostic and public health microbiology).

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CHARACTERIZATION OF DERMATOPYTES AND NON DERMATOPYTES ISOLATED FROM PATIENT WITH ONYCHOMYCOSIS, ATTENDING AT RANK DERMATOLOGY CLINIC, ADDIS ABABA, ETHIOPIA.

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Approved by the Examining Board

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Internal Examiner	Signature

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ABBREVIATIONS/ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BSC	Biosafety cabinet
CAF	Chloramphenicol
DST	Drug susceptibility test
DM	Diabetes mellitus
GM	Gentamycin
HIV	Human Immune Deficiency Virus
SFI	Superficial Infection
SDA	Sabrouad Dextrose Agar
KOH	Potassium Hydro-Oxide
SPSS	Statistical Packages for Social Sciences
NDM	Non-Dermatophytes molds
LPCB	Lacto-Phenol Cotton Blue
PDA	Potato Dextrose Agar
PH	Power of Hydrogen Ion
PPE	Personal protective equipment

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Abstract

Background: Onychomycosis a chronic nail infection caused by dermatophyte, yeast, and non-dermatophyte molds, is one of the most usual forms of people disease, Onychomycosis in adults contribute around 50% of wholly nail illnesses. Nail mycoses give until 2-9% global and its occurrence is regularly growing mainly in poor state. Even though Onychomycosis is minor illness, it affects patient psychology and also it is expensive disease by means of absenteeism from job due to seeking medication and also it has job discomfort. Regardless of recent advanced in the development of anti-fungal drugs and therapy Onychomycosis is still difficult to treat conventional treatments often fail and it has been suggested that many reasons such as age, peripheral vascular disease, nail growth, poor drug penetration into the nail lesions and fungal growth patterns adversely affect clinical result.

Objective: This research was designed to assess the prevalence of Onychomycosis and spectrum of fungal etiologic agent's associate with the nail mycosis among patients attending at Rank dermatologic clinic from February-July 2019 G.C Addis Ababa, Ethiopia.

Material and methods: The present study was a single institutional cross-sectional study was conducted among a total of 200 patients visiting dermatology clinic from Feb 2019 to July 2019 at Rank dermatology clinic in Addis Ababa. Nail scrapings that was collected from study participants following standard procedure. A small amount nail scraps specimen was put on a slide then added droplet of 20% KOH and stay for a minimum of 5 minutes, then detection was performed by direct microscope for the positive or negative of fungal pathogen. The rest of nail scraped of every specimen was inoculated on culture media. At same time culture processed for every positive or negative result of detection. Every nail scraps specimen was inoculated on two petredishe Mycosel agar and Sabouraud's dextrose agar prepare with antimicrobial antibiotics but not added cycloheximide. All culture media performed based on the manufacture's guide and inoculated media stay at room temperature (25-30 C^{0}) aerobically and put the inoculated plate by

inverted part up to six weeks. The processed media which incubate in the incubator were checked 3 times per week for the presence or absence of any growth. Culture growth supposed for dermatophytes were sub-cultured onto potato dextrose agar to generate pure isolate. Molds were found by investigative macroscopic and microscopic analysis. Microscopic detection of Molds was done by small amount from growth culture putting to new slide and added lactophenol cotton blue reagent then cover by a cover slip and examined by microscope. Yeast investigation of C. albicans by performed by germ tube production. Data was evaluated by performing excel and SPSS version 20 software.

Results: Out of 200 nail scrap samples processed, 161(80.5%) yielded significant Onychomycosis of which 106(65.8) obtained from female patients and 55(34.2%) were from male patients. The ages of research participant were from 2 to 72 year. From 200 study participant, 61(30.5%) fungal element was detected by direct microscopy whereas 161(80.5%) nail scraps specimen was isolated by culture and Six specimens become negative for culture whereas positive by KOH detection. Non dermatophyte were the main pathogen that contribute 99(61.5 %) after that mixed pathogen in 26 (16.1%) patient and then dermatophytes were identified in 22(13.7%) next by yeasts that contributed 14(8.7%) of the isolates. *Aspergillus fumigatus* was the predominate fungal pathogen identified from non-dermatophytes and from dermatophyte, *T.mentagrophyte* was the leading fungal pathogen and also *Candida albican* was the chief fungi identify from yeast.

Conclusion: The prevalence Onychomycosis was high, that was 161/200 (80.5%). However non-dermatophytes were the most common causative agents for Onychomycosis, dermatophyte and yeasts fungi also representing the reason for Onychomycosis are diverse. Clinical diagnosis, direct microscopy and culture isolate are essential for appropriate analysis. These highlight the need for nationwide study on the spectrum.

1. INTRODUCTION

1.1 Background

Onychomycosis is nomenclature define as mycotic disease of nails. Dermatophytes, yeasts, and non-dermatophyte molds became suggest as the causative agents [1].

Onychomycosis represent around 2-9% of the overall people of worldwide [2] and it contribute half of wholly nail abnormalities [3, 4] in addition, it is one of superficial disease [5, 6]. Even though this Onychomycosis is only just severe, such excessive occurrence and the related illness for example physiological effect, not relax for working, life time injury, transmit of the disease to other people and longtime of taking medication and its expensive becoming crucial community health problem [3, 4].

The prevalence of Onychomycosis particularly caused by non-dermatophyte is increasing [7].

The global rate of Onychomycosis is growing and a various of reasons contribute to this nail infection for example a rise in the incidence of chronic illness for instance diabetes and HIV that decrease the immune- situation of person, high taking of medicine decrease immunity, high contact to beauty treatment, becoming elderly of people and shared bathe area, the utilization of narrow foot covering documented as reason for increase of the Onychomycosis [8-12].

Even though the actual prevalence of nail mycoses is undetermined (i.e., widespread numbers in the study is very inconstant), the generality of nail infection and causative agents of the disease are sufficiently study around the globe. but, only a one research that is exclusively carried out nail infection in Ethiopia. Consequently, more studies are required as the prevalence and etiological agents vary from time to time and place to place. Social and economic limitations and shortage of trained personal (mycologist) have been considered as major bottle neck for the investigation, at the end, the primary objective of this study was to evaluate the prevalence of Onychomycosis and the etiological agents.

1.2 Statement of the problem

Onychomycosis defined as fungal infection of the nail. Prevalence studies in many countries, around the world indicate that Onychomycosis show around 50% of wholly nail abnormality and 30% is nail infection from superficial disease. Its prevalence is regularly growing specially in developing nations. Even though Onychomycosis is a minor disease, it has psychological problem and because of high morbidity, it causes loss of job times and it takes long time to treatment so that it become expensive. Nail mycoses being chiefly by dermatophytes but; non dermatophyte fungi are being highly indicated in produce nail mycoses [23].

The spreading of nail infection and its etiologic differ from one area to the other. Reasons for this are reduced blood flow distribution, shared washing, DM, injury of nail, hard to keep correct nail cleanliness and long-lasting using tobacco etc. when nail damage by fungal pathogen lead to long-lasting reservoir which reason for skin mycotic infection and an aesthetic problem and influence the bodily, emotional, community and work-related discomfort of the client [24].

The prevalence of nail mycoses and the chief causative ones are unwell identified in Ethiopia. So, assessing the public burden of Onychomycosis and it's causative thing in Ethiopia is very essential**.** So that the goal of this study was that of assessing the prevalence of nail mycoses and the ethological agents in a sample.

1.3 Significance of the study

The outcome of this research will used as a ground information for epidemiological analysis of Onychomycosis in Ethiopia. Studying of the prevalence Onychomycosis gives important data on the amount of the disease widespread and helps to know disease managing technique. The study also helps the concerned bodies to formulate guidelines for choosing an effective antibiotic therapy and can be used as a source document like DST. Evaluating of the prevalence of nail mycoses is essential to control the extent of the medicinal issues and get-well knowledge about the causative agent and changes over time.

2. LITERATURE REVIEW

Onychomycosis is defined as nail infections commonly happen by dermatophytes, yeast and non-dermatophytes molds and it contribute around 50% of all nail disease [9]. Characterization of dermatophytes and non-dermatophytes isolated from patient with Onychomycosis profiles have been studied by various researchers across the globe in different times. This chapter reviews these studies.

