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The Prevalence of Fungal Keratits in Selected Health Institutions Addis Ababa, Ethiopia

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The Prevalence of Fungal Keratits in Minilik II Memorial Hospital Addis Ababa, Ethiopia

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Contents

List of Table

Abbreviation and acronyms

AAU	Addis Ababa University
CI	Confidence Interval
CLSI	Clinical Laboratory Standards Institute
DMLT	Department of Medical Laboratory Technology
DRERC	Department Of Research and Ethical Committee
PI	Principal Investigator
PKP	Post Keratoplasty
QC	Quality Control
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
SDA	Saboraud Dextrose Agar
WHO	World Health Organization

Abstract

Background: Mycotic keratitis is an important cause of corneal blindness all over the world. Geographical location and climate are known to influence the profile of fungal diseases. While there are several reports on mycotic keratitis from developing and developed countries, the prevalence of mycotic keratitis and its etiologic agents in Ethiopia few is available.

Objective: To demine the prevalence mycotic keratitis and spectrum of fungi implicated in causes mycotic keratits

Material and methods: Corneal scraping was obtained under aseptic conditions with a sterile 21 gauge needle, following the instillation of a local anesthetic by an ophthalmologist. Corneal scraping was inoculated directly onto Sabouraud Dextrose Agar in C-shaped streaks and incubated at 25^{o}C aerobically for four weeks. Cultures of mycelia fungi were identified by examining macroscopic and microscopic characteristics of their colony. Yeasts were identified by employing conventional biochemical and assimilation test procedures and using chromagar Candida culture medium (Becton Dickinson) as per the instruction of the manufacture.

Result: Out of 153 cases of microbial keratitis investigated, fungi were recovered from 69 patients giving fungal keratins prevalence of 45.1%. Of these 29 (42%) were female and 40 (58%) were male patients. 68% and 17 % of fungi were isolated from rural and urban patients respectively. Fungal recovery rate was the highest (47%) in age groups of 25-44. Similarly the recovery rate of fungi was higher in farmers in which out of 33 farmers, fungi were isolated in 30 patients. Trauma was the leading predisposing factor. A total 77 fungal isolates belonging to 12 genera were recovered in this study. Of the total isolates mycelia fungi were the most common isolates accounting 64 (83.1%) of the total isolates. *Fusarium* and *Aspergillus species* were the two predominate mycelia fungi accounting 21(27.3%) and 19(24.8 %) of the total isolates respectively, with together comprising 52.1% of the total isolates. *Cladosporium spp and* Scedosporium *sp* accounted 6(6.5%) and 5(5.2%) respectively. Yeast isolates accounted only 15.6% of the total fungal isolates.

Conclusion A high prevalence of mycotic keratitis**,** highlight the need for nationwide study on the mycotic keratitis and precise identification of the causative fungus and institution of appropriate treatment strategy

1. INTRODUCTION

1.1 Background

Microbial keratitis is predominantly an opportunistic, serious ocular infectious disease that can lead to significant vision loss and ophthalmic morbidity [1]. Fungi are one of the most common infective organisms responsible for this morbidity [2]. The first case of fungal keratitis was reported by Leber in 1879 [3.] Since then, it has been recognized as a major global public health problem particularly in developing nations located in tropical and subtropical regions [4, 5, 6]representing up to 6-50% of all cases of culture proven infectious keratitis [7]. Development and widespread use of broad-spectrum antibiotics and steroids [7] , trauma to the eye [7, 8, 9,11], frequent and prolonged use of contact lenses [11], seasonal variation [12] and ocular surface disease [11] underlying diseases that compromise the immune mechanism of the host [13, 14] have been identified as major factors that contribute to the increasing number of fungal keratitis.

More than 100 fungal species belonging to 56 genera have been implicated in mycotic keratitis [7, 15, 16, 17, 18, 19] the most frequently isolated being**,** *Aspergillus*, *Fusarium, Curvularia, Helminthosporium, Alternaria, Penicillium Candida* and *Bipolaris species*. The diversity of fungi isolated from mycotic keratitis, however, varies with geographical regions studied [20, 21, 22]. Fungal keratitis is reported more frequently from regions with a warm, humid climate and/or with an agricultural economy [20, 21]. Yeast, especially *Candida sp*, predominate in temperate regions, [16, 23, 24] while tropical isolates are most frequently filamentous fungi such as *Aspergillus sp* or *Fusarium sp.* [20, 21, 25, 12]. Filamentous organisms are associated with infections following trauma with vegetable- contaminated matter [8, 9, 10], whereas yeast infections typically occurs in eyes with preexisting ocular surface disease [26]

Ethiopia being a developing nation located in the tropic with a warm, humid climate and agricultural lead economy appears to fall into the category of regions with high prevalence of mycotic keratits. However, like many African countries the public health importance of mycotic keratitis in Ethiopia is poorly known. Lack of routine fungal culture and experts in the field in health institutes are incriminated as the major highest priorities.To this effect conducting research to understand the actual magnitude of mycotic keratitis ant its etiological agents appears to be of the highest priority. Thus, this study was designed to determine spectrum of fungi implicated

in cause the disease. Findings from this study will provide up-to date information on keratitis for evidence-based action aimed at reducing the morbidity of the infection

1.2 Statement of the problem

According to the World Health Organization, corneal diseases are a major cause of vision loss and blindness, second only to cataract in overall importance [27]. It is estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new cases of corneal blindness annually and fungal keratitis is one of the most severe eye diseases worldwide that may lead to blindness, and ophthalmic morbidity especially in the agricultural countries with tropical and temperate climates**.** [28] the most common predisposing factor in most of the cases is trauma [8, 9] Widespread abuse of broad spectrum antibiotics and steroids and increasing use of corneal contact lenses [7, 11] corneal surface disorders, dry eye, bullous keratopathy, and exposure keratitis are also associated with the development of ulcerative keratitis . One report from South India found that 44% of all central corneal ulcers are caused by fungi [29].

