ORIGINAL ARTICLE

Comparison of KOH Mount & Fungal Culture in the Diagnosis of Onychomycosis

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ABSTRACT

Objective: Comparison of potassium hydroxide mount and mycological culture in the diagnosis of onychomycosis.
Study design: Cross-sectional, comparative study.
Setting: Study was carried out from September 2009 to July 2010, on one hundred twenty four patients in the Department of Microbiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), who were clinically diagnosed as a case of Onychomycosis with the collaboration of Dermatology department, JPMC.
Method: Specimen were taken from the department of dermatology, JPMC and processed for the diagnosis by potassium hydroxide mount and mycological culture in the department of Microbiology, BMSI. Potassium hydroxide mounts was microscopically evaluated for the presence of thread-like branching structures (hyphae) or beaded spherical structures (spores). If present, it was considered as a positive test. Mycological culture was done using Sabouraud's dextrose agar at 25ºC and at 37ºC. Observation for growth was done periodically for 4 weeks; if growth was present then the pathogen was identified by cultural characteristics and microscopy.
Results: KOH mount showed fungal element in 76 specimens while in 52 patients the fungal culture was positive.
Conclusion: KOH mount was more effective than fungal culture.
Key words: Onychomycosis, KOH mount, Fungal culture

INTRODUCTION

Nails are an appendage of the skin. It is a horn-like envelop covering the dorsal aspect of the terminal phalanges of fingers and toes and are made of a tough protein called keratin[1].

It consists of the following structures: proximal and lateral folds, cuticle, matrix, nail plate (commonly called the nail), nail bed, and hyponychium. The cuticle is the horny layer of the proximal nail fold. The matrix is responsible for the production of the cells that become the nail plate. The distal, visible part of the matrix is called the lunula[2].The nail plate is the largest structure of the nail unit and grows by sliding forward over the nail bed, where upon the distal end becomes free of the nail bed[3].

A healthy nail is aesthetically appealing and protects the distal phalanx, the fingertip, and the surrounding soft tissues from injuries. It also serves to enhance precise delicate movements of the distal digits through counter-pressure exerted on the pulp of the finger[4]. It act as a counterforce when the end of the finger touches an object, thereby enhancing the sensitivity of the fingertip[5]. Onychomycosis is the invasion of the nail plate caused by any fungus. The infection may be due to dermatophytes, non dermatophytes mold or yeast[6]. The term Tinea unguium can be applied only if the infection is due to a dermatophyte[7].

Incidence of onychomychosis is increasing worldwide in spite of improved personal hygiene and living environment and now it is not considered merely a cosmetic problem[8].

It has an important impact on the life of affected individuals. Psychosocial and emotional effects resulting from the disease may affect quality of life[9].

Fungal nail disease is common in people with other nail problems (e.g., a history of nail trauma), in immunocompromised persons (e.g., those with diabetes mellitus or human immunodeficiency virus infection or those taking immunosuppressive medications), in persons with peripheral vascular insufficiency[10]. The condition is associated with Tinea pedis in one third of cases[11].

Adults have infection rates 30 times higher than children. The reasons for this increase in the prevalence of onychomycosis in adults in comparison to children may include (i) above mentioned conditions that may be present in adults, (ii) more exposure to fungi because of

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slower nail growth in adults as compare to children and (iii) larger nail surface for invasion[12,13].

In a small percentage of persons, onychomycosis may also be caused by a genetic defect that causes an alteration in immune function[14].

MATERIALS AND METHODS

The study design was cross-sectional comparative study which was carried out on one hundred twenty four patients who were clinically diagnosed as a case of Onychomycosis during the period from September 2009 to July 2010. Descriptive statistics for KOH mount and mycological culture (with frequency and percentage) of the type of fungus and organisms and comparison between these two methods were evaluated. Data was collected and results tabulated. All patients suspected with clinical diagnosis of Onychomycosis, irrespective of age or sex referred by Dermatology Department JPMC was included in the study. With the exception of those clinically diagnosed for Onychomycosis, all other lesions were excluded.

