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A COMPARISON OF POTASSIUM HYDROXIDE AND CALCOFLUOR WHITE FOR RAPID DIAGNOSIS OF SUPERFICIAL MYCOSIS

Medical Microbiology

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ABSTRACT

Background: Dermatomycosis constitute a group of superficial fungal infections of keratinised tissue-skin, hair and nails, caused by dermatophytes, Candida and Malassezia. Superficial mycosis is commonly seen in hot, humid climate countries like India, Sri Lanka etc. **Objectives:** To compare potassium hydroxide (KOH) and calcofluor white (CFW) for rapid diagnosis of superficial mycosis. **Methodology:** The study was conducted from September 2020-September 2022 at Kempegowda institute of medical sciences, Bangalore. All cases with clinical features suggestive of superficial fungal infection, were subjected to mycological work-up in the department of microbiology. Specimens like skin scraping, hair, and nail were collected and microscopically examined using 10%, 20% and 40% KOH respectively for fungal filaments. All the samples were also stained using CFW. Culture was put up on Sabouraud dextrose agar medium with and without cycloheximide. **Results:** There were a total of 4499 clinically suspected samples in the study. The infection was found to be common in male than female patients & most common affected age group being 21-30 years. The KOH was positive in 54.63%, CFW was positive in 57.83% and culture in 31.07% samples. Of the positive cultures, 57.77% were dermatophytes, 35% Candida species and 7.22% Malassezia species. **Conclusion:** In the present study CFW was more sensitive when compared to KOH and culture.

KEYWORDS

Dermatophytes; Sabouraud dextrose agar; Potassium hydroxide; Calcofluor white stain

INTRODUCTION

Superficial mycoses are commonly occurring fungal diseases prevalent in most part of the world and in tropical country like India. It is a fungal disease infecting skin, hair and nail. This group includes candidiasis, dermatophytosis and pityriasis versicolor.[1]

The microscopic examination is generally done by potassium hydroxide (KOH) and calcofluor white fluorescent stain (CFW).[2] Direct microscopic examination of specimens in suspected cases of superficial mycosis provides early detection when compared to culture, which may take days or weeks.

KOH examination is very simple, fast and most cost-effective technique used for the diagnosis of fungal infections .[3]The CFW is a very sensitive technique which helps in the identification of fungal elements, even in small quantities.[2]

KOH is a keratolytic reagent but does not provide color contrast and requires skilled expertise to report.[4] The CFW application for rapid detection of fungi was first introduced by Hageage and Harrington in clinical mycology[5]. CFW is a non-specific, white fluorochrome stain having ability to bind particularly on cellulose and chitin contained in the fungal cell wall. Cotton fibers fluoresce strongly and therefore to be differentiated from fungal hyphae and depending upon the filter being used, fluoresces either blue white or an apple green colour. The fluorescent staining description for diagnosis of superficial mycoses was made by Chick and Behar 1991.[5]

Cultures are more specific and is the gold standard method for the diagnosis of superficial mycosis and can also help in identifying species.

Objective

The aim of this study was to compare KOH, CFW and Culture in the diagnosis of superficial mycoses and to determine their sensitivity and specificity.

MATERIALS AND METHOD

Methodology

Study Design: Retrospective study

Sample Population: Patients from the outpatient department of Dermatology, Kempegowda Institute of Medical Science and Research Centre, Bangalore with clinical features suggestive of superficial fungal infection, were referred to the department of Microbiology, from September 2020 to September 2022.

Sample Size: A total of 4499 patients with clinical features suggestive of fungal infection were selected.

Methodology: Depending on the site involved, samples like skin scrapings, hair plucking, nail clippings and from undersurface of the nail bed were collected on a slide. All samples were subjected to KOH mount, CFW and fungal culture. Rapid screening of the specimen was done by CFW fluorescent stain method and by KOH microscopy using fluorescent and compound microscope respectively.

Sample Collection: Following aseptic precautions with 70% povidone iodine and then with alcohol, skin was scraped in a wedge shape from inflamed region to healthy region using number 22 scalpel blade. Hair was plucked with epilating forceps, nail was clipped and material from underneath the nail bed was scrapped. All the samples were collected in the mycology section of the department of Microbiology.

Because of the paucity of fungal element in the clinical specimen, maximum amount of clinical material was collected.

Preparation of CFW

A 1% (w/v) stock solution of CFW in deionized water was prepared with gentle heating to help dissolve the powder. The resulting solution was colorless. This stock solution was diluted to 0.1% for routine use. To maintain the stability of the solution, as it may vary depending on the source of the powder, the solution was stored in the dark at room temperature. The mixture of CFW solution and KOH is unstable and hence cannot be stored. So, CFW solution was mixed with KOH solution just before use.[3]

Staining Procedures

KOH mount: 10%, 20% and 40% KOH wet mounts were prepared for skin, hair & nail respectively. The samples were incubated at 37 °C for 20-30 min for skin scrapings, 30 min to 1 hour for hair and 2-4 hours for nail samples. To look for the presence of branched septate hyphae/arthrospores, the wet mounts were screened first under low power 10X and if present, confirmed by high power 40X of compound microscope. Samples were reported positive for fungal filaments if any of the above structures were seen.[6](Figure 1, 2 and 3)

Figure 1

Figure 2

Figure 3

KOH mounts showing refractile, branched, septate hyphae & arthrospores seen under low & high power of bright field microscope.