More than 70 species of fungi have been reported as pathogenic to human cornea; the most frequently isolated pathogen varies with the geographical area studied. Mycotic keratis is a major problem of developing countries that primarily depend on agriculture. It has been found to account for 6% to 50% of all the cases of ulcerative keratits [7] to this end; it appears to be mycotic keratitis could be a major problem in Ethiopia. Hence, to institute appropriate measures for prevention, accurate diagnosis, and effective treatment of fungal keratitis, a study that determine the prevalence and the etiologic of mycotic keratitis in Ethiopia are of the highest priority.

1.3 Significance of the study

- The results obtained in this study may be used as a baseline data for epidemiological studies of mycotic keratitis in the country.

- Knowledge of the prevalence mycotic keratitis, provides relevant information on the extent of the disease epidemic, helps to identify infection control mechanisms and selection of appropriate antibiotics for empiric treatment
- Fungal isolates obtained in this study can be used for further study such as drug susceptibility study

2. LITERATURE REVIEW

Fungal keratitis is a very serious, potentially sight-threatening corneal infection which most commonly develops in patients after trauma or those with a compromised corneal surface [28] to this effect the prevalence, the etiologic agent and the predisposing factors of mycotic keratitis are studied in different parts of the world.

In USA study by Lisa J.Keay e tal(2011) [30] involving 733 cases shows that most cases were confirmed by culture from corneal scraping (n = 693) or biopsies (n = 19); 16 cases were diagnosed by microscopic examination of corneal scraping alone; and 5 cases were diagnosed by confocal microscopy alone. Some 268 of 733 cases (37%) were associated with refractive contact lens wear, 180 of 733 cases (25%) were associated with ocular trauma, and 209 of 733 cases (29%) were associated with ocular surface disease. No predisposing factor was identified in 76 cases (10%). Filamentous fungi were identified in 141 of 180 ocular trauma cases (78%) and in 231 of 268 refractive contact lens-associated cases (86%). Yeast was the causative organism in 111 of 209 cases (53%) associated with ocular surface disease. Yeast accounted for few cases of fungal keratitis associated with refractive contact-lens wear (20 cases), therapeutic contact-lens wear (11 cases), or ocular trauma (21 cases)

Similarly 24 cases of culture positive fungal keratitis were reviewed by Marco e tal (2000) [31] Out of 24 study subjects fourteen patients (58.3%) were female. Predisposing factors recorded in this study in their descending order were chronic ocular surface disease (41.7%), contact lens wear (29.2%), atopic disease (16.7%), topical steroid use (16.7%), and ocular trauma (8.3%). Half of the cases (12 eyes) had corneal infections caused by yeast, and the other half by filamentous fungi. Of the fungal isolates *Candida albicans* was the most commonly isolated organism (45.8%), followed by *Fusarium sp* (25%).

Jones,D.B. e tal (1970) A study in South Florida. [32] Shows that the fungi isolated were *Fusarium solani* (11 cases), F. *oxysporum* (1), F. sp. (17), *Aspergillus fumigatus* (2), *A.* sp. (1), *Candida albicans (2), Curvularia lunata* (1), *Macrophoma* sp. (1), *Pénicillium citrinum* (1) and *Phialophora verrucosa* (1). The infection occurred most commonly in young men with a history of mild outdoor trauma. Two *F.* infections were in diabetics and 2 in pregnant women. The *C. albicans* infections were in abnormal eyes

In Southeast Brazil a study by Marlon e tal 2009 [33] indicated that ocular trauma occurred in 40% of cases (27). The causing agents were *Fusarium sp* in 67%, *Aspergillus sp* in 10.5% and *Candida sp* in 10%

Study at Shahid Sadoughi Hospital (Yazd, Iran) was carried out by M. R. Shoja and M. Manaviat (2004)(34) involving 80 cases The predominant predisposing factors were corneal trauma (39%), ocular diseases (34.1%), previous ocular surgery (10%) and use of contact lens (9.8%) it revealed that 40% of cases were culture- positive. *Staphylococcus epidermis* (21.9%), *Staphylococcus aureus* (18.8%) and *Streptococcus pneumonia* (15.6%) were the most common isolates. Fungi were isolated in five eyes

Ashok e tal (2010) [35] in Pakistan study involving 315 cases and it indicated that fungi were found to be the frequent cause of suppurative corneal ulcer following agriculture trauma and Candida albicans being the most commonly isolated pathogen. *Candida albicans* was the most frequent organism which was isolated in eighty (69%) patients

Study by JADHAV e tal (2013) [36]. Involving 271 patients and corneal scarpings were collected and fungi microscopy and culture was done. Of 271 patients investigated fungal etiology was established in 68 (25.9%) of which 48(70.58%) were males. Among the fungal isolates, filamentous fungi, i.e 53(77.94%) were predominant. Among these 24(45.28 %) were *Aspergillus flavus* followed by *A. fumigates* 9 (16.98%) and *Fusarium spp.* 8(15.9%). Of the 15 isolates of yeasts, non Candida *albicans* were identified in 9(60%) cases.

The prevalence of mycotic keratitis in north Indian by Parmjeet kaur (2011) [37] was studied and 72 (43.6%) corneal scrapings were positive on direct examination and 65 (39.3%) on culture. Most common fungal isolates were *Aspergillus species* (50%), *Candida* (20%), *Fusarium* (15%), *Penicillium* and *Curvularia* (9.7%), *Paecilomyces and Mucor* (5%). *Exserohilum and Exophiala* (1.3%) each.

Fadzillah Mohd-Tahir e tal (2012) [38] In Malaysia studied involving 47 patients and demonstrated that the incidence of fungal keratitis has increased each year from 2007 to 2011 by 12.50%, 17.65%, 21.21%, 26.83%, and 28.57%, respectively. The most common predisposing factors were injury to the eye followed by use of topical steroid, and preexisting ocular surface disease. *Fusarium species* were the most common fungal isolated, followed by *Candida species*.