The prevalence and risk to acquire Onychomycosis in mature of people in Spain was investigate in 2000 showed that one thousand case were clinically diagnosis and specimen from nail scraps were obtained from study participant having indicators for fungal nail infection. Prevalence of nail infection was 5.7%. In this study the predominant causative agent was *Trichopyton rubrum* next *by Ttrichopyton tonsurans* then *Trichopyton mentagrophytes* were isolated [10].

Similar study done in Mexico City general hospital out of 70 patients, 34 were men and 36 women, sub ungual and total dystrophic Onychomycosis were the common clinical types (55.1% and 33.7%, respectively). 58 fungal pathogens were identified. Dermatophytes account 48.6% and the predominant pathogen was *trichophyton rubrum* (37.1%). twelve fungal pathogens were identified from yeast. *c. Albicans* and *c. Parapsilosis were* the most common. And 6 non-dermatophytes were identified [11].

The prevalence, epidemiology and risk factors for nail mycosis studied. 109 hemodialysis clients were joined. Onychomycosis was diagnosed in 26.6% of hemodialysis patients. In diabetes patient for Onychomycosis, 68.9% of nail mycoses was isolated from DM client. [12].

Similar study done to check the prevalence nail mycoses from finger nail. 37 % participant were positive from forty-five suspected patients. From which 17.78% were direct microscopy positive for fungal element and negative for direct microscopy were 20.0%.and the predominant pathogen were yeasts that contribute (64.71%), then dermatophytes (17.65%). (11.76%) mixed infection was isolated. Fungal pathogen mostly happened in the mid age, from 31up to 40 years old, because of trauma at the job area and in female, by reason of their wet job [13].

The Epidemiological, clinical and cultural study of Onychomycosis study on 64 patients with Onychomycosis exposed that distal sub-ungual Onychomycosis (DSO) was the commonest type Onychomycosis contribute 47(78.35%) this was followed by candida Onychomycosis10(16.6%),

proximal subungual Onychomycosis (3.34%) and superficial white Onychomycosis1(1.71%). The most isolated fungal pathogen was *Trichophyton rubrum* in 22(35%) then *Trichophyton mentagrophytes* was the next one which was 6(10%) and 3 (5%) fungal pathogen was identify from non-dermatophyte [14].

Another prevalence study done on Onychomycosis in eleven population-based and 21 hospital-based studies was evaluated, it presented that fungal nail infection was the chief in toenails and predominant fungal pathogen found in male. Dermatophyte was the principal fungal group that was 65.0%. from dermatophyte the dominant fungal pathogen was *T. rubrum*. Yeast was the next fungal pathogen that was 21.1% and the least was mold that was 13.3% [15].

An observational and descriptive on the epidemiology of and therapeutic approach to Onychomycosis in Goiana Brazil, from 7,852 patients Onychomycosis positive by clinically analysis was 28.3%. Above 45 years old women who having disease exposed more probability of having nail infection. This infection more found in the feet and the most common fungus was *Trichophyton rubrum* [16].

Another Study done in Brazil by 2004 among 2273 patients with nail infection showed that 1282 cases had confirmed Onychomycosis, the principal causative agent were dermatophytes and yeast. *C.albicans* was the predominant fungal pathogen from yeast that contribute 492 (38.4%). from dermatophyte, *T.rubrum* was the chief that account in 327 (25.6%) and followed by *T. mentagrophytes* that was 258 (20.1%). [17].

Prevalence of nail mycosis and causative agent in South India documented that from ninety-five clinical suspected patients, thirty-eight (40 %) were identify. The main found fungal group was dermatophytes which in 20 (52.6%) followed by molds identify in 18 (47.4%). From dermatophyte *T. mentagrophytes* were the dominant one and from mold the principal fungal pathogen were species of *Fusarium and Aspergillus* [18].

A study done on nail mycoses in Eastern India established that from a total of 249 clinical suspected patients, which in 126(50.6%) have nail mycosis. The predominate fungal pathogen were dermatophyte which was (55.9%). From dermatophyte *Trichophyton rubrum* was the most found that was (65.9%). and from yeast *Candida albicans* was the most common fungal pathogen (79.2%). The least etiological agent was non-dermatophyte that was 15.5% [19].

Similar study done on clinically diagnosis patient for nail mycosis were 150 from which 66.6% were positive for nail mycosis. In this investigation males have greater Onychomycoses. 21-30 years old was the predominate infected age group. Finger nails were more infected by fungal pathogen than toe nail. Dermatophyte the most common (62.68%) follow by non-dermatophyte (29.85%) the least was yeast (7.46%). *T. rubrum* was the predominant fungal pathogen from dermatophyte [20].

Study in Pakistan Punjab Medical College 100 clinically suspected cases, the diagnosis was confirmed by mycologist. Variant clinical manifestation was noted and connected with fungal pathogens. A total of 100 study participant 72 were female and male were 28%. 50% specimen from finger nail and 23% sample found from toenails and 27% of nail scraps from finger and to nail. The main fungal pathogen was Candida (46%), the next was dermatophytes that was (43%) from dermatophyte Trichophyton rubrum the predominate (31%), the least fungal pathogen was non-dermatophyte molds that was (11%) [21].

Prevalence of non-dermatophyte molds in clients appeared with abnormality in nails involving 32 patients in Egypt. In this study the main pathogen was non-dermatophyte (59,4%) follow by dermatophytes. Among non-dermatophyte *Aspergillus* species were predominant fungal pathogen contribute 47%. Dermatophytes were 5(15.6%) and *M. canis* and *T. violaceum* become the main pathogen from dermatophytes.3(9.4%) fungal pathogen were isolated from Yeasts [22].

Study conducted in Ethiopia by Teklebirhan and Bitew, 2015 conducted a study on clinically suspected patient from which the prevalence of Onychomycosis was 51.1%. Dermatophyte were the main fungal pathogen the next fungal pathogen was non-dermatophyte molds and the yeast were also found. [23].

3. OBJECTIVE

3.1 General objective

The objective of this research is to determine the prevalence of Onychomycosis and the spectrum causative agent implicated in Onychomycosis at Rank dermatology clinic, from February up to July 2019 G.C.

3.2 Specific objectives

- To assess the prevalence of Onychomycosis and type of causative agent from February up to July 2019 G.C at Rank dermatology clinic Addis Ababa, Ethiopia.
- To determine the association of Onychomycosis from different independent variables from February up to July 2019 G.C at Rank dermatology clinic Addis Ababa, Ethiopia.
- To determine and compare causative agent involve in causing Onychomycosis from February up to July 2019 G.C at Rank dermatology clinic Addis Ababa, Ethiopia.

4. HYPOTHESIS

The prevalence of Onychomycosis in the investigation place become increase.

5. MATERIALS AND METHODS

5.1 Study area

The study was done at Rank Dermatology Clinic. It is a private dermatology clinic in Addis Ababa Ethiopia and provides dermatology health care services to patients. The clinic has general laboratory have 5 department including Mycology laboratory in this laboratory approximately 20 mycological tests done daily and found Ethio china street, Addis Ababa, Ethiopia.

5.2 Study design and period

A descriptive cross-sectional analysis was performed in Rank Dermatology. The study was carried out from February up to July 2019 G.C.

5.3 Population

5.3.1 Source population

The Source populations were patients seen at Rank dermatology clinic during the study period.

5.3.2 Study population / target population

Study Population was those patients who were referred to mycology department laboratory with medical sign and symptom of nail mycoses at Rank Dermatology Clinic during the study period.

5.3.3 Sample technique and sampling size

A convenient sampling method was performed to take in study participants who meet the inclusion criteria which were limited by time from February to July 2019. A total of 200 study participants were enrolled in the study. Based on to a cross sectional research done at Tikur Anbessa Hospital, the prevalence of Onychomycosis was 16.1%. so, the procedure of the sample size is showed as below:

$$N = \frac{(Z\alpha/2)2*(p)*(1-p)}{d^2}$$

$(Z\alpha/2)2$ = at 95% confidence interval Z value ($\alpha = 0.05$) = 1.96

P = proportion and occurrence of Onychomycosis16.1% (0.16)

n = the sample size

d = margin of error at 5% (0.05)

$$N = \frac{(1.96)2(0.161)(1-0.161)}{(0.05)^2} = 207$$

10 % non-response rate = $\frac{10 \times 207}{10}$ =21, Sample size was therefore 207 + 21 = total sample size was 228.

