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RESEARCH ARTICLE

A COMPARISON OF CFW STAIN, KOH AND CULTURE FOR THE LABORATORY DIAGNOSIS OF SUPERFICIAL MYCOSES

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ABSTRACT

The purpose of this study was to compare KOH, culture and CFW stain in the diagnosis of superficial mycoses and to determine their sensitivity, specificity and predictive value. Clinically diagnosed 100 superficial fungal infection cases (males 56 and Female 44) age ranged 25 to 55yrs formed the study group. This is in collaboration with department of Dermatology, Victoria Hospital Bangalore. The study was conducted in Microbiology department AIMS BG Nagara. Samples from skin, nail and hair were collected as per individual symptomotology. Samples were processed using CFW stain, KOH and culture. ***Result*:** Of the 100 cases, T.*corporis* (62) was the commonest clinical type followed by T.*cruris*(14), onycomycoses(12), T.*versicolor*(5), T.*pedis*(4), T.*capitis* (2) and T.*faceie*(1). KOH was positive in 82, culture in 78 and CFW in 84 case. Culture isolates were dermatophytes in 64 (82.05%) and yeast like fungi in 14 (17.94%). T.*rubrum* (64.06%) was the commonest followed by T.*gypseum* (17.18%), T.*mentagrophyte*(14.06%), T.*tonsurans*(1.56%), T.*canis*(1.56%), and T.*violaceum* (1.56 %). Among the yeast like fungi isolates were Candida spp (78.57%) and Malssisia furfur (21.42%). ***Discussion and Conclusion:*** Considering culture as gold standard, KOH showed sensitivity of 95.12%, specificity 73.33%, PPV 90.69 NPV 84.61 and CFW stain showed sensitivity of 100%, specificity 78.57, PPV 92.85, NPV 100. CFW stain has the advantage compared to KOH and culture, being rapid (30-60sec), simple, reliable, easy visibility at low power, with high sensitivity and specificity and inexpensive if fluorescent microscope is available in the laboratory.

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INTRODUCTION

The use of calcoflour white stain (CFW) in clinical mycology was first described by Hageage and Harrington, has found extensive use in this area for rapid detection of microorganisms (Hageage *et al.,* 1984). CFW is a nonspecific fluorochrome stain that bind to fungi and depending upon the filter system employed, fluoresces either an apple green or blue white colour when exposed to UV light. The first description of a fluorescent staining for the diagnosis of superficial mycoses was made by Chick and Behar (Harington *et al.,* 1991; Panasit *et al.,* 2006). Superficial mycoses are commonly encountered fungal diseases prevalent in most part of the world and tropical country like India. It is a fungal disease infecting skin, nail and hair. This group includes dermatophytosis, Pitryasis versicolor and candidiasis. The aim of the present study was to compare KOH, culture and CFW stain in the diagnosis of superficial mycoses and to determine their sensitivity, specificity and predictive value.

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MATERIALS AND METHODS

The study included 100 randomly selected, clinically suspected cases of superficial fungal infection patients attending the outpatient department of Dermatology of Victoria Hospital, Bangalore and AIMS of BG Nagara, both being tertiary care hospital .Out of 100 cases 40 were from urban and 60 rural area. The study was done from Jan 2015 to Dec 2015. After taking detailed history and clinical examination for the type, site of lesion, hair, nail and skin samples were collected as per individual symptomatology. Fresh cases who had not received any antifungal treatment were included for the study. An exclusion criterion was cases that were on treatment. Ethical clearance was obtained from the institution and verbal consent from each patient was taken. The samples were collected in a small white paper and processed in the Microbiology department of AIMS BG Nagara. Each sample was processed with 10-20% KOH mount, KOH+CFW stain and for culture. Direct microscopy:

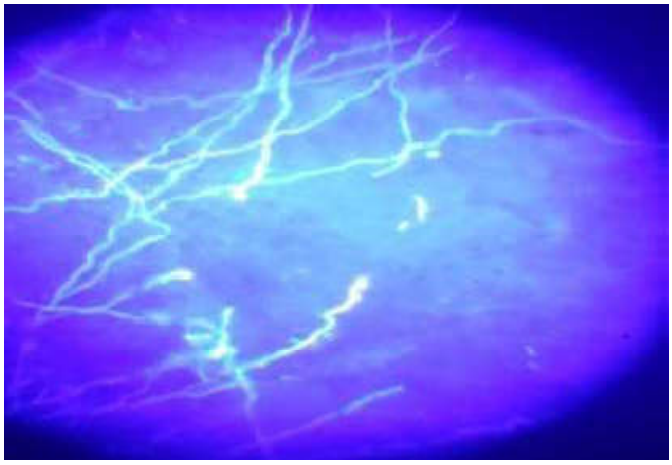
1. Wet mount preparation of the samples were performed using 10 -20% KOH and were examined under microscope for the presence fungal elements.