A study conducted by Anupma Jyoti Kindo e tal (2009) [39] in SRMC involving 45 patients. Out of 45 corneal ulcers cultured, 10(22%) were found to be bacteria, 20(44%) were found to be fungi, 3(6.7%) were found to be mixed with bacteria and fungi, and the remaining 12(26.7%) were found to be culture negative. The predominant fungal pathogens isolated were *Aspergillus fumigatus* 8 (40%) followed by *Fusarium solani* 7 (35%). cases of *Curvalaria spp*(2),and one each of *Hormonema dermatioidis*, *Alternaria alternata* and *Scytallidium infestans*.

Study by Jin Cao e.tal (2014) [40] in Central China shows the prevalence of presenting corneal diseases was 0.8%, while the prevalence of infectious keratitis was 0.148%. The prevalences of viral, bacterial, and fungal keratitis were 0.065, 0.068, and 0.015%, respectively. There were no significant differences found between the prevalence of viral (accounting for 43.6%) and bacterial (accounting for 46.2%) corneal ulcers. cases of Acanthamoeba keratitis were not found.

Pham Hong Nhu etal (2012) [41] in Vietnam shows 687 fungal keratitis patients was diagnosed and 363 fungal strains were isolated from these patients. The predominant fungal species isolated was *Fusarium spp*. (39.6%), followed by *Aspergillus spp*. (25.9%). No yeast species was isolated. A large proportion of the patients was in the middle decades of life (41-60 years; 51.8%) and worked as farmers in rice fields. Corneal ulceration most often occurred after a superficial corneal injury with organic materials.

There are also studies conducted in Africa which indicates the severity of the disease across the continent

A multicentre study in Ghana and southern India by A K Leck e tal (2002) [42]. 1090 patients were recruited with suspected microbial keratitis. Corneal specimens collected from all study patients were investigated by direct microscopy and culture. Among microbial isolates (fungi and bacteria) 42% in both regions was accounted by filamentous fungi of which: *Fusarium* and *Aspergillus species* were the commonest fungal isolates.

Fayemiwo e tal (2013) [43] in Nigeria**.** The scrapings were subjected to wet preparation with 10.0 % KOH, Gram staining and Giemsa staining to rule out inclusion bodies. The diagnosis of Keratomycosis was made on the basis of the recognizable and characteristic appearance of fungal hyphae and fruiting bodies under microscopy. The media with no obvious growth after 3-4 weeks of incubation were regarded as negative. A total of 48 specimens from patients with suspected diagnosis of Keratomycosis were included in the analysis. The patients consisted of 42 (87.3%)

males and 6 (12.5%) females. The age at diagnosis ranged; from 3 to 73 years with a mean of 36.46 years and a median of 35.5 years. The prevalence of Keratomycosis among this group of patients in this hospital was 8.4 %. *Candida albicans and Fusarium spp* were the fungal isolates in these patients as it occurred in 4.2 % (2/48) of them respectively

Study conducted by TRG Pode,D L Hunter (2002) [44] in northern Tanzania Forty four corneal ulcers were seen. Organisms were cultured from 24 of the 44 ulcers (54.6%). Fifty per cent of positive cultures were fungal. Fungal growth had been predicted by positive microscopy for fungal elements in nine of the 12 cases that grew fungi (75%), and there was never positive fungal microscopy without fungal growth. fungal keratitis accounted for 50% of culture positive cases of microbial keratitis in northern Tanzania, with the majority of these cases (42%) yielding pure fungal isolates on culture. These figures are similar to those published from West Africa, where 56% of microbial keratitis was caused alone or in part by fungi. The most common genus of fungus isolated was that of the filamentous fungus *Fusarium.*

A cross-sectional survey by Zelalem (2014)[45]in central Ethiopia involves 735 children. The most common ocular morbidity encountered was conjunctivitis (35%), then ocular trauma (11.8%), refractive error (11.4%) and trachoma (7.6%). Bilateral visual impairment (UCVA < 6/18 in the better eye) was found in 119 children, and the causes were refractive errors (47.1%), keratitis/corneal opacity (16%), amblyopia (14.3%), ocular trauma (11.8%), cataract (6.3%), Glaucoma (2%) and uveitis (2%).

A cross sectional study by Alemayehu N(2004)[46] in Ethiopia Addis Ababa Four hundred eighty five samples were processed for culture and a total of 235 strains were isolated, with a positive culture yield of 47.4%. The most common etiologic agents isolated from both hospitals were *S. aureus* 57(24.3 %), followed by *S. pnuemoniae* 49 (21%), Coagulase-negative staphylococci 25(10.6%), *H.* xi *influenzae* 22 (9.4%), *Psuedomonas* spp 20(8.5%), *H. aegyptius* 12 (5.1%), *K. pneumoniae* 11 (4.7%), *Moraxella* spp 8 (3.4%), *N. meningitides* 4 (1.7%) and other bacteria 15 (6.3%). The gram-positive bacteria constituted 136 (57.9 %) of the total bacterial isolates. The fungal pathogens isolated were *Fusarium* spp 3 (1.3%) and *Aspergillus fumigatus* 3 (1.3%) followed by *C. albicans* 1(0.4 %). All the fungal pathogens were isolated from keratitis except for one *Aspergillus* isolate from a case of blepharitis

3. OBJECTIVE

3.1 General Objective

To demine the prevalence mycotic keratitis and spectrum of fungi implicated in causes mycotic keratits

3.2 Specific objective

- To determine the spectrum of fungi associated with mycotic keratitis in the study site
- To determine the most common predisposing factors
- To determine the most frequent isolated fungi

3.3 Hypothesis

The prevalence of mycotic keratitis from patients at Minillik II Memorial Hospital is very high.

4. MATERIALS AND METHODS

4.1 Study area and period

The study was carried out in Minilik II Memorial Hospital, Addis Ababa, Ethiopia from January, 2015- July 2015. The hospital is located in Addis Ababa, Ethiopia. The hospital is one of the dedicated centers in the city for the management of infectious keratitis and runs both out-patient and in-patient services. Willing to participate in the study, a presumptive diagnosis of infectious keratitis following an appropriate complaint made at the clinic visit, and no history of antifungal therapy within two weeks prior to the attendance were the inclusion criteria.

4.2 Study Design

A single institutional hospital based cross-sectional study was conducted to determine the prevalence of mycotic keratitis and their predisposing factors in Minilik II Memorial hospital.