One hundred twenty four patients who were clinically diagnosed as a case of onychomycosis were included in the study. Specimen were taken from the department of dermatology, JPMC and processed for the diagnosis by potassium hydroxide mount and mycological culture in the department of Microbiology, BMSI.

Potassium hydroxide mounts were microscopically evaluated for the presence of thread-like branching structures (hyphae) or beaded spherical structures (spores). If present, it was considered as a positive test.

Mycological culture was done using Sabouraud's dextrose agar at 25ºC and at 37ºC with and without antibiotics. Observation for growth was done periodically for 4 weeks; if growth was present then the pathogen was identified by cultural characteristics and microscopy. Non-probability convenience sampling technique was applied.

RESULT

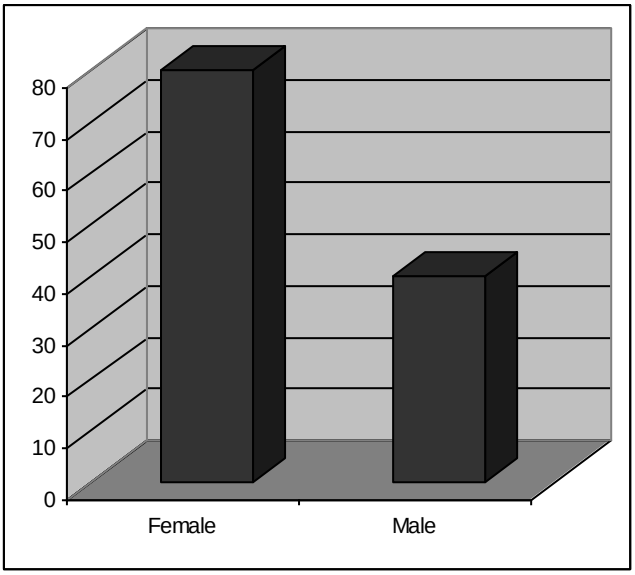
Demographic profile in our study was among these one hundred twenty four patients eighty two (66.13%) were female and forty two (33.87%) were male. Age range was from 14 to 80 years. Maximum patients were between the age ranges of 14-24 years. One hundred thirteen (91.13%) patients were between 14-50 years of age while only eleven (8.87%) were more than 50 years of age. Among these six patients were between 51-60 and four patients were 61-70 years while only one patient was 80 years of age.

KOH mount shows fungal element in seventy six specimens while no fungal elements was seen in forty eight specimens.

Fungal culture was positive in fifty two patients while negative in seventy two patients with dermatophytes in 26, Candida in 16 and non-dermatophyte moulds (NDM) in 10 patients which includes Fusarium in 4 samples, Chrysosporium spp in 2 samples, Aspergillus fumigatus in two samples, Aspergillus niger in one sample,Scopulariopsis spp in one sample.

Trichophyton rubrum was the commenst and candida were the second most common organisms isolated in the study.