5.4 Measurement

5.4.1. Dependent Variables

- Prevalence of the disease of the Onychomycosis
- Types of causative agents for Onychomycosis

5.4.2. Independent Variables

- Age
- Sex

5.5 Inclusion and Exclusion Criteria

5.5.1 Inclusion criteria

All study participant with sign and symptom of nail mycoses and provide consent to participate in the study were included in the specified period of study.

5.5.2 Exclusion criteria

Clinically suspected but not permission to give agreement to join in the designated investigation were excluded.

5.6 Data collection procedures

5.6.1 Demographic data

Age and Sex variables was collected from laboratory request form got by the patient.

5.6.2 Sample collection and transportation

The present study was carried out February 2019 to July 2019 at Rank Dermatology clinic. 200 sample of nail scrapings were found from clinically suspected by Onychomycosis. Specimen of

nail scraps were found by swapped infected nails by 70% alcohol then scrapping infected nails by sterilized blade. Samples of nail scraps were put on sterilized plastic petri-dishes and send to microbiology laboratory of school for culture processes. Agreement form was signed by the parents for those patients ≤ 18 years old to found nail scraps sample. For above 18 years old, Samples of nail scraps were found by after finding written informed agreement from clinical suspected patient.

5.7 Laboratory analysis

5.7.1 Sample collection procedure

A total of 200 nail scrap samples from suspected patients was analyzed at the microbiology laboratory of Addis Ababa University during the specified period. Scrapings infected nails from suspected patient with Onychomycosis of previously swapped with 70% (v/v) ethanol. Its procedure performed by the main researcher and personnel of laboratory by sterilized razor blades and transferred into sterilized plastic petri-dishes. Each plate was being appropriately recorded with name, age, sex and date of collection of the patent.

5.7.2 Direct microscopy and Culture

5.7.2.1 Direct microscopy (KOH)

A small amount nail scraps specimen was put on a slide then added droplet of newly prepared 20% KOH and stay for a minimum of 5 minutes, then detection was performed by direct microscope for the positive or negative of fungal elements like budding yeast cells, the Pseudohyphae, the hyphae and the arhthroconidia.

5.7.2.2. Culture

All Sample of study participant was streaked to duplicate plates of Sabouraud's dextrose agar having antimicrobial antibiotics but without cycloheximide and Mycosel agar. All culture media performed based on the manufacture's guide and inoculated media stay at room temperature (25-30 C ^{0}) aerobically and put the plate by inverted part. Inoculated plates which found in the incubator checking 3 times per week to know the present of any growth and incubate it minimum for a month to determine culture negative result. Macroscopic and Microscopic method used to diagnosis culture characteristics (texture, rate of growth, topography, and pigmentation) of mold

by level of species or genus of the growth culture. yeasts were examined by routine investigative technique [15]. Guidebooks and manual [15-17] were used as reference resources in the diagnostic time. urease test was performed for in the differentiation of *T.tonsurans, T.violaceum,* and *T.rubrum.*

5.8 Data quality assurance

The culture samples were transported on time to the diagnostic center. The whole apparatus was checked their work or not. Sterility test was done by incubated 2 % of ready media for a week and check the growth of any contamination, if there was any growth it was be rejected and done it again. Performance test was done before regular analysis was done by using known sample. Before regular examination pretest was performed. I was following SOP to assure laboratory investigation.

5.9 Data processing and analysis

Data entry and analysis was performed using SPSS statistical software version 20 (Statistical Package for Social Sciences, SPSS). Results were analyzed by counts and percentages by means of Statistical methods. During analysis frequencies of the different variables was done, cross tabulations was performed to compare frequencies. Descriptive statistics was used to define the study members in relation to relevant variables. Variables that were expression a significant association was selected for further analysis. P-value less than 0.05 was considered as statistically significant. Lastly, the outcomes performed on words, table and chart.

5.10 Ethical consideration

All ethical considerations and obligations were duly addressed and the study was conducted after the approval of Department Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences then a letter updating to Rank dermatology clinic and authorization was found from rank dermatology clinic to get sample from clinical suspected patient. Written consent and assented was obtained from participants at Rank Dermatology clinic before data collection. Study participant was given full authority to be enrolled in the research and stop participation at any moment through the investigation time All the data gathered from the eligible subjects was given identification code to maintain data security.

6. RESULTS

6.1 Demographic analysis

A total of 200 cases were collected and involved in the study. Male contributes 64(32%) and female 136(68%) which shows 1:2 ratios. The age of the study group was found to be between 2-72 years of age. From the study participant the age 25-44 appeared in highest proportion (n=93, 46.5%) in both genders shown below (table.1)

Table.1 Gender and age distribution of study participant attending at Rank Dermatology Clinic, Addis Ababa, Ethiopia, 2019

Age	Sex		Grand Total
	F (n, %)	M (n, %)	
1-14	15(11.1)	8(12.3)	23(11.5)
15-24	39(28.8)	10(15.3)	49(24.5)
25-44	67(49.6)	26(40.0)	93(46.5)
45-64	14(10.3)	13(20.0)	27(13.5)
>65	1(0.74)	7(10.7)	8(4.0)
Grand Total	136(68%)	64(32%)	200(100.0)

6.2 Prevalence of Onychomycosis

The overall prevalence of Onychomycosis in this study was **161/200 (80.5%).** 106(65.8%**)** were obtained from female of clinical suspected and the rest 55(34.2%) from male. Most Cases of Onychomycosis were 69(42.9%) recorded among age group of 25-44 years followed by the age group 15-24 years 42(26.1%).

Table.2 Prevalence of Onychomycosis patients in Rank Dermatologic Clinic, Addis Ababa, Ethiopia, 2019 (n=200).

Age	No of samples Processed	No of samples with Onychomycosis	No of samples without Onychomycosis
1-14	23(11.5%)	22 (13.7%)	1(2.6%)
15-24	49(24.5%)	42(26.1%)	7(17.9%)
25-44	93(46.5%)	69(42.9%)	24(61.5%)
45-64	27(13.5%)	22(13.7%)	5(12.8%)

≥ 65	8(4.0%)	6(3.7%)	2(5.1%)
Total	200	161 (80.5%)	39(19.5%)

Detection and Isolation method fungal causative agent from eligible clients seen at Rank dermatology clinic, Addis Ababa, Ethiopia (n=200). Out of 200 samples 61(30 .5%) were positive by microscopy and but later found that 161 (80.5%) were positive by culture,139(69.5%) and 39(19.5%) were negative in microscopy and culture respectively.

Table 3. Detection and Isolation rates of fungal pathogens among clients from Feb -July 2019 G.C at Rank dermatology clinic, Addis Ababa, Ethiopia. (n=200).

Microscopy Result`	**Culture result**		**Total Result**
	Culture positive	**Culture Negative**	
Positive	55(27.5%)	6 % (3%)	61(30.5%)
Negative	106(53%)	33 (16.5 %)	139(69.5%)
Total	161(80.5%)	39 (19.5%)	200

Table 4. Spectrum of fungal isolates among patients attending at Rank dermatology clinic, Addis Ababa, Ethiopia, 2019, (n=200).