4.3 Population

Source population

Source population of the present study was all patients who presented with clinically presumed fungal keratitis with corneal ulceration

Study population

Patients from which clinical samples were taken within the study period

4.4 Sampling Size and Sampling Technique

The world wide prevalence of fungal keratitis ranges from 6-50 %.[7] the colonization rate of fungal species among infectious keratitis patients of Minillik II Memorial and Police Hospital was found to be 7.2%[46]

So by taking the prevalence of 7.2 P= (0.072), a confidence interval of 95% (Z= 1.96 where z is a statistic for a level of confidence) and a sampling error of 5% (d=0.05), the total sample size (n) will be calculated using the formula $n = Z\alpha/22 \times P \times (1- P)/d2$, and substituting the values provides, the sample size of n = 103. Therefore a total of 103 participants will be enrolled using consecutive sampling technique.

4.5 Data Collection and Processing

Specimen collection

Corneal scraping was taken by ophthalmologist after a written informed consent and ascent obtained from adult participants and from the guard for the participants less than the age of 18 years. Corneal scraping was obtained under aseptic conditions with a sterile 21 gauge needle, following the instillation of a local anesthetic by an ophthalmologist and the sample is picked with sterile cotton tip. Material obtained from scraping was inoculated directly onto Sabouraud Dextrose Agar (SDA) plates supplemented with chloramphenicol (Oxoid, Basingstoke, UK) in C-shaped streaks and transported at appropriate temperature with a minimum of delay for microbiological investigation to Mycology Laboratory of Addis Ababa University, Department of Medical Laboratory Sciences

Identification of isolate

Culture plates were examined twice a week for any fungal growth. Cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topography and pigmentation of the front and the reverse side of the culture were employed for the macroscopic identification. Microscopic identification of mold isolates was performed by placing pieces of a colony from SDA to clean microscopic slide and staining with lactophenol cotton blue. After placing a cover slip, each preparation was observed microscopically. Yeasts were identified by employing conventional biochemical and assimilation test procedures and using chromagar Candida culture medium (Becton Dickinson) as per the instruction of the manufacture.

4.6 Measurement

Dependent variables and Independent variables

Dependent variables: fungal isolates.

Independent variables: Socio-demographic factors (age, sex), occupation predisposing factors.

Inclusion Criteria and Exclusion Criteria

Inclusion criteria

All eye patients come with clinically presumed fungal keratitis with corneal ulceration during the data collection period and provide consent for participation

Exclusion criteria

Patients who have been treated with antimicrobial agents less than two weeks before obtaining corneal scrapings and patients unable to consent to participate in this study were excluded

4.7 Operational Definition of Terms

Fungal keratitis: - is predominantly an opportunistic, serious ocular infectious disease caused by fungi and affects cornea that can lead to significant vision loss and ophthalmic morbidity

Corneal ulcer: - is an inflammatory or more seriously, infective condition of the cornea involving disruption of its epithelial layer with involvement of the corneal stroma

Mycelia fungi: - are eukaryotic microorganisms classified as members of the fungus kingdom with 1,500 species currently identified

Yeast: - Yeast is single-celled eukaryotic microorganisms that are classified, along with molds and mushrooms, as members of the Kingdom Fungi.

Chrome agar: - is a novel, differential culture medium that is claimed to facilitate the isolation and presumptive identification of some clinically important yeast species.

Sugar assimilation:- is biochemical identification used for the assessment of the ability of yeast to utilize carbohydrates based on the use of carbohydrate- free yeast nitrogen base agar and observing for the presence of growth around carbohydrate impregnated filter paper disks after an appropriate period of incubation

Germ tube: - The germ tube test is a simple, reliable and economical procedure for the presumptive identification of *Candida albicans*

PKP (postkeratoplasty):- post corneal transplantation.

4.8 Data quality control

All data quality control tools (pre-analytical, analytical and post-analytical stages) of quality assurance that were incorporated in standard operating procedures (SOPs) of the microbiology laboratory were strictly followed. Adequate specimen was collected using appropriate equipment and method. The specimen was kept free of contamination. All materials, equipment and procedures were adequately controlled. Culture media was tested for sterility and performance. The performance of equipments (autoclave, incubators and refrigerators) was monitored by using standard procedures. The data were checked for completeness and representativeness prior to entry.

4.9 Statistical Analysis

Information from the laboratory analysis was cleaned, coded; double entered in Excel and analyzed using SPSS (Statistical Package for Social Sciences) version 20. Tables and graphs were used to describe the results in Percentage ,and frequency. Categorical variables will be summarized by proportions and percentages. Comparisons of proportions between groups were done by chi-square test, and the significant level set at $p < 0.05$.

4.10 Ethical consideration

The study was conducted after it was ethically reviewed and approved by the Department Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences, College of Health Sciences, and Addis Ababa University. Permission was obtained from Menillik II Memorial hospital. Informed written consents were obtained from participants before data collection. The respondent was given the right to refuse to take part in the study .All the information obtained from the study subjects were coded to maintain confidentially

4.11Dissemination of results

After conducting the research, the results of the study will be submitted to Department of Medical Laboratory Sciences (DMLT) Addis Ababa University (AAU). Oral presentation of the thesis will be made. Reports will also be submitted Minilik II Memorial Hospital, annual conferences of professional societies and other concerned bodies. Since it is said that scientific work is incomplete until published, the manuscript will be submitted to peer reviewed journals for publication.