Sex distribution

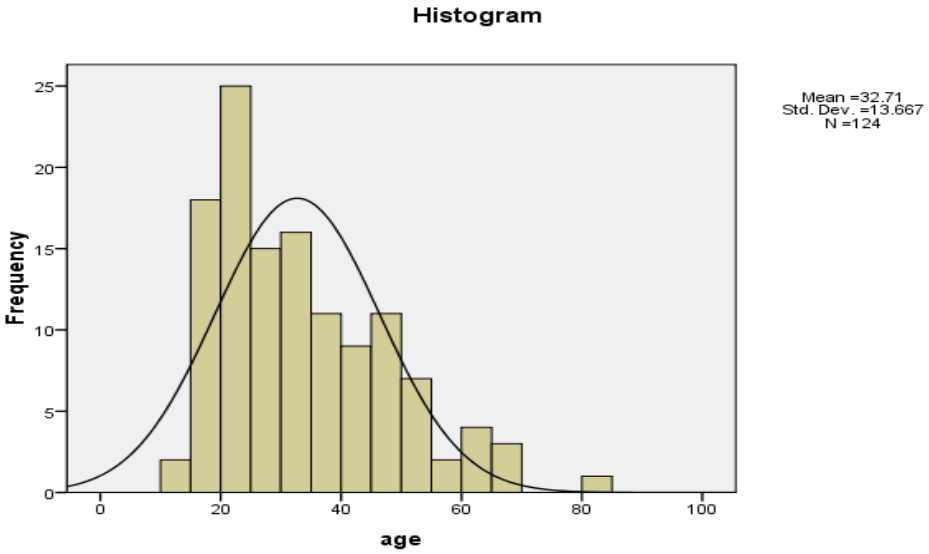


Frequency (Gender)

Valid	Frequency	%	Valid %	Cumulative %
Male	42	33.87	33.87	33.87
Female	82	66.13	66.13	100.0
Total	124	100.0	100.0	

Age

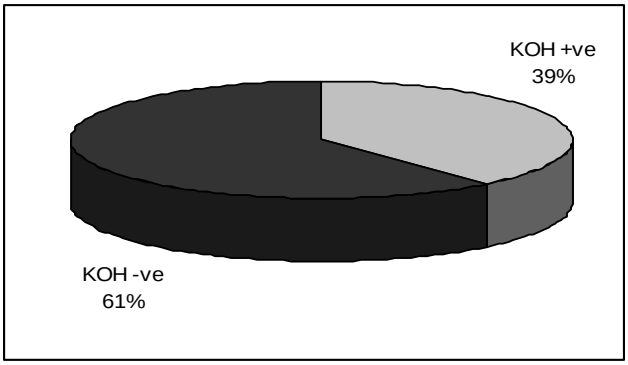
N	**Valid**	**124**
	Missing	**0**
Mean		32.71
Median		30.00
Mode		24
Std. Deviation		13.667
Variance		186.777