	Single identify	**mix with other fungi**	**Total identified**
Non dermatophyte			
Alternaria SPP.	2	3	5
Aspergillus SPP.	6	-	6
Aspergillus fumigatus	31	4	35
Aspergillus niger	7	4	11
Cladosporium SPP.	8	6	14
Cladosporium Trichoids	2	1	3
Curvularia SPP.	1	2	3
Epicoccum SPP.	1	-	1
Exophiala werneckii	1	-	1
Fusarium SPP.	11	1	12
Geotrichum SP	1	1	2
Mucor	2	-	2
Penicillium SPP.	13	3	16
Rhizopus	1	-	1
Rhizopus SPP.	6	1	7
Scedosporium SPP.	1	-	1
Scopulariopsis SPP.	4	2	6
Scytalidium dimidiatum	1	-	1
Non dermatophyte Sub total	**99**	**28**	**127**
Dermatophytes	**Single (Pure) identify**	**Mix with other fungi**	**Total identify**
Microsporum audouinii	2	4	6
Microsporum Ferrugineum	1	-	1
Trichophyton mentagrophytes	6	5	11
Trichophyton Soudanense	2	3	5
Trichophyton tonsurans	2	1	3
Trichophyton verrucosum	5	1	6
Trichophyton violaceum	3	1	4
Trichophyton rubrum	1	2	3
Dermatophytes Sub total	**22**	**17**	**39**
Yeast	**Single (Pure) Identify**	**Mix with other fungi**	**Total identified**

Candida SPP	1	4	5
Candida tropicalis	1	1	2
Candida albicans	12	4	16
Yeast Sub total	**14**	**9**	**23**
Total	**135**	**54**	**189**
	Single (Pure) Identify	**Mix with other fungi**	**Total Identify**
Mixed Fungal species			
Cladosporium SPP.+ Microsporium audionii	-	1	1
Alternaria SPP. + Candidia SPP.	-	1	1
Aspergillus fumigatus + Tricophyton rubrum	-	1	1
Aspergillus fumigatus + Microsporum audouinii	-	1	1
Aspergillus niger + Candidia Tropical + Scopulariopsis SPP.	-	1	1
Bipolaries + Alternaria SPP.	-	1	1
Candidia albicans + Asparigillus niger	-	2	2
Candidia albicans +Aspergillus niger + Geotrichem SPP.	-	1	1
Cladosporium SPP. + Aspergillus fumigatus	-	1	1
Cladosporium SPP.+ Trichophyton violaceum	-	1	1
Cladosporium Trichoides + Penicillum SPP.	-	1	1
Curvularia SPP. +Trichophyton mentagrophyte	-	2	2
Fusarium SPP. + Aspergillus fumigatus	-	1	1
Microsporum audouinii + Cladosporium SPP.	-	1	1
Microsporum audouinii + Alternaria SPP.	-	1	1
Rhizopus SPP.+ Trichophyton soudanense	-	1	1
Trichophyton mentogrophyte + Candida SPP.	-	1	1
Trichophyton soudanense + Candidia SPP.	-	2	2
Trichophyton verrucosum +Candida albican + Pencillium	-	1	1
Tricophyton mentagrophytes + Pencillum SPP.	-	1	1
Trichphyton tonsurans + Scopulariopsis SPP	-	1	1
Tricophyton mentagrophytes + Cladosporium SPP.	-	1	1
T.rubrum+ Cladosporium SPP. + Scopulariopsis SPP.	-	1	1
Total	**0**	**26**	**26**

6.3 Fungal pathogens isolated in the study subjects

Species of fungal causative agents were found to be from non-dermatophyte molds, yeasts, and dermatophyte among 200 patients at Rank Dermatologic clinic stated below in figure1. non-dermatophytes were the common and highest in occurring from a total of 161 isolated 99(61.5 %)

next with mixed pathogen in 26 (16.1%) patient and dermatophytes were isolated in 22(13.7%) and yeasts contributed 14 (8.7%) of the identify.

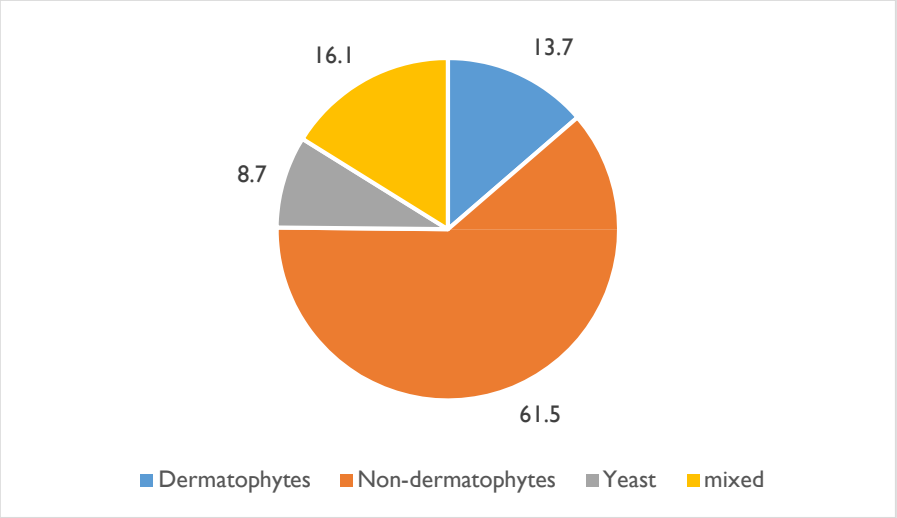

Figure.1 Proportion of causative agents from Onychomycosis from clinical suspected patient seen at Rank dermatology clinic, Addis Ababa, Ethiopia, 2019, (N=161).

Table 5. Gender Characterization of fungal pathogen from clinically suspected seen, from Feb-July,2019 G.C at Rank dermatology clinic, Addis Ababa, Ethiopia (n=161).

Fungal Pathogen	**Male**		**Female**		**Total**	
	Frequency	%	Frequency	%	Frequency	%
Mixed	7	12.73	19	17.9	26	16.15
Alternaria SPP.	1	1.82	1	0.9	2	1.24
Aspergillus SPP.	3	5.45	3	2.8	6	3.73
Aspergillus fumigates	14	25.45	17	16.0	31	19.25
Aspergillus niger	1	1.82	6	5.7	7	4.35
Candida SPP.	0	0.00	1	0.9	1	0.62
Candida tropicalis	1	1.82	0	0.0	1	0.62
Candidia albicans	4	7.27	8	7.5	12	7.45
Cladosporium SPP.	3	5.45	5	4.7	8	4.97
Cladosporium Trichoids	0	0.00	2	1.9	2	1.24
Culuvaria SPP.	1	1.82	0	0.0	1	0.62
Epicoccum SPP.	0	0.00	1	0.9	1	0.62
Exophiala werneckii	0	0.00	1	0.9	1	0.62
Fusarium SPP.	4	7.27	7	6.6	11	6.83
Geotrichum *SPP.*	1	1.82	0	0.0	1	0.62
Microsporum audouinii	0	0.00	2	1.9	2	1.24

Microsporum Ferrugineum	1	1.82	0	0.0	1	0.62
Mucor	0	0.00	2	1.9	2	1.24
Penicillium SPP.	3	5.45	10	9.4	13	8.07
Rhizopus	0	0.00	1	0.9	1	0.62
Rhizopus SPP.	1	1.82	5	4.7	6	3.73
Scedosporium SPP.	1	1.82	0	0.0	1	0.62
Scopulariopsis SPP.	2	3.64	2	1.9	4	2.48
Scytalidium dimidiatum	1	1.82	0	0.0	1	0.62
Trichophyton mentagrophytes	1	1.82	5	4.7	6	3.73
Trichophyton Soudanense	2	3.64	0	0.0	2	1.24
Trichophyton tonsurans	0	0.00	2	1.9	2	1.24
Trichophyton verrucosum	1	1.82	4	3.8	5	3.11
Trichophyton violaceum	1	1.82	2	1.9	3	1.86
Trichophyton rubrum	1	1.82	0	0.0	1	0.62
Total	55	100.00	106	100.0	161	100.00

Table.6 Characterization of fungal isolate in age group from patients seen at Rank dermatology clinic, Addis Ababa, Ethiopia, (N=161).