5. RESULTS

5.1 patient characteristics

During the study period one hundred fifty three (153) patients having the clinical diagnosis of microbial keratitis were examined of which 90 (58.8%) were male and 63 (41.12%) were female patients. The ages of study subjects ranged from 9 -91 years with a mean age of- years. Majority of patients 64 (41.8%) were in the age groups 25 to 44 years. Males 90 (58.8%) were more affected than females 63 (41.1%) Table 1

Table 1. Distribution of patients with corneal ulcer according to age groups (n=153)

Age (years)	Males (n, %)	Females (n, %)	Total (n %)
1-14	11(12.2%)	15(23.8%)	26(16.9%)
15-24	26(28.8%)	12(19.04%)	38(24.8%)
25-44	41(45.5%)	23(36.5%)	64(41.8%)
45-64	8(8.8%)	12(19.04%)	20(31.7%)
>65	4(4.4%)	1(1.58%)	5(3.26%)
Total	90(58.8%)	63(41.1%)	153(100%)

As shown in table 2 majority of patients 84(54.9%) was from rural areas, whereas 69 (45.1%) were from urban population (p= 0.04). Thirty three (21.6%) patients were farmer by profession (p= 0.002) while 23 (15.01%) laborer, followed by students 20 (13.1%) and unemployed 17(11.1%).

Table 2 Distribution of patients with corneal ulcer according to occupation and residences (n=153)

Occupation	Frequency	Percentage	Positive	%
Farmers	33	21.6	30	43
Students	20	13.1	8	12
Trades man	7	4.6	6	9
Laborers	23	15.0	13	19
Unemployed	17	11.1	12	17
Carpenter	7	4.6	0	0
Driver	7	4.6	0	0
Cleaner	9	5.9	0	0
Secretary	6	3.9	0	0
Surveyor	9	5.9	0	0
Teacher	15	9.8	0	0
Total	153	100.0	69	100
Residence				
Rural	84	55	47	68
Urban	69	45	22	32
	153	100	69	100

In this study, the predominant predisposing factor for corneal ulcer was trauma, which was present in 54 (70.1%) of cases, followed by use of topical steroid 7 (7.8 %), together comprising 77.9% of all the cases.

Table 3. Distribution of patients with corneal ulcer according to risk factors (n=153)

Risk factor	Total observed	Fungal Isolate					
		Yeasts		**Filamentous fungi**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
ocular disease	7	4	21	1	1.7	5	6.5
Contact lens	4	0	0	2	3.4	2	2.6
Diabetes	7	1	5.2	4	6.8	5	6.5
Perforated cornea	2	0	0	0	0	0	0
Poor operation	1	0	0	1	1.7	1	1.3
Post-operation	5	1	5.2	1	1.7	2	2.6
Trauma	91	8	42.1	46	79.3	54	70.1
Undefined	26	1	5.2	0	0	1	1.3
Use of topical steroids	10	4	21	3	5.17	7	7.8
Total	153	19	100	58	100	77	100

Out of 153 cases of microbial keratitis investigated, fungi were recovered from 69 patients giving fungal keratits prevalence of 45.1%. Of these 29 (42%) were female and 40 (58%) were male patients. 47(68%) and 22(31.8 %) of fungi were isolated from rural and urban patients respectively. Fungal recovery rate was the highest 33(47%) in age groups of 25-44. Similarly the recovery rate of fungi was higher in farmers in which out of 33 farmers, fungi were isolated in 30 of them.

Table 4 Demographic characteristics of 69 cases of culture-proven fungal keratitis (n=69)

Demographic peculiarities		**N (%)**	**Fungal isolates (n, %**
Sex	Female	63(41.1%)	29(42)
	Male	90(58.8%)	40(58)
Age	1-14	26(16.9%)	6(8.6)
	15-24	38(24.8%)	22(31)
	25-44	64(41.8%)	33(47)
	45-64	20(31.7%)	8(11.5)
	>65	5(3.26%)	-
Residence	Rural	84(55)	47(68)
	Urban	69 (45)	12(17)
Occupation*	Farmers	33(21.6)	30(43)
	Laborer	23(15)	13(19)
	Unemployed	17(11.1)	12(17)
	Students	20(13	8(12)
	Trades man	7(4.6)	6(9)

*Occupation in which fungi are isolated

A total 77 fungal isolates belonging to 12 genera were recovered in this study. Of the total isolates mycelia fungi were the most common isolates accounting 64 (83.1%) of the total isolates. *Fusarium* and *Aspergillus sp* were the two predominate mycelia fungi accounting 21(27.3%) and 19(24.8 %) of the total isolates respectively, with together comprising 52.1% of the total isolates. *Cladosporium sp and Scedosporium sp* accounted 6(6.5%) and 5(5.2%) respectively. Yeast isolates accounted only 15.6% of the total fungal isolates.

Table 5. Spectrum of fungi isolates from patients with mycotic keratitis

S.no	Fungal isolates	Pure culture	Mixed culture	Total isolates	% of the total
6	*A. fumigates*	3	-	3	4.0
7	*A. niger*	10	1	11	14.3
5	*A.flavus*	4		4	5.2
8	*A.terreus*	1	-	1	1.3
3	*Acremonium sp.*	3	-	3	4.0
4	*Alternaria sp.*	1	-	1	1.3
1	*C. albicans*	8	3	11	14.3
9	*Cladosporium spp.*	5	-	5	6.5
10	*Curvularia spp.*	1		1	1.3
11	*Fusarium oxysporium*	5	1	6	7.8
12	*Fusarium solani*	14	3	17	22.1
14	*Paecilomyces spp.*	0	1	1	1.3
13	*Penicillium spp.*	6	-	6	7.8
15	*Rhizopus spp.*	1	-	1	1.3
2	*Rhodotorula sp.*	-	1	1	1.3
16	*Scedosporium sp*	1	3	4	5.2
17	*Sepedonium sp.*	1	-	1	1.3
	Total	64	13	77	100

6. DISCUSSION

During the study period one hundred fifty three (153) patients having the clinical diagnosis of microbial keratitis were examined. Of 153 patients 90 (58.8%) were male and 63 (41.12%) were female patients indicating that male patients were strongly affected than female patients. A high prevalence rate of infectious keratits in male patients in the present study was well within the reported range. A prevalence rate of 57.5%, 64.2%, 71% and 61.3% were reported by earlier similar studies (Shoja and Manaviat) [34] (Behboody and Mohammadi) [47], (Ormerod , Hertzmark) [48] , (Srinivasan) [15]. The result of this study showed the highest frequency of corneal ulcer was recorded in age group of 25-44 years that is similar to the observations made by another study (Derek) [49]. This study also showed that farmers were more affected than patients working in other occupations. This finding is in good agreement with similar studies conducted [47]. In this study, as in another study Schaefer Frederic [50] trauma was the leading predisposing factor accounting 59.5%.