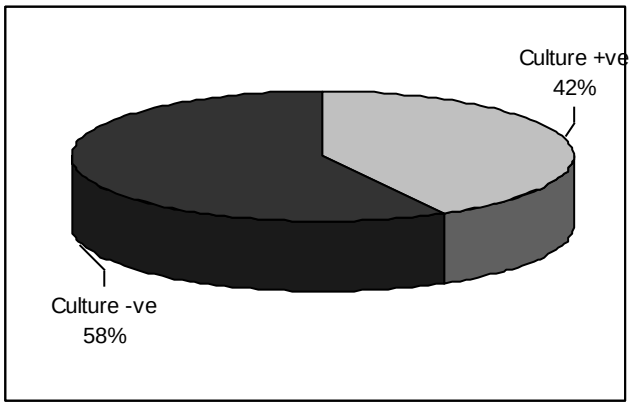


Comparison of KOH & Culture methods

Test	+ve	-ve	%age
KOH Mount	76	48	61.30
Fungal culture	52	72	41.93

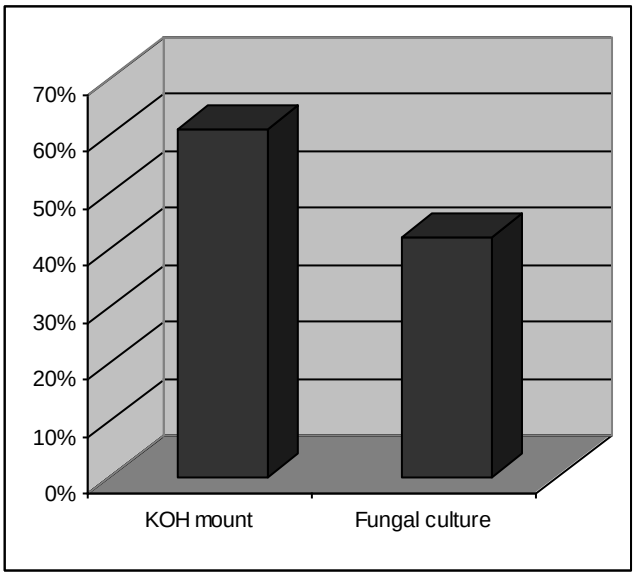
KOH mount





Comparison of KOH & Culture methods

Fungal culture



Out of the 124 patients, direct microscopy with KOH mount and mycological culture showed positive results in 76(61.3%) and 52(41.93%) patients respectively. Hence yield of KOH mount is more than mycological culture in the diagnosis of onychomycosis.

DISCUSSION

Onychomycosis is the invasion of nail plate by fungus which may be due to dermatophytes, non-dermatophyte molds or yeast. It affects approximately 5% of the population world wide and accounts for up to 50% of all nail diseases. It occurs in all ages but children have infection rates lower than the adults[12].

It is necessary to diagnose the infection with some laboratory evidences before treating them with antifungal drugs, where duration of treatment is long and may have some serious side effects.

Female preponderance (66.13%) was observed in our study with a male to female ratio of 1:1.95 while Elewski[15] showed a male preponderance. Bokhari et al[16] observed 72% female while male were 28% in their study. The study was conducted in Department of Dermatology, King Edward Medical College/Mayo Hospital, Lahore, This is a local study and comparable with our study, while Cohen et al.[2] included the male gender as a general risk factor for onychomycosis.

The reason for the female preponderance may be; because majority of the patients belonged to low socioeconomic class so they can not afford the treatment especially when it is not producing any problem apart from an ugly look. Male usually do not care for their diseases producing only aesthetic problem. Male attended the dermatology clinic after a long duration of the disease when they had some secondary infection. Females are very much conscious about their beauty and outlook. Nails are a part of the look, particularly in young age they are very sensitive for the problem. Also the females are usually involved in using water in kitchen, in cleaning of house, washing clothes, using detergents especially those who are very poor and work as housemaid. These may be the reasons that in our study female preponderance was found and majority of the patients were of younger age.

In our study the age range was from 14 years to 80 years with a mean of 32.58 years. Maximum patients were between the age ranges of 14-24 years and were forty six. Thirty six were between 25-35 years of age, thirty one between the ages of 36-50 years. 113 (91.13%) patients were between 14-50 years of age while only eleven (8.87%) were more than 50 years of age. Among these six patients (4.84%) were between 51-60 and four (3.22%) were 61-70 years and only one (1%) patient was of 80 years of age. Minimum age was 14 years while maximum was 80 years. Elewski[15] in his study observed that among 200 patients suffering from the disease, none was younger than 20 years and almost 24% of those patients aged 70 years or older had the disorder. Similarly in another study by Elewski and Charif [17] found 28.1% of the patients aged 60 years or older for onychomycosis, versus 1.1 and 2.9% for those aged 10 to 18 years and 19 to 30 years, respectively and explained for the age-related increase in onychomycosis due to the reasons already mentioned. As we have mentioned above 91.13% patients were between 14-50 years of age in our study and only 8.87% were more than 50 years of age. Similarly, in a local study conducted by Bokhari et al (1999) the mean age in seventy-two women was 32.6±14.8 years) while in 28 men (mean age, 40.6±15.8 years) which is again consistent with our study.

Cohen et al.[2] described the general risk factors for any type of onychomycosis which are very much consistent with our study as we found these factors present in most of the patients.