Calcofluor White: Two drops of calcofluor white stain (comprising 1g/L calcofluor white and Evans blue) was added to the specimen on the slide. The slide was then left to stand for 10 minutes and was examined under fluorescence microscopy using blue light excitation (300-400 nm for the emission wavelength with excitation at around 355 nm) and looked under 10X and 40X of fluorescent microscope for apple green filaments.[7] (Figure 4)

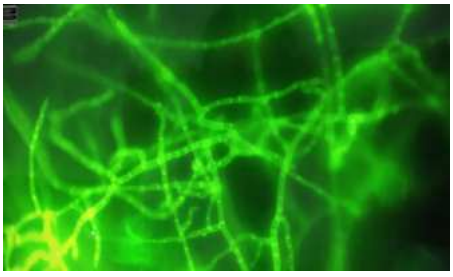

Figure 4- Apple green branched septate filaments under fluorescence microscope

Fungal Culture: Two tubes of Sabourauds dextrose agar (SDA) with chloramphenicol and with & without cycloheximide were used for the growth of fungus. The material was directly inoculated onto the surface of SDA media and incubated aerobically at room temperature (~25 °C) for 3 weeks. The tubes were examined daily during the first week and twice weekly during the next two weeks. The isolates were identified by standard laboratory procedures (LPCB) and in case of any doubt in morphology, was confirmed by slide culture.[3] If KOH/CFW showed spaghetti and meat ball appearance, an additional two tubes of SDA were inoculated, one of them with overlay of olive oil.

RESULT

Out of the 4499 patients, direct microscopy showed fungal filaments in 2458 (54.63%) in KOH mount, 2602 (57.83%) in CFW mount. Fungal culture showed growth in 1357 (31.07%) patients. (Table 1)

Of the total, positivity was seen more in males, 2891 (64.3%) were male and 1608 (35.7%) were female patients. Most affected age group was 21-30 years, which is 2997 (66.6%) patients.

Based on clinical diagnosis, 50.08 % cases were of Tinea corporis, 41% of Tinea cruris, 22 % cases showed both T.corporis and T.cruris, followed by onychomycosis in 21% , Tinea capitis in 11%, and least was Tinea mannum in 3% of the cases.

Growth in fungal culture showed 784 (57.77%) dermatophytes, 475 (35%) Candida species and 98 (7.22%) Malassezia species.

Among dermatophytes, Trichophyton rubrum was isolated in 52.4% followed by Trichophyton mentagrophytes in 44%. Among the Candida species, Candida albicans was isolated in 322 (67%) followed by Candida tropicalis in 153 (32.2%).

A total 139 clinical cases of Tinea versicolor were seen, out of which 121 were positive by KOH and 130 were by CFW stain and only 98 were culture positive.

Table 1. Comparison of the performance of KOH and CFW stain considering culture as gold standard

Test	Total Positive	Total Negative	Total
KOH	2458	2041	4499
CFW	2602	1897	4499
CULTURE	1357	3142	4499

DISCUSSION

Superficial fungal infection forms a large group of patients attending the Dermatology outpatient department. Indian subcontinent has a varied topography, high temperature, body sweating and humid climate which facilitates the acquisition of fungal infections. Identification of fungi causing superficial mycosis is necessary, to find out the source of infection, for treatment and to exclude other skin disorder which mimic superficial fungal infections making laboratory diagnosis essential.[8]

In our study, out of the 4499 patients, males (64.3%) and females (35.7%), this male preponderance could be explained by the fact that males tend to have more outdoor activities, are less concerned about personal hygiene or appearance than female counter part. A similar male predominance is also reported in study done by Shakthi R et al with male 62% and females 38%.[3]

The commonest clinical type was T.corporis (62%), which is corroborated with other study done by Vijaya D et al with T.corporis 62%.

Our direct microscopy with KOH mount, calcofluor mount and mycological culture showed positive results in 2458 (54.63%), 2602 (57.83%), 1357 (31.07%) respectively. Similar to studies done by S Manick Dass et al showed KOH (56%), CFW (63.33%) and Culture (39.33%) respectively.[6]

Commonest dermatophyte was T.rubrum (52.4%) similar to study done by Vijaya D et al showing T.rubrum (64.06%). This species might have developed increased virulence and better adaption to hard keratin of skin, hair and nail leading to its increased prevalence.[8]

In case of Candida species, C.albicans was 322 (67%), C. tropicalis was 153 (32.2%), similar was isolated by Vijaya D et al C.albicans being (54.54%) C.tropicalis [5] (45.45%).[8]

CONCLUSION

Superficial mycosis is the commonest fungal infection occurring more commonly in males than females. T.corporis is the commonest clinical type and T.rubrum is the commonest fungi. CFW stain and KOH together has higher positivity rate than culture.

Using fluorescent microscopy, is less eye straining & large number of samples can be easily screened for the presence of fungal filaments.

Minimum time required for screening the sample in KOH mount takes 3-4 minutes for screening each slide whereas in CFW preparation, slides can be viewed or screened easily on low power objective (10X) and it can be completed within 1 minute. Background or debris material can be easily differentiated using CFW whereas artifacts can be easily taken as false positive in KOH. Hence, CFW is a quick, reliable, more sensitive and specific method than KOH mount.

Overall the use of fluorescent provides an accurate alternative for the detection of fungal elements in clinical specimens in a routine laboratory setting.

Culture is taken as gold standard because of high specificity and it is the only confirmatory test in routine use for identifying the species causing dermatophytosis.[8]

Conflicts of interest – There are no conflicts of interest.
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