Due to increasing incidence in past three decades and insignificant responses to antifungal agents (Bagyalakshmi et al) [51], fungal keratitis has become one of the leading causes of visual loss in many developing countries, where the large numbers of the population are farmers. Although mycology has undergone remarkable changes by taking full advantages of spectacular developments in molecular biology, and chemistry to improve the understanding of phenotypic and genotypic characteristics of fungi, fungal keratitis remains a diagnostic and therapeutic challenge to ophthalmologist. The difficult matters lie in establishing a clinical diagnosis, isolating the etiologic fungal organisms in the laboratory, and treating the keratitis effectively with topical antifungal agents. [31]

Out of one hundred fifty three patients with infectious keratitis attended Minilik II Memorial Hospital the prevalence of fungal keratitis was found out to be 45.1%. Prevalence's of fungal keratitis of 30.4%, 37.5% and 36.8% were reported in similar studies conducted by Pursuant Garg et al [52], Shokohi et al [53] and Narsani et al [54] respectively. A similar study conducted at University Hospital of Taiwan in 2004 reported a prevalence of fungal keratitis in only 13.5% of 476 patients with microbial keratitis Fong et al [55] which is quite lower than our study. In contrast to our study Mirshahi et al [56] and Javadi et al [57] reported prevalence 83% of fungal keratitis which is much higher than our study. This variation in the prevalence of fungal keratitis confirms

the regional difference of fungal keratitis. In line with other studies [18, 58, 59], males (58%) were predominant affected in the present study. The increased risk in males in our study was probably due to their more active involvement in outdoor activities, which consequently increases their vulnerability to this blinding disease. The result of this study showed the highest frequency mycotic keratits (47%) was in the age groups of 25 to 44 years that was more or less similar to the observations made by Upadahyay, in Nepal [60], as well as in others series [26, 50]. Our finding with regard age was also the same to the scenario in many developing countries such as south India [61], north China [15] and southeast Brazil [5]. This also may be explained by the fact that the subjects ages 25-49 in this study are the main force of the manual works, especially agricultural works, and more involved in outdoor activities.

In terms of occurrence, risk factors and therapeutic approaches, two basic types of mycotic keratitis are recognized, namely, keratitis due to filamentous fungi and keratitis due to yeast-like and related fungi (dimorphic fungi).There appears to be a strong geographical influence on the occurrence of the different forms of mycotic keratitis. The proportion of corneal ulcers caused by filamentous fungi has shown a tendency to increase towards tropical latitudes, whereas in more temperate climates, fungal ulcers appear to be uncommon and to be more frequently associated with *Candida species* than filamentous fungi [4]. This is evident by the present study in which, of the total number of fungal isolates mycelia fungi were the most common accounting 64 (83.1%) of the total isolates. *Fusarium* and *Aspergillus species* were the two predominate mycelia fungi accounting 21(27.3%) and 19(24.8 %) of the total isolates respectively, with together comprising 52.1% of the total isolates. This was followed by *Cladosporium spp and* Scedosporium *sp* accounted 6(6.5%) and 5(5.2%) respectively. *Fusarium* spp. as the predominant species is reported from South Florida and Ghana [62], southeast Brazil [5], north China [15], Malaysia [63] where the climate is warm and humid like Ethiopia. This is contrast to most reports of *Aspergillus spp*. from north India [18] and *Candida* from some developed countries [8, 16]. In the present study yeast isolates accounted only 15.6% of the total fungal isolates. An overwhelming majority of patients had predisposing factors, either local or systemic, that favored the development of keratitis. Ocular trauma was the most common underlying risk factor accounting 78% of fungal keratits.

7. Conclusion and Recommendation

The prevalence of mycotic keratitis was found to be very high. *Fusarium* and *Aspergillus species* were the dominant fungi recovered in this study. Mycotic keratis was dominant in rural population and trauma was the leading predisposing factor. Clinical suspicion by the ophthalmologists should be confirmed by microbiological finding prior to initiating corticosteroids. Precise identification of the causative fungus and institution of appropriate treatment strategy could save the blindness of the eye

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Annexes

Annex I English Versions of Participant Information Sheet

My name is TIHITNA KIBRET. I am a laboratory technology postgraduate student at Addis Ababa University. Now I am conducting a study entitled The prevalence of fungal keratits in minillik II memorial hospital, Addis Ababa, Ethiopia You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction

The topic of this study is the prevalence of fungal keratits in Minilik II, memorial hospital Addis Ababa, Ethiopia. Since fungal keratitis is the major opportunistic health problems in the world, the result of the study can be helpful in planning and intervention to solve the problem in our country. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet.

Expected from participants

As a participant of this study, you are expected to give corneal scraping. Being asked to give sample does not necessarily mean that you have the disease. When you are found to be positive for the micro-organisms, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required

You will spend 5-7 minutes until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will be done using sterile 21 gauge needle, that will have no effect and you will not get any risk as the sample will be collected by well trained professional

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access 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 benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems fungal keratitis. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:

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

Annex II Amharic Versions of Participant Information Sheet

እኔ **ትህትና ክብረት** በአዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ፣ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ሊይ እገኛለሁ፡፡ እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ በጥናቱ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃሁ፡፡

መግቢያ

የጥናቱ ርዕስ በአይን ላይ በስታ የሚያመጡ ፈንገሶች ስርጭት በ ምኒልክ መታሰቢአጠቃላይ ሆስፒታል ኢትዮጵያ'' በሚል ርዕስ እያጠናሁ እገኛለሁ፡፡ ይህ ጥናትም በተሳታፊ ሙለ ፈቃዮኝነት ላይ ተመስርቶ በአይን ላይ በስታ የሚያመጡ ፈንገሶች ስርጭት መጠቆም ያስችላል፡፡