Gianni et al[18] found direct microscopy was positive in 59.3% nail specimens, histological examination was positive in 54.6% samples and fungal culture was positive in 90 cases (52.9%), showing a dermatophyte in 45, a yeast in 23 and a mold in 22 samples. The histological examination was positive in 94(54.6%) samples. In above study 45 (50%) out of 90 positive samples were dermato-phytes and 45(50%) were non-dermatophytes. In 45 non dermatophytes, 23(25.55%) were yeast and 22(24.44%) were molds. In our study dermatophytes were 26(50%) while non-dermatophytes were

26(50%) in number out of 52 isolated organisms. In non-dermatophytes 16(30.77%) were yeast while 10(19.23%) were mold. In our study the percentage of the organisms isolated is consistent with the study of Gianni et al[18]. Also direct microscopy was positive in 59.3% in Gianni et al[18]. Study which is very much consistent with our study while positive culture is 41.93% in our study which is less than that of Gianni et al and was 52.9%.

In the study by Bokhari et al[16] conducted in Lahore, *Candida* was the most common pathogen (46%), followed by dermatophytes (43%); *Trichophyton rubrum* (31%), *T. violaceum* (5%), *T. mentagrophytes* (4%), *T. tonsurans* (2%), and *Epidermophyton floccosum* (1%) and nondermatophyte molds (11%); *Fusarium* (4%), *Scopulariopsis brevicaulis* (2%), *Aspergillus* (2%), *Acremonium* (1%), *Scytalidium dimidiatum* (1%), and *Alternaria* (1%) while the fungi which were isolated in our study; *T. rubrum* (32.5%), *T. mentagrophytes* (17.5%), *T. tonsurans* (2.5%), *Candida* (22.5%), *Fusarium spp (10%), Chrysosporium spp.* (5%), *Aspergillus fumigatus* (5%), *Aspergillus niger* (2.5%), *Scopulariopsis spp* (2.5%). Dermatophytes were found 43% which is comparable with our study in which the dermatophytes were 50%. Among these dermatophytes Bokhari et al [16] isolated *Trichophyton rubrum* 31% which is consistent with our study and it was 32.69%.

Weinberg et al.[19] evaluated 105 cases of onychomycosis with four diagnostic tests, which were KOH mount, culture; PAS and calcofluor white stain, and found KOH method more sensitive than the culture which was also found in our study.

Lilly et al. [20] compared the effect of KOH interpreted both by dermatologist (KOH-CLINIC) and a laboratory technician (KOH-LAB); KOH with dimethyl Sullfoxide (KOH-DMSO) and with chlorazol black E (KOH-CBE); culture with DTM, with mycobiotic medium; and histopathologic analysis using PAS stain. They KOH-CBE with 94.3% sensitivity. Dermatophyte test medium was the least sensitive test (57.3%).

Shenoy et al[21] who showed KOH mount was positive in 53% and culture in 35% and sensitivity of KOH mount was 64% while of mycological culture was 42% which is consistent with our study.

KOH mount is simple rapid and inexpensive. Experience is required to interpret the mount[22]. There exists a possibility of false negative results at a rate of approximately 5 to 15% by KOH. Direct microscopy is often time-consuming because nail debris is thick and coarse, and hyphae are usually sparse[15]. However, because information concerning the accurate identification of the specific pathogen is not available through KOH alone, mycological culture continues to remain the indisputable 'gold standard' of mycological diagnostics. It is the only test in routine use that can identify the species of the fungus. False negative results with culture are high and based on experience of the laboratory. It may be due to: (a) If nail sample contains only non-viable organisms. (b) If insufficient sample is collected. (c) When nail sample is clipped distal to growth. (d) When sample is not crushed before the test[19].

Our study is comparable with the study of Shenoy et al[21] who showed KOH mount was positive in 53%, culture in 35% and are very much consistent with Shenoy et al[21] and Reisberger et al[23].

CONCLUSION

From the data it was observed that KOH mount is more effective than fungal culture in the diagnosis of Onychomycosis.

RECOMMENDATION

Mycological culture has a higher specificity, but it has lower sensitivity as compared to direct microscopy by KOH mount. Direct microscopy by KOH mount is not an alternate test to mycological culture. It is a simple and sensitive screening test and can be performed as an OPD procedure prior to treatment. Therefore KOH staining should be performed routinely as a standard of practice in the Dermatology OPD for the diagnosis of onychomycosis.

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