Fungal Pathogen	1-14	15-24	25-44	45-64	> 65	Total
Mixed	2	6	13	3	2	26
Alternaria SPP.	0	0	1	0	1	2
Aspergillus SPP.	0	1	4	0	1	6
Aspergillus fumigates	6	8	12	5	0	31
Aspergillus niger	1	0	4	2	0	7
Candida SPP.	0	0	0	1	0	1
Candida tropicalis	0	0	1	0	0	1
Candidia albicans	0	4	7	1	0	12
Cladosporium SPP.	1	0	4	3	0	8
Cladosporium Trichoids	1	0	1	0	0	2
Culuvaria SPP.	0	0	0	1	0	1
Epicoccum SPP.	0	0	1	0	0	1
Exophiala werneckii	0	1	0	0	0	1
Fusarium SPP.	3	4	2	1	1	11
Geotrichum SPP.	0	0	0	1	0	1
Microsporum audouinii	1	0	0	1	0	2
Microsporum Ferrugineum	0	0	1	0	0	1
Mucor	0	0	1	1	0	2
Penicillium SPP.	2	7	4	0	0	13

Rhizopus	0	1	0	0	0	1
Rhizopus SPP.	0	3	3	0	0	6
Scedosporium SPP.	0	1	0	0	0	11
Scopulariopsis SPP.	0	1	3	0	0	4
Scytalidium dimidiatum	0	0	1	0	0	1
Trichophyton mentagrophytes	0	4	2	0	0	6
Trichophyton Soudanense	0	0	0	1	0	1
Trichophyton tonsurans	0	0	2	0	0	2
Trichophyton verrucosum	3	1	1	0	0	5
Trichophyton violaceum	0	1	2	0	0	3
Trichophyton rubrum	0	0	0	1	0	1
Total	20	43	71	22	5	161

6.4 Predictors of Onychomycosis for study participant

In this study bivariate and multivariate logistic regression were computed for socio demographic characteristics age group from 25-44 years of age were found to be 0.120 times more at risk of developing Onychomycosis compared to age group less than 15 years of age. Regarding risk of getting Onychomycosis there is no significant association with gender which means both genders had equal risk.

Table.7 Socio Demographic as a predictor of Onychomycosis Attending at Rank Dermatology clinic from February to Julye 2019 in Addis Ababa, Ethiopia**.**

Covariates		Culture results Negative N (%)	Culture results positive N (%)	P-Value	COR (95%C. I)	Odds Ratio	AOR (95%C. I)
Sex	Male	9(23.1)	55(34.2)		1.00		1.00
	Female	30(76.9)	106(65.8)	0.137	0.57(0.25-1.30)	0.52	0.52(0.123-1.23)
Age in years	<15	1(2.6)	22(13.5)		1.00		1.00
	15-24	7(17.9)	42(26.1)	0.192	0.08(0.02-2.18)	0.238	0.23(0.028-2.05)
	25-44	24(61,5)	69(42.9)	**0.043**	**0.81(0.015-0.94)**	**0.120**	**0.81(0.015-0.93)**
	45-64	5(12.8)	22(13.7)	0.135	0.14(0.06-1.16)	0.183	0.38(0.02-1.69)
	≥ 65	2(5.1)	6(3.7)	0.112	1.72(0.80-4.71)	0.125	0.16(0.010-1.62)

7. DISCUSSION

This research was conducted with the objectives of predicting prevalence of nail mycoses and causative agents at Rank dermatology clinic, Addis Ababa, Ethiopia. The variance of Onychomycosis in distribution may be because of Onychomycosis to differ from one environment to other, mainly by variance in nature of climate, difference in the distribution also based on people age, job, predisposing reasons, community status, life style and regularity of journey. Another causes for difference in the prevalence because of don't seek medical attention [26].

A total of 200 cases 68% were from females the rest 32% were males and this is nearer to the research done in Pakistan and Ethiopia [21,23]. But contradiction with the findings of other study in Pakistan [20]. The most abundant of Onychomycosis in our research that was 25-44 years old. The nearest finding in Ethiopia in which the mid age is the most common of Onychomycosis that was 25-44 years old [24].

In our finding of the 200 participants of nail scrapings were collected, fungal pathogen (Mold, mixed, dermatophyte and yeasts) were identified in 61(30.5%) patients nail scraps by direct detection (microscopy) and 161(80.5%) samples of nail scraps were culture positive. Six nails scraps become negative for culture, but fungal element seen by direct microscopy.

The finding of our culture result show that like the previous studies of Mexico that was 83%. The result of the current investigation in by means of culture result was totally not similarity with the result of a research in Brazil which were 28.3%. In this study, A total of 200 study population 61 (30.5%) were microscopy positive and 161 (80.5%) were culture positive. The finding of the current investigation had similarity with the result of other study [13] and [24] However, our finding shown that culture isolation was more sensitive than KOH detection, we recommend that KOH detection is more useful than isolation of culture because of it doesn't need special culture setup laboratory, no need of expensive equipment like BSC and reagent. And, it takes short period of time, in diagnosis of fungal pathogen [23].

Our study shows that from 161 fungi isolated by culture non-dermatophytes accounted the highest that was 99(61.5 %) followed by mixed 26 (16.1%) and dermatophyte fungi 22(13.7%) and yeasts were the least that contribute 14(8.7%). The isolation rate of non-dermatophytes, mixed,

dermatophyte and yeasts obtained in the current study was similar with those of previous studies of Egypt and India that was non-dermatophyte molds was the predominant. A study in Egypt reported that non-dermatophyte was identify that was (59.4 %) from that *Aspergillus SPP* was predominate fungal pathogen which in (47%) next by dermatophytes that contribute (15.6%). Then yeasts that accounted (9.4%) [22]. A study done in India documented that non-dermatophytes were major causative agent that account (35.3%), next by dermatophytes that was (18.6%). and yeasts were contributing that (10 %) [27]. On the other hand, many studies reported that the prevalence of dermatophyte or yeast were greater than non-dermatophytes. Study in Pakistan in 100 clinically suspected cases Candida was the most common pathogen (46%), followed by dermatophytes (43%) and non-dermatophyte molds (11%). in Eastern India the etiological agents were dermatophytes (62.68%), NDM (29.85%), yeasts (7.46%). In our study the predominant non-dermatophyte molds was *Aspergillus fumigatus* followed by *Penicillium species* and *Fusarium species* (19.3%) (8.1%) (6.2%) respectively. This is a good agreement with earlier studies in Egypt and India that was *Aspergillus species* being the commonest isolates accounting 47% and 30% respectively [22,27].

The present study discovered that mixed fungal pathogen agents isolates accounting (15.5%) and the most prevalent species were *cladosporium SPP*. from non-dermatophyte. Among derma tophyte *Trichophyton mentagrophytes* was the predominant by Mix with other fungi. *Candida SPP* and *Candida albicans* were the predominant from yeast by mix with other fungi This result agreed with the study reported [24].

This research also revealed that *Tricophyton. mentagrophyte* was the chief fungal pathogen from dermatophyte (3.73%), next by *Tricophyton. Verrucosum* (3.11%). The finding was similar from study done in south India [18]. However, other studies in brazil, Pakistan and North India (16,20 and 21) reported *T.rubrum* was the predominant dermatophyte.

In our study the principal yeast were *C.albican*. and the outcome is concordant with study done in India (79.2%) [19], brazil (38.4%) [17].

The rate of identification from total of identification in our investigation were from most abundant to least were, *Aspergillus fumigates31*(19.25%), *Penicilliumspecies*13(8.07%), *C.albicans*12(7.45%), *Fusarium species*12(6.83%), *Cladosporium species* 8(4.97%),*Aspergillus*

niger 7(4.35) ,Aspergillus species 6(3.73%), *T.mentagropyte* 6(3.73%), Rhizopus species 6(3.73%), *T.verrucosum* 5(3.11%) ,*Scopulariopsis* species 4(2.48%),Trichophyton *violaceum* 3(1.86%),*Cladosporium Trichoids* 2(1.24%), *Trichophyton tonsurans* 2(1.24%),*Microsporum audouinii* 2(1.24%), *T.Sudanese* 2(1.24%),*Alternaria* 2(1.24%), *Exophiala werneckii* 1(0.62%),*Curvularia species* 1(0.62%),*Scedosporium* species 1(0.62%),*Rhizopus* 1(0.62%),*Geotrichum*1(0.62%),*T.rubrum*1(0.62%),*Scytdedion dimidiatoum* 1(0.62%),*Aspergillus niger1*(0.62%),*Candida species* 1(0.62%), *Candida tropicalis* 1(0.62%), respectively.