ከጥናቱ ተሳታፊ የሚጠበቁ

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ከአይን ውስጥ ናሙና ተጠርጎ እንዲወሰድ መስማማት ይጠበቅቦታል፡፡ የጤና ባልሙያ ከእርሰዎ ናሙናዉን ይሰበስባል፡፡ ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች የእርሶን ማንነት የማይገልጹ ማስረጃዎችን ማለትም ስም፣ አድራሻና የመሳሰለት መረጃዎች ሳይጨምርና ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር በመጠቀም ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል፡፡ ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም፡፡ በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈሊጊውን ህክምና ያገኛሉ፡፡

የመረጃዉ ሚስጥራዊነት

ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ፡፡ ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍ-ቃል ባለዉ የኮምፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

ተሳታፊዉ የሚያጠፋዉ ጊዜ

የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 5-7 ደቂቃ ያስፈልጋል፡፡

በጥናቱ በመሳተፍ የሚስከትላቸዉ ችግሮች

ናሙና በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልቦትም፡፡ ሆኖም ናሙናዉ በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል፡፡

በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች

ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደመሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም$ ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ላሉ በሽተኞች በመረጃ ላይ የተመረተ ህክምና ለመስጠት ያግዛል$ ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ፡፡

የጥናቱ ተሳታፊዎች መብት

ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተና ተሳትፎዎን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ፡፡በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቛረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም፡፡ ከዚህም በተጨማሪ ጥናቱን

በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠቅና ገለፃ የማግነት መብት አለዎት፡፡ የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል፡፡

ግንኙነትና ጥያቄ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥዉ ድንገተኛ ችግር ወይም ጥያቄ ካለኦት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ተመራማሪ፣ **ትህትና ክብረት**

(ቢ.ኤስ.ሲ) ሞባይል +251912992834, ኢ-ሜይል፤ askulove2005@gmail.com

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል! የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

አማካሪ፣ አዳነ ቢተዉ (ፒ.ኤች. ዲ) ሞባይል +251911039162, ኢ-ሜይል፤ bitewadane@gmail.com

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል! የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ፤ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ;

ስልክ-+251112755170

ከዚህ በታች የሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መስማትዎን እና መገንዘብዎን የሚያሳይ ነው፡፡ ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ፣ የተሳትፎ ጉዳትና ጥቅሙ፣ የመተው፣ የማቋረጥ፣ መብትና ነፃነት እንዳለዎት ይረዱ፡፡ ተስማምተዋል? የጥናቱን መግለጫ አንብቢያለሁ/ ሰምቻለሁ እናም ተረድቻለሁ፡፡ መመሪያው ምን እንደ ሆነና በእኔ ምን ሊከሰት እንደሚችል ተረድቻለሁ፡፡ በጥናቱ ላይ ለመሳተፍ፤

እስማማለሁ ---------- አልስማማም ----------

Annex III English Versions of Consent form

This page contains an agreement signature to participate in the study entitled with prevalence of Fungal Keratins in MinilikII memorial hospital, Addis Ababa, Ethiopia. So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study "prevalence Of Fungal Keratits in Minilikii terriary hospital Addis Ababa Ethiopia" and I can communicate with the investigators when I want them.
2. I know that the information/ specimen that I will give used for this study only.
3. I understand that, all the information given for the study and the results are confidential.
4. I understand that I will not get any money for my participation.
5. I understand that I have a right to stop from participation any time in the study.
6. I understand all the information which is explained by specimen collector.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Evidences of the agreement

1._____

2._____

N B Please directs any questions or problems you may encounter during this study to:

Tihitna Kibret

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Mobile: +251912992834

Email:askulove2005@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170 24

Annex IV Amharic Versions of Consent form

የተሳታፊ ስምምነት ቅጽ

ይህ ገጽ “prevalence of mycotin keratits in minilikII memorial hospital” ማለትም በአይን ላይ በስታ የሚያመጡ ፈንገሶች ስርጭት በ ምኒልክ መታሰቢአጠቃላይ ሆስፒታል ኢትዮጵያ” በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነዉ፡፡ በመሆኑም እባክዎን ከዚህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ፊርማዎን ከታች በተሰጠው ቦታ ሊይ እንዱፈርሙ እጠይቃለሁ፡፡

1. እኔ በአይን ላይ በስታ የሚያመጡ ፈንገሶች ስርጭት በ ምኒልክ መታሰቢአጠቃላይ ሆስፒታል ሆስፒታል ኢትዮጵያ” የሚለዉ ጥናት አላማ በደንብ ተገንዝቤአለሁ፡፡
2. ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ፡፡
3. ሁለም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ፡፡
4. በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ፡፡
5. በጥናቱ ያለመሳተፍ እንዱሁም በማንኛዉም ጊዜ የማቃረጥ መብት እንዳለኝ አዉቄአለሁ፡፡
6. ሁለም መረጃዎች በአስተባባሪዉ/ዎች ተገልጾልኝ በደንብ ተረድቻለሁ፡፡

የተሳታፊ ፊርማ፡ ----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

በስምምነቱ ወቅት የነበሩ ምስክሮች

1._____

2._____

ይህንን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ትህትና ክብረት

ሞባይል፡ +251912992834

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል

የጤና ሳይንስ ኮሌጅ፤

አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይሌ፤ askulove2005@gmail.com

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

ስልክ፡ +251 112 75 51 70

Annex V English Version of Ascent form for less than 18 Years Participants

This page contains an agreement signature for family members or other adult guard for participants under the age of 18 years to participate in the study entitled with "prevalence of fungal keratitis from eye patients in Minilik II memorial hospital, Addis Ababa, Ethiopia".

For this study samples will be collected using sterile swab. The sample collection procedure does not induce pain or does not have any danger.

The sample collected will be used only for the study purpose and the result is strictly kept confidential and if the disease causing agent is found in your sample the results will be communicated to your physician for the appropriate management.

You will get no financial benefits. Participation in the study is completely voluntarily. You can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits.

Do you allow the (name) to participate in the study?