Table 1. Distribution of fungi isolated in relation to clinical types

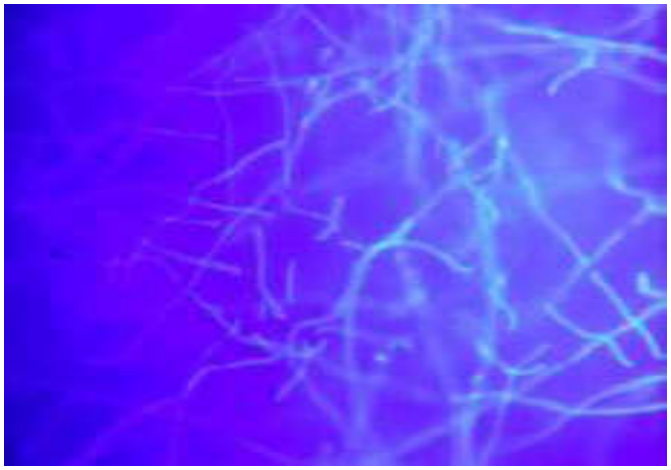
Clinical type Fungi	T.corporis No 62	T.cruris No 14	Onychomycoses No 12	T.versicolor No 5	T.pedis No 4	T.faceie No 1	T.capitis No 2	Total 100
T.*rubrum*	31	6	3	0	1	0	0	41
Tgypseum	11	0	0	0	0	0	0	11
T.*mentegrophyte*	8	1	0	0	0	0	0	9
T.*violaseum*	0	0	0	0	0	0	1	1
T.*tonsurans*	0	0	1	0	0	0	0	1
M.*canis*	1	0	0	0	0	0	0	1
Candida spp	0	4	5	0	2	0	0	11
Malassesia furfur	0	0	0	3	0	0	0	3
Total +ve	51	11	9	3	3	0	1	78
Percentage+ve	82.25	78.57	75	60	75	0	50	78

Table 2.Comparison of the performance of KOH and CFW stain considering culture as gold standard

Test	Total +ve	Total-ve	False+ve	False-ve	Sensitivity	Specificity	PPV	NPV
KOH	82	18	8	4	95.12	73.33	90.69	84.61
KOH+CFW	84	16	6	-	100	78.57	92.85	100



Calcofluor white stain preparation showing hyphal structures under fluorescent microscope



Calcofluor white stain preparation showing hyphal structures under fluorescent microscope

2. CFW Stain: 10% KOH was placed on a slide +clinical sample to which CFW stain was added (Avinash medicals, Bangalore)and examined under low power of fluorescent microscope using blue light excitation filter (300-400nm). A Candida albicans positive control for CFW stain was prepared for each batch of preparations being examined. Stained preparations of C.albicans stored in a wet box at room temperature in the light, remained stable for at least 72 days (Panasit *et al.,* 2006).

Culture: Samples were cultured on Sabouraud dextrose agar (SDA) with chloramphenicol, SDA with chloramphenicol and cycloheximide and Dermatophyte test medium (DTM). T.*versicolor* samples were inoculated onto SDA with overlay of olive oil. Tubes were incubated at $37^0$and 25^0 for a period of four weeks. Isolates were identified by standard methods (Hamer *et al.,* 2006).

RESULTS

Study group included of 56 males and 44 females age ranged from 25-55 Years. Of the 100 cases studied 40 were from urban and 60 from rural area. Out of 100 samples KOH in 82%, CFW in 84% and culture were positive in 78%.

Table 1 above shows the distribution of fungi isolated in relation to clinical types.