Regarding to age, the proportion of Onychomycosis was frequently occurring between age 25-44 (46.5%) and next by age 15-24(24.5%). consistent finding done in India and Ethiopia [5, 24]. In This study from 200 enrolled client's females contributed 68% and males 32%. Age of enrolled clients were between 2-75 years of age. The average age lies between 25-44 years this finding was similar with study conducted in Ethiopia from 303 clinical suspected patient male contribute (33.1%) the rest (66.9%) were females. In further research males were increased at risked by Onychomycosis than females [10, 20]. various research show that females were high at risk than males which good agreement with other documented study [21,23&24]. however, our study revealed nail mycosis not related with Gender, but females contributed (68%). This might be that frequent use of beauty salon, high related with water, applying hard cleaners which can be the reason for nail injury. Health looking for Females are more than males regarding nail mycoses due to cosmetic reasons [24] Though, some research finding reported that males were vulnerable than females because males do have tendency nail injury and using closed shoes [25].

To treat Onychomycosis, only clinical investigation is not enough. It was shown that clinical diagnosis and laboratory investigation are essential to get good result for patent. It is useful to do both direct microscopic examination and culture to get advance finding. After finishes these studies, other associated factors for nail mycosis will be broad field of study and I will advise that larger study shall be done in the future for treatment guideline and policy change.

8. STRENGTHS AND LIMITATIONS OF THE STUDY

8.1 Strengths

- ☐ The outcomes of these research will be used as baseline data for further research. In our study, we report probable fungal causative agent included.
- ☐ The study documents all possible sites of Onychomycosis and potential fungal pathogens.

8.2 Limitations

Because of economic limitation and other constraints risk factor for Onychomycosis of study participant where not collected and drug susceptibility test was not done this will be our active site in future study.

9. CONCLUSION AND RECOMMENDATIONS

Conclusion

The identification rate of fungal pathogen that reason for Onychomycosis was increased that was 161(80.5%) out of 200 study participants. However, Non-dermatophytes were the principal fungal pathogen 99(61.5 %). Mixed, dermatophytes and yeast also were source of causative agent for nail mycosis which contributes 16.1%, 13.7 % and 8.7% respectively. health seeking behavior of females to dermatology clinic with clinical sign and symptoms of Onychomycosis was greater in number and positive diagnosis than males. Aspergillus fumigatus was the predominant pathogen identify from nail scraps.

Recommendations

The below list of recommendations was made based on the findings of the present study:

- ☐ We recommend and encourage clinical diagnosis, direct microscopy and at same time fungal culture together are important for establishing appropriate diagnosis and treatment.
- ☐ As our study has showed that the prevalence of Onychomycosis seems to be high as compared to previous research so that, large scale study on the spectrum and drug sensitivity pattern for this fungal infection is recommended.
- ☐ In addition, we suggest this study gives baseline information, hence, we recommend further study.

10. REFERENCES

1. Weitzman I, Summerbell RC. Thedermatophytes. *ClinMicrob Rev*.1995; 8:240-59.

2. Scher P. Onychomycosis: A significant medical disorder. *J Am AcadDermatol*. 1996; 35: S2-S5.

3. Gupta AK, Jain HC, Lynde CW, Macdonald P, Cooper EA, Summerbell RC. Prevalence and epidemiology of Onychomycosis in patients visiting physicians' offices: a multi centercanadiansur vey of 15,000 patients. *J Am AcadDermatol*. 2000; 43:244-8.

4. Gupta AK, Jain HC, Lynde CW, Watteel GN, Summerbell RC. Prevalence and epidemiology of unsuspected Onychomycosis in patients visiting dermatologists' offices in Ontario, Canada--a multicenter survey of 2001 patients. *Int J Dermatol.*1997; 36:783-7.

5. Sharma S, Capoor MR, Deb M, Ramesh V, Agrawal P. Epidemiologic and Clinico mycologic profile of Onychomycosis from north India. *Int J Dermatol.* 2008; 47:584-7.

6. Veer P, Patwardhan NS, Damle AS. Study of Onychomycosis: Prevailing fungi and pattern of infection. *Indian J MedMicrobiol.* 2007; 25:53-6.

7. Chander J. Dermatophytosis. In: Chander J,editor. Textbook of Medical Mycology. 2nded. *New Delhi: Mehta Publishers;* 2002. P.100-101.

8. Gupta AK, Jain HC, Lynde CW, Watteel GN, Summerbell RC. Prevalence and epidemiology of unsuspected onychomycosis in patients visiting dermatologists' offices in Ontario, Canada--a multicenter survey of 2001 patients. *Int J Dermatol.*1997;36:783-7.

9. Elewski BE, Charif MA. Prevalence of Onychomycosis in patients attending a dermatology clinic in north eastern Ohio for other conditions. *Arch Dermatol* .1997; 133: 1172–1173.

10. Sofia P, Maria J, Margarita G, Alba G, Antonio R, Amalia p. Prevalence and risk factor of tinea unguium and tinea pedis in General hospital Spain. *journal of clinical microbiology*. 2000; 38:3226-3230.

11. Patricia MG, Francisca HH, Luis JM, Yanni PM, Erika CM, Elva BM, et al. Onychomycosis incidence in type II diabetic mellitus patient in Mexico.*journal of copathologia*. 2008; 166:41-45.

12. Kuvandik G, Çetin M, Gultekin G, Horoz M, Duru M. The prevalence, epidemiology and risk factors for Onychomycosis in hemodialysis patients.BMC. *Infectious Diseases* 2007.

13. Gelotar P, Vachhani S, Patel B, Makwana N.The Prevalence of Fungi in Fingernail Onychomycosis. *Journal of Clinical and Diagnostic Research,* 2012.

14. Reddy KN, Srikanth BA, Biradar PM. Epidemiological, Clinical and Cultural Study of Onychomycosis. *American Journal of Dermatology and Venereology* 2012;1(3):35-40.

15. Sigurgeirsson B, Baran R. The prevalence of Onychomycosis in the global population – A literature study. *European Academy of Dermatology and Venereology*. 2013; p 1-12.

16. Souza LK, Fernandes OF, Passos XS, Costa CR, Lemos JA, Silva MR. Epidemiological and mycosis in Goiana Brazil. *Journal Blackwell verlag.Gmbh* mycosis.2010; 53:68-71

17. Di Chiacchio N, Suarez MV, Madeira CL, Loureiro WR. An observational and descriptive study of the epidemiology of and therapeutic approach to onychomycosis in dermatology offices in Brazil. A Bras Dermatol. 2013; 88:1-12.

18. Beena, Bhavana MV, Babu PRS. Onychomycosis: Prevalence and its etiology in a Tertiary Care Hospital, South India. *Int J Health Sci Res.* 2013; 3:81-85.

19. Satpathi P, AcharA,Banerjee D, Mait A, Sengupta M, AnjuMohata A.,Onychomycosis in Eastern India - study in a peripheral tertiary care Centre. *Journal of Pakistan Association of Dermatologists*. 2013; 23:14-19.

20.Mohammed Azam B, Ljaz H, Mohammad J, Tahir S, Shahbaz A, Khawar K. Onychomycosis in Lahore Pakistan. *international journal of dermatology* 1999; 38:591-595.

21. Lone R, Showkat HI, Bashir D, Khursheed S, Sarmast AH. Clinico-Mycological Pattern of Onychomycosis a single center one-year study in Kashmir-North India. Eur *J Gen Med* 2013; 10:150-153.

22. Batawi M, Araot H, Shoeib S, Bosseila M, Fangary M, Helmy AS. Prevalnce of non-dermatophyte molds in patients with abnormal nails, *Egyptial Der*. 2006;2 (1): 1-12.

23.Teklebirhan G, Bitew A. Profile of Dermatophyte and Non Dermatophyte Fungi in Patients Suspected of Dermatophytosis. *American Journal of Life Sciences*. 2015; 3: 352-357.

24. Wolde S, Bitew A. Prevalence risk factor and spectrum of fungi in patients with Onychomycosis in Addis Ababa, Ethiopia. A Retrospective Cohort studies. *Hindawi Journal of tropical medicine.*2019; 6:1-7.

25.Nourchène T, Cyrine D, Dalenda E, Mustapha R, Mourad M, Najla S. Prevalence Etiology and Risk Factors of Tinea Pedis and Tinea Unguium in Tunisia *J Infect Dis Med Microbiol* 2017.