Allow Participation

Signature of the participant: _____

Signature of the Guardian:_____

Address of the participant: _____

Date: _____

Evidences of the agreement

1._____

2._____

Please direct any questions or problems you may encounter during this study to:

Please direct any questions or problems you may encounter during this study to:

Tihitna Kibret

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Mobile: +251912992834

Email:askulove2005@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170 24

Annex VI Amharic Version Ascent form for under 18 Years Participants

ይህ ገጽ “prevalence of fungal keratitis from eye patients in minilik II memorial hospital, Addis Ababa, Ethiopia”,” ማለትም በአይን ላይ በስታ የሚያመጡ ፈንገሶች ስርጭት በ ምኒልክ መታስቢአጠቃላይ ሆስፒታል በሚል ርዕስ እድሜያቸው ከ18 አመት በታች ሆሆናቸው ተሳታፊዎች ስምምነት የመጠየቂያ ቅጽ ነው፡፡

ናሙና የምንወስድበት መሳሪያ ንጽህናው ሙሉ በሙሉ አስተማማኝና ከዚህ በፊት ጥቅም ሊይ ያልዋለ ነው፡፡ ናሙና በምንወስድበት ጊዜ የሚኖር ህመም የሚፈጥር ስሜትም ሆነ አደጋ የሚያስከትል ሂደት የለውም፡፡

ለጥናቱ የሚወሰደው ናሙና ለጥናቱ አላማ ብቻ ይውላል፡፡ የናሙናው ውጤት ምስጥራዊነት የተጠበቀ ሲሆን በናሙናው ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡ በጥናቱ ላይ በመሳተፍዎ ምንም የገንዘብ ክፍያ አያገኙም፡፡ በጥናቱ ለመሳተፍ የመፍቀድም ሆነ ያለመፍቀድ እንዱሁም በማንኛዉም ጊዜ የማቋረጥ መብት አለዎት፡፡

(ስም) በጥናቱ እንዲሳተፍ/ እንድትሳተፍ ይፈቅዳሉ?

ፈቃዮኛ ከሆኑ፤

የተሳታፊ ፊርማ፣---------- የፈቀደው ግለሰብ ፊርማ፣----------

አድራሻ፣---------- ቀን፣----------

በስምምነቱ ወቅት የነበሩ ምስክሮች

1._____

2._____

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ትህትና ክብረት

ሞባይል፡ +251912992834

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል

የጤና ሳይንስ ኮሌጅ፤

አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይሌ፤ askulove2005@gmail.com

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

ስልክ፡ +251112755170

Annex VII: Laboratory test Procedure for Specimen Collection and Processing

Sample collection, processing, inoculation, and identification will carried out using different microbiological methods described by different researchers [64]

A Collection and processing of corneal scraps

1. The procedure is explained to the patient.
2. The patient is positioned comfortably at the slit-lamp.
3. The patient must be instructed to keep both eyes open during the procedures as blinking will only add to discomfort.
4. Local anesthetic eye drops are instilled to the affected eye to minimize ocular discomfort and facilitate the corneal scraping procedure.
5. A sterile platinum loop or a sterile needle is used to scrape the base of the ulcer with care. This is to ensure that the infective material is reached as the micro-organisms may lie deep or at the edge of the ulcer.
6. The collected material is plated on the growth media
7. At the end of the procedure, the patient is given instruction in appropriate care, i.e., hand washing, lid hygiene and instillation of an antibiotic.
8. All specimens are clearly and correctly labeled before being sent to the microbiological laboratory.
9. Incubate at 25 ^{0}c for 4 weeks. Plates with no growth after 4weeks will be discarded
10. Use a pure colony for the identification of the species using standard conventional mycological methods. Macroscopic and Microscopic
11. Examine under 10X microscope with lacto phenol cotton blue

B Identification Tests for Yeasts

1. Germ Tube Test

The germ tube test provides a simple, reliable and economical procedure for the presumptive identification of *Candida albicans*. About 95% of the clinical isolates produce germ tubes when incubated in serum at 35^0C for 2.5-3 hours. Germ tubes represent the initiation of a hypha directly from the yeast cell. Germ tube formation is influenced by the medium, inoculums size and temperature of incubation.

Procedure:

1.1. Two to three fresh colonies of yeast were inoculated into 0.5 ml of human serum in a small tube.

1.2. Incubate at 37º C for 2-3hrs.

1.3. After desired period of incubation, place a loop-full of culture on a glass slide and overlaid with a cover-slip and observe under microscope for germ tube formation [42].

1.4. The appearance of small filaments projecting from the cell surface confirmed formation of germ tubes [43].

C. Reagent Preparation

1. **Lacto Phenol cotton Blue (LPCB)**

Formulae: Distilled water 20.0 ml.
Lactic acid 20.0 ml.
Phenol crystals 20.0 g.
Cotton blue 0.05 g.
Glycerol 40.0 ml.

Dissolve phenol in the lactic acid, glycerol, and water by gently heating. Then add aniline blue.

Purpose: Used for wet mount preparations of fungal cultures.

2. Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin

Approximate Formula per Liter Purified Water

Pancreatic Digest of Casein.....5.0 g

Peptic Digest of Animal Tissue.....5.0 g

Dextrose.....40.0 g

Agar.....15.0 g

Chloramphenicol.....0.05 g

Gentamicin.....0.05 g

Storage Instructions: store plates in the dark at 2 – 8°C ready for use.

For slopes: Dispense 10 ml. amounts into test tubes.

PRINCIPLES OF THE PROCEDURE

Sabouraud Dextrose Agar is a peptone medium supplemented with dextrose to support the growth of fungi. The peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Chloramphenicol is a broad-spectrum antibiotic which is inhibitory to a wide range of gram-negative and gram-positive bacteria. Gentamicin is an amino glycoside antibiotic that inhibits the growth of gram-negative bacteria.

For slopes: Dispense 10 ml. amounts into test tube. Autoclave 121^{o}C/15 minutes. Store at RT. Final pH 7.0 at 25^{0}C.

Annex VIII: Declaration

Title of Project: prevalence of Fungal Keratins in Minilik II hospital, Addis Ababa, Ethiopia. I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Principal investigator:

Name: **Tihitna Kibret (BSc)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Advisor:

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

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

_____