DISCUSSION

Superficial fungal infection forms a large group of patients attending the Dermatology outpatient department of tertiary care Hospital of Bangalore and BG Nagara. The temperature in these parts is very high during summer. The high temperature and body sweating facilitates fungal growth. Indian subcontinent has a varied topography and the hot and humid climate is highly favourable for the acquisition of fungal infections (Kaur *et al.,* 2015). The importance of identifying fungi causing superficial mycosis is important, to find out the source of infection, for treatment and to exclude other skin disorder which mimic superficial fungal infections making laboratory diagnosis essential (Parameshwari *et al.,* 2015). Males (56%) preponderance may be explained by the fact that males tends to have more outdoor activities, increased perspiration and are less concerned about personal hygiene or appearance than female counterpart. Male predominance is also reported in other studies (Kaur *et al.,* 2015).

The commonest clinical type was T.*corporis* (62%), which is corroborated with other studies (Parameshwari *et al.,* 2015; Vyoma chudasma *et al.,* 2014; Aruna vyas aggarwal *et al.,* 2002; Bhavasar Hitenra *et al.,* 2012). KOH dissolves most cellular debris readily without affecting the chitinous cell wall of fungi. KOH was positive in 82%, others have reported from 53% to 91% (Kaur *et al.,* 2015; Parameshwari *et al.,* 2015; Bhavasar Hitenra *et al.,* 2012; Bhagra *et al.,* 2014; Das *et al.,*

2008; Shenoy *et al.,* 2018). The reason may be, in the present study all were fresh cases, the technique used in the collection of samples and examining under microscope may play role in positive findings. KOH preparation often contains artifact which are difficult to distinguish from fungal elements thus limiting interpretation (Hamer *et al.,* 2016). KOH showed sensitivity of 95.12%, Specificity 73.33%, PPV 90.69, and NPV 84.61. In the present study culture was positive in 78%, others have reported from 20 to 61 % (Kaur *et al.,* 2015; Parameshwari *et al.,* 2015; Bhavasar Hitenra *et al.,* 2012; Bhagra *et al.,* 2014; Das *et al.,* 2008; Shenoy *et al.,* 2018). Of the fungi isolated dermatophytes were in 64(82.05%), *Candida* spp in 11(14.1%) and Malassezia spp in 3(3.84%). T.*rubrum* (64.06%) was the commonest among dermatophyte which is similar to others repor (Kaur *et al.,* 2015; Bhavasar Hitenra *et al.,* 2012; Lone *et al.,* 2013; Veer *et al.,* 2007; Alvarez, 2014) T.*rubrum* as the most common dermatophyte, suggesting that it might have developed increased virulence and better adaption to hard keratin of skin, hair and nail leading to its increased prevalence (Kaur *et al.,* 2015; Bhavasar Hitenra *et al.,* 2012). Of the Candida spp isolated 6(54.54%) were C.albicans and C.*tropicalis* 5 (45.45%). In cases of T.*versicolor* only 3 were culture positive, but other 2 were positive by KOH and CFW stain. Isolation of M.*furfur* is difficult. CFW stain was positive in 84% cases .Four of the KOH negative were positive by CFW stain and culture. CFW staining offers excellent visualization of morphology of pathogenic fungi, when clinical material is very scanty (Chander, 2009). Abdelrahman has reported CFW showed sensitivity of 88% and KOH in 72% (Abdelrahaman *et al.,* 2016). KOH sensitivity of 95.12%, specificity 73.33%, PPV 90.69 NPV 84.61. CFW stain showed sensitivity of 100%, specificity 78.57%, PPV 92.85, NPV 100.

The combined staining method of KOH with CFW stain makes full use of the properties of KOH to digest impurities and the high sensitivity of CFW stain to detect the fungi, thereby enhancing the rate of correct diagnosis (Whihong Zhang *et al.,* 2010). It is important that the expertise of the examiner is a key factor for interpreting the result of KOH and CFW stain, as our laboratory is specialized in mycologial diagnosis, the positives will be high compared to others.As treatment is normally prescribed in empirical form, without any confirmation, it will be more appropriate to know the etiological fungi before the initiation of antifungal therapy to have an effective and rapid cure and to treat as per the report to have an more effective and rapid cure (Aruna vyas *et al.,* 2013).

Conclusion

Superficial mycoses isthe commonest fungal infection and T.*corporis* is the commonest clinical type. T.*rubrum* is commonest fungi .CFW stain and KOH together has higher positivity rate than culture.

Conflicts of interest

Authors had no conflicts of interest to declare in relation to this article.

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