26.Venkatesh VN, Swapna Kotian. Prevalence of Onychomycosis in and around Karwar,Uttara Kannada, *Journal of international medicine and dentistry.*2016; 3(3):126-133

27.Raghavendra KR, Devendra Y, Akshay K, Mukul S, Jitendra B, Anita E. The non-dermatophyte mold: Emerging as a leading causes Onychomycosis in south east Rajastahn, *Indian dermatology online journal* 2015;6: 92-97.

Annex I: Operational Explanations

Dermatomycosis: - disease of the skin and it bring by fungal pathogen of dermatophytes, non-dermatophyte and yeast.

Mycoses: -it is infection of fungal pathogen it appears inside of the body or outside portion of the body.

Dermatophytes: - pathogen of fungal that need keratin for development. These fungi can reason for disease of the nail, hair and skin. This fungal pathogen transmitted by through attachment among other animals, people, soil, and from fomites.

Dermatophytosis: - it is the diseases of nail, hair and skin it happens by a dermatophyte.

Non-dermatophyte molds: - it is fungal pathogen and it is filamentous which are mainly obtained in nature as soil and plant.

Tinea unguium/Onychomycosis: -is defined as fungal disease of nails it happen by fungal pathogen of dermatophytes, yeasts or non-dermatophytes mold.

Tinea/ringworm: - fungal disease of the nail, skin or hair mainly appear by dermatophyte.

Yeast: - it is the numerous small, single-celled fungi that reason for illness.

Annex II: Materials, Equipment's and Culture media

Equipment's obtainable at the study place

- Autoclave
- Incubator
- Microscope
- Glass ware
- Bio safety cabinet (LEVEL II)
- Refrigerator
- Hot air oven
- Balance

Culture media and Items accessible

- Culture media
- Reagents
- Plate
- Stains
- Disinfectants
- Personal protective equipment
- Antibiotic

Annex III: Sample collection, storage and transportation

Clean nail with alcohol sponge. Scrape and discard outer part of nail. take scrapings from inner nail and transfer in envelope or between glass slides. Send a whole nail, if it has been removed, in a sterile screw cap container. Store and transfer at room temperature. Processing Nails necessity be ground in a mortar before inoculated on a medium.

Annex IV: preparation of reagent (formula of reagent)

1. 10% KOH

Method of the preparation

- Potassium hydroxide 10g
- Purified Water 80 ml
- Glycerol 20ml

Liquefy the KOH in purified water, after that add glycerol and combine up to liquify then Filter sterilizes. keep in sterilized amber bottle. store for 3 months.

Purpose: To get clear morphology by digest the debris for instance tissue cells in a specimen to become simply demonstrated.

Principle: KOH reagent not destroy fungal element. Glycerol lengthens time duration by avoiding crystallization and keep the sating slides for short period of time.

Procedure

1. put a droplet of 10% KOH to nail scrap on slide. Cover by Coverslip.

- Gentle heating may aid in dissolving fragments

- If sample is not dissolving easily, wait 15-30 minutes to liquify

2. Examine performed (X10) and (X40) power magnification for the positive or negative of fungal element

2. Lacto Phenol cotton Blue (LPCB)

Reagent for LPCB

- Cotton blue 0.05 g.
- Lactic acid 20 ml.
- Glycerol 40.0 ml.
- purified water 20 ml
- Phenol crystals 20.0 g.

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Procedure of LPCB preparation is First mix phenol in the lactic acid, distilled water and glycerol by lightly heating. After that mix with aniline blue.

2.Sabouraud Dextrose Agar (SDA) mix Chloramphenicol

Estimated preparation of SDA by one Liter of distilled Water

- Dextrose 40.0g
- Peptic 5.0g
- Agar 15.0g
- Chloramphenicol 0.05g

Keep the reagent at temperature of 2–8°C in the dark area.

SDA Principles: Sabouraud Dextrose Agar is a peptone medium added with dextrose to help the development of microorganism. Dextrose gives a power for the development of fungi the peptones are bases of nitrogenous development factors. Chloramphenicol is antibiotic which suppress the growth of any bacteria. The prepared media ready for Autoclave for 121^{O}C and15 minute and then Keep it at room temperature. 40 ml of Prepared media dispensed on sterilized plate.

3. Mycosel agar

Purpose of mycosel agar

This media used to identify fungal pathogen (especially dermatophytes) from contaminated sample It also suppress bacteria and most saprophytic fungi. The other purpose is to determine Cycloheximide resistance of fresh isolates as a screening test for pathogenic fungi.

Method of preparation of Mycosel agar

36 g of mycosel agar suspend in one litter of distilled water. Mix well, after that Heat with regular agitation up to medium boils, to totally mix the powder then Autoclave the media at 118°C for 15 minutes. Don't do more heating and check sample of the ended product for performance using stable, typical control cultures and autoclave prepared media at 118 ^{O}C for 15 minutes then dispense 40ml prepared media for each plate.

4. Potato dextrose agar (pda)

It used to Sporulation medium for fungi and for perform slide culture. 39g Potato dextrose agar mix by one litter purified Water, make to a boil to mix. And make Cool to 5^{O}C. then Autoclave at121 ^{O}C/15 minutes. make Cool. Pour plates (label "PDA"). Shrink wrap plates individually. Put at 4 ^{O}C.

5. Germ tube test

The aim of germ tube test is a fast test for the presumptive detection of C. albicans. and Bovine serum is a reagent a few amounts can be used as a working solution and can be keep at 2 - 8^{o}C. Stock solution can be dispensed into small tubes and keep at. -20^{O}C. Required material are new microscope slides, cover slips, tubes (13 x 100 mm) and Pasteur pipettes.

Germ tube test Procedure

1. add three droplets of serum in a small glass tube.

2. by means of a Pasteur pipette touch a colony of yeast and gently emulsify it in the serum. The Pipette can be left in the tube.

3. keep at 35°C to 37°C for up to 3 hours but not more than 3 hours.

4. put droplet of the serum to a slide for diagnosis

5. Cover by Cover slip and observe microscopically using x 40 objective

Annex V: Participant information sheet (for adult)

My name is Feruza Osman I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am studying a characterization of dermatophytes and non dermatophytes isolated from Patient with Onychomycosis, at Rank dermatology clinic, Addis Ababa Ethiopia. You are requested to join in my study. Please read the next statements and ask any not understand points before you decide to participate. If you allow to be included in this research, I would like to ask you to sign on a file to show your agreement; participate accordingly and give clinical specimen. Participation in this research is exclusively voluntarily. If you are not allowed to join, u can withdraw at any time, there will be no consequences and you will obtain get all the services given in the Laboratory. If you agree to participate, you must sign on the consent form and you may get a copy of this information sheet.

Requirement from participants

As a participant of this investigation, you are expected to give sample of nail scrap. Being asked to give nail scraps sample does not necessarily mean that you have the infection. When your result to be positive for the Onychomycosis, you will be telling by the health worker and get correct treatment. But your name, address will not be disclosed rather an identification code will be taking.

Time need for participating

You will take 10 up to15 minutes until the nail scrap is found and the agreement is signed

Risks of participant

Specimen collection will have no effect and you will not get any risk.

Confidentiality

The data in your records is strictly confidential. All data that you give and the results from your sample will be used for this research only. limited numbers of health worker will have access to the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation

By participating, you will get no financial advantage. Even if there is no direct advantage due to participation in this research. The result of the study is useful for better understanding of the problems of Onychomycosis. You will also get all the findings of the analysis and connected to your physician for the appropriate management.

Rights of participants

Your involvement is totally your agreement and have right to stop or dis continue from the research at any time. Not voluntary to join will not loss any service of clinic or any other advantage. You can get your outcomes of diagnosis.

Communication

Just in case if you want to ask any un clear or not understand ideas and uncertainty about the investigation, communication addresses are:

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Your signature below shows that you have read /or listened, and understand the information given for you about the research. Before you sign, please understand aim of the study, procedure, risks and advantage of participation, right to stop or withdraw, confidentiality and privacy, and who to contact.

I have read /or listened to the description of the research and I understand what procedures are and what will happen to me in the investigation.

Agree to participate? Yes_____ No_____

Annex VI : ቅጽ የተሳታፊ ስምምነት ገጽ

ከ ራሴ የሚወሰደዉ የምርመራ ናሙና ለጥናቱ

አላማ ብቻ እና ብቻ እንደሚዉል ተረድቻለሁ፡፡

የምርመራ ናሙና ዉጤት ሚስጢራነቱን የጠበቀ መሆኑን ተገንዝቤአለሁ፡፡

ተሳታፊ በመሆኔ ምንም የገንዘብ ክፍያ እንደማይሰጠኝ ተረድቻለሁ፡፡

ያለመሳተፍ እንዲሁም በየትኛውም ጊዜ የማቋረጥ ሙሉ መብት እንዳለኝ ተገልንዝቢያለሁ፡፡

መረጃዎች በጥናቱ አስተባባሪ/ዎች ተገልጸልኝ በደንብ ተረድቻለሁ፡፡

ፊርማ፡_____

አድራሻ፡_____

ቀን፡_____

ጥያቄ ቢኖርዎት በሚከተለዉ አድራሻ ያሳውቁን፡፡

Feruza osman **ሞባይል፡** +251911812640

ኢ-ሜይል፤ seidferu@gmail.com

ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ፡፡ ስልክ+251112755170

Annex VII: English versions of participant information sheet (for mothers or guardians)

- You are asked to participate in a research to be conducted by MSC student at Addis Ababa University, college of health sciences, School of allied health science, Department of medical laboratory science, please understand the following statements and ask any not understand points before you approve to participate.
- As a participant of this research you are expected to approve nail scrap from your child. Which In extra you are expected to get answers for few questions about yours and your child health and socio demographic conditions. You need to know that the findings might be conversed with appropriate personals out of this institution. But the name of you or your child, address and phone number will not be disclosed and rather than examination code will be used in such conditions.
- You will stay 10-15 minutes until the sample is taken collected and the consent is signed.
- There are no anticipated risks to your child.
- All data that you give and the findings from your child specimen will be used for this research
- Only professionals will have access to the information.
- Since this research is Msc student study, there will not be payment for participants. Even if there is no direct advantage due to participation in this study, the result of the study is useful for better understanding of the problems of investigation. You will also obtain all the findings of the analysis and connected to your physician for the appropriate management.
- You can right to withdraw your child from the investigation at any time and all the services taken in the institution will not be discontinued. You have also welcomed if you have any question for further clarifications about the research. You can get the findings of the analysis.

In case if you have any questions, not understand ideas and unclear about the study, communicate the researcher by: Feruza Osman (BSc),+251911812640

Email- seidferu@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU, +251911039162

For extra information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170.

Your signature below express that you have read /or listened, and understand the data providing for you about the study. Before you sign, please understand aim of the investigation, procedure, risks and advantage of participation, right to can refuse or withdraw, confidentiality and privacy, and who to communicate if you have question. I have read /or listened to the description of the research and I understand what procedures are and what will happen to me in the research.

Agree to participate?

Yes_____ No_____

Annex VIII: Amharic versions of participant information sheet (for mothers /guardians)

አዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ, የአላይድ ጤና ሣንይስ ት/ቤት, የሕክምና ላቦራቶሪ ሣንይስ ክፍል / አሳዳጊዎች የተዘጋጀ መረጃ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ወይም ያዳምጡና ግልጽ ያልሆነዉን/ትን ማንኛዉም ሃሳብ/ጥያቄ ይጠይቁ፡፡

መግቢያ

እርስዎ ና ልጅዎ በዚህ ጥናት ላይ የሚኖራችሁ ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ተቋም የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡ ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

•በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙና ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚወሰድና ለጥናቱ እንዲዉል መስማማት ይጠበቅብወታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ተቋም ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን እንዲሁም የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል ልጅዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለርስዎና ስለልጅዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨ ማሪ ጥያቄዎች መልስ መ ስጠት ይጠበቅብዎታል፡፡

•የተዘጋጀዉን መጠይቅ ለመ ሙ ላት፤የስምምነት ቅጹ ላይ ለመፈረምና ናሙ ና ለመስጠት ከ 10-15 **ደቂቃ ያስፈልጋል፡፡**

•ናሙ ና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡

•ናሙና የሚሰበሰበዉ ከልጅዎ ከተወሰደ ናሙና ሃኪሙ ያዘዘለት ቤተ-ሙከራ ተሰርቶ ሲያልቅ የተረፈዉ ስለራሶና ስለልጅ የሰጡት ማንኛዉም መረጃና ከሰጡት ናሙና ለጥናቱ ብቻ ነዉ፡፡ ይህን የጥናት ማህደር ሊመለከቱ የሚችሉት ውስን የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ በተጨማሪም ስለ እርሶና ስለ ልጅዎ ያለውን የትኛውም አይነት መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር መረጃ ማህደር ውስጥ ይቀመጣል፡፡

•ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡

•በጥናቱ ውስጥ ያላችሁን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጅን ከጥናቱ በማግለል ምክንያት የሚቀርበት ምንም አይነት የተቋሙ አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱ በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለበት።

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።። ፈሩዛ ኡስማን +251911812640

Email- seidferu@gmail,com

አማካሪ: አዳነ ቢተው (ፒኤችዲ), DMLT, AAU +251911039162

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርስቲ የጤና ትምህርት የህክምና ላቦራቶሪ ክፍልን በ ስልክ ቁጥር፡ +251112755170 እባክዎ ያነጋግሩን ።

Annex IX: English versions of consent form (for mothers/guardians)

•Code number_____

•Name of mother/guardian for participant_____

•I have been informed about the study which is aimed to assess "characterization of dermatophytes and non-dermatophyte isolated from patient with onychomycosis attending rank dermatology clinic

"For this research nail scrap will be collected from my child which is taken for the child's own diagnostics test and the left-over nail scraps will be taken for the research after the requested test is done. The aim of the research was mainly described to me.

I am also known that all the data is to be saved confidential. Also, I understand of my duty and right to keep hold of data, decline to cooperate and can make my child withdraw from the study.

•It is so u get knowledge and information that I provided the learnt agreement voluntarily to the investigator to use the nail scrap taken from my child for the study. In addition, I can do the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the advantage of participation is to get the findings of analysis from my child sample measured for free via the counselor.

•I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

•Participant's mother/guardian signature /finger print _____

•Name of deponent _____sign _____

(For mothers unable to read) Name of counselor _____signature _____

Date _____

Annex X: Amharic versions of consent form (for mothers/guardians)

•የተሳታፊወች ስምምነት ማረጋገጫ ቅጽ

•የሚስጥር ቁጥር_____

•የተሳታፊዉ ልጅ እናት/ አሳዳጊ ስም _____

•እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ ስለሚሰራው ጥናት በቂ ገለጻ ተደርጎልኛል። ለጥናቱም ከልጆች የተወሰደ ናሙና ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚያስፈልግ ተገልጾልኛል። የጥናቱን አላማዎችም ተረድቻለሁ። በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።

•ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው።ናሙና ለምርምር እንደሚውልም ተረድቻለው ።በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ።የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ። በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ/ ተረድቻላዉ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

•የተሳታፊዉ ወላጅ ወይም አሳዳጊ ፊርማ / የጣት አሻራ _____

•የምስክር ስም 1 _____ **ፊርማ** _____

2 _____ **ፊርማ** _____

☐ **(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)**
የአማካሪ ነርስ ስም -_____**ፊርማ** _____

ቀን _____

Annex XI: Demographic and clinical data record format

Demographic and clinical information request Paper Addis Ababa University Collage of Health Sciences Department of Medical Laboratory Science demographic and documented for characterization of dermatophytes and non-dermatophytes isolated from patient with Onychomycosis, joining at Rank dermatology clinic, Addis Ababa, Ethiopia.

i. Sample ID
ii. Age
iii. Sex:

1 male

2 females

iii clinical manifestations

Microscopic (KOH) result

1 Fungal element seen

2 No fungal element seen

Culture result_____

Annex XII: Declaration

I the undersigned, agree to receive all responsibilities for the scientific and ethical conduct of the study project. I was communicating to my advisor and take the essential comment and support during the investigation. I was discussing to my advisors about all sponsors involved in this research.

Name: **Feruza Osman Abdulkadir (BSc.)**

Signature _____

Date of submission _____

Approval of the Advisor

Name: **Adane Bitew (MSc, PhD, Associate Professor)**

Signature _____

